Investigations into the control of placental steroid production

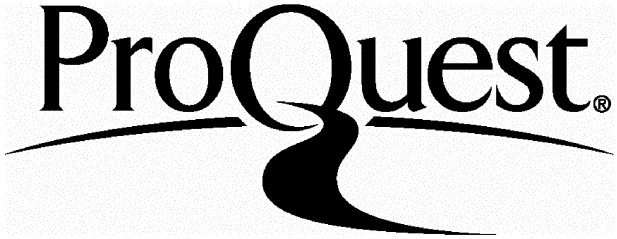
A thesis submitted for the
Degree of Philosophy of the University of London

by

Leonie Anne Shepherd

Department of Obstetrics and Gynaecology
University College London

Submitted 1997



ABSTRACT

During normal human pregnancy there is a gradual rise in plasma progesterone and oestrogen levels with a steep rise in oestriol concentrations a few weeks before term. Taken in conjunction with the known effects of progesterone and oestrogens on the myometrium these changes suggest that the regulation of the production of these steroids by the placenta may be important in maintaining uterine quiescence and in initiating labour, respectively.

The aim of this study was to assess the effect of corticotrophin releasing hormone, adrenocorticotrophic hormone and the insulin like growth factors I and II, on the placental production of progesterone and oestrogens. Placental minces and isolated cytotrophoblast cells were incubated with various steroid precursors at 37°C with/without these substances. The production of progesterone, oestrone, oestradiol and oestriol was measured by radioimmunoassay.

The effects of these substances on the enzyme systems, cholesterol side chain cleavage, 3β-hydroxysteroid dehydrogenase, aromatase and 17β-hydroxysteroid dehydrogenase were studied. There was very little cholesterol side chain cleavage activity in isolated cells when 25-hydroxycholesterol was used as a precursor but 3β-hydroxysteroid dehydrogenase, aromatase and 17β-hydroxysteroid dehydrogenase were active when pregnenolone, androstenedione and 16α-hydroxyandrostenedione respectively were used as precursors. Androstenedione inhibited the production of oestriol by isolated cells when 16α–hydroxyandrostenedione was used as a precursor.

The addition of NADPH to placental tissue explants resulted in significantly raised progesterone and oestradiol production but not oestrone or oestriol. The results suggest that the energy supply to the enzyme systems responsible for the production of progesterone and oestradiol is rate limiting for the overall reaction.

There was no consistent significant effect of corticotrophin releasing hormone, adrenocorticotrophic hormone or the insulin like growth factors on progesterone, or oestrogen production in either tissue explants or isolated cytotrophoblast cells under the experimental conditions used in this study.

Table of contents

Table of contents

Chapter 1. Introduction

Chapter 2. Materials and Methods

Chapter 3. Results

Chapter 4. Discussion

List of figures

List of tables

Acknowledgements

I would like to express my gratitude to my supervisor Miss G. C. L. Lachelin for her time, help and encouragement throughout this project. I am also indebted to Dr H. H. G. McGarriggle for his constructive advice and the time he has devoted to reading through various drafts of this thesis.

I would also like to express my appreciation to Professor Rodeck for allowing me to undertake this study in his department and to all my colleagues who have supported me whilst I have been doing this research. I would especially like to thank Dr Diana Mansour, Kay Dalby and Dr John Morrison for their help in obtaining the placental tissue used in this study.

My heartfelt gratitude also goes to all my family and friends who have helped me in their various ways throughout the years. In particular I would like to mention my mum and Tom for their unfailing encouragement and support of my academic pursuits, and Sue and Helena who supplied a sometimes much needed sympathetic ear. Most especially, my thanks go to my husband David, without whose understanding, patience, love and broad shoulders I could never have finished this thesis.

I dedicate this thesis with love to my dad.

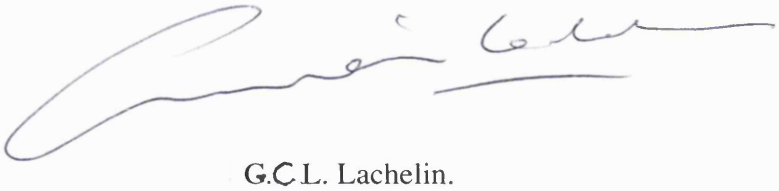
Personal statement

All the work in this thesis (except for that which is specifically cited in the methods) was performed solely by the candidate and is original.

L. a. Shepherd

Leonie Anne Shepherd.

Certification by the supervisor



G.C.L. Lachelin.

Abbreviations

The following abbreviations have been used in the text:

A	androstenedione
ACAT	acyl-CoA-cholesterol acyl transferase
ACTH	Adrenocorticotrophic hormone
16α-OHA	16 alpha-hydroxyandrostenedione
16α-DHEA	16 alpha hydroxydehydroepiandosterone
16α–OHE1	16 alpha-hydroxyoestrone
16α-OHT	16 alpha-hydroxytestosterone
3β–HSD	3 beta-hydroxysteroid dehydrogenase isomerase complex
17β–HSD	17 beta-hydroxysteroid dehydrogenase
cAMP	cyclic 3', 5'-adenosine monophosphate
CO	carbon monoxide
CO_2	carbon dioxide
CRF	corticotropin releasing factor
^0C	degrees centigrade
CSCC	cholesterol side chain cleavage
CV	coefficient of variance
DHEA	dehhydroepianrosterone
DHEAS	dehhydroepiandosterone sulphate
DNase	deoxyribonuclease
DOC	sodium deoxycholate
DMEM	Dulbeccos Modified Eagles Medium
f	fempto (prefix)
g	grams
HBBS	Hanks Balanced Salt Solution
hr(s)	hour(s)
25-OHcholesterol	25-hydroxycholesterol
HMGCoA	3-hydroxy-3-methylglutaryl Co A
HCG	human chorionic gonadotrophin
HPL	human placental lactogen
IGF-I	insulin like growth factor I
IGF-II	insulin like growth factor II
l	litre
LDL	low density lipoprotein
m	minutes
μ	micro (prefix)

mg	milligram (prefix)
mIU	milli international units
µl	microlitre
ml	millilitre
mRNA	messenger ribonucleic acid
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide reduced form
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate reduced form
n	nano (prefix)
ng	nanogramme
nm	nanometers
E2	oestradiol
E3	oestriol
E1	oestrone
p	pico (prefix)
P	progesterone
P-450cscc	cytochrome P-450 cholesterol side chain cleavage enzyme
t	time
TCA	trichloroacetic acid
%	percentage
RCF	relative centrifugal force (gravities)

Note on reference citation

When a paper has been presented by a group of authors all surnames are initially reported in the text, thereafter only the first author is mentioned.

Introduction

1.1. Historical perspective

Knowledge about steroid synthesis in human pregnancy has burgeoned since the beginning of this century. Increasingly sophisticated experimental techniques have enabled the isolation and characterization of the steroids present during gestation, whilst the development of radioactive tracers and the perfusion of isolated placentae have helped in the elucidation of the biosynthesis and metabolism of these compounds.

At the beginning of the century it was known that destruction of the corpus luteum in the rabbit resulted in pregnancy loss. This suggested that the corpus luteum was secreting a compound necessary for the maintenance of pregnancy. In 1929 Corner and Allen obtained a crude extract from the sow corpus luteum and showed that it was capable of maintaining pregnancy in ovariectomised rabbits (Corner and Allen 1929; Allen and Corner 1929). This hormone was later purified and characterized as progesterone by a number of researchers (cited by Davis, Plotz, LeRoy, Gould and Werbin 1956). Later studies showed that progesterone was converted to pregnanediol glucuronide, a substance which had previously been isolated from the urine of pregnant women (Browne, Henry and Venning 1937; Venning and Browne 1940). Pregnanediol levels in the urine of pregnant women were found to rise with advancing gestational age and to fall after parturition (Browne et al 1937; Bacman, Leekly and Hirschmann 1940; Bradshaw and Jessop 1953; Appleby and Norymberski 1957), suggesting that the fetus or placenta was involved in the production of progesterone.

Evidence that the increasing progesterone produced during pregnancy was secreted by the placenta came from a variety of sources. Pregnant women who had Addison's disease or had been adrenalectomised were known to produce normal levels of this compound, indicating that the maternal adrenal was not involved in the production of

progesterone (Samuels, Evans and McKelvery 1943; Knowlton, Gilbert, Mudge and Jailer 1949; Venning, Sybulski, Pollak and Ryan 1959). Other studies showed that after the first few weeks of gestation very little progesterone arises from the ovaries or corpus luteum (Browne et al 1937; Tulsky and Koff 1957). Although progesterone had been isolated from human placentae and research showed that progesterone could be produced by this tissue this was not conclusive evidence that it was the major site of production. (Diczfalusy 1952; Pearlman and Cerceo 1952; Salhanick, Noall, Zarrow and Samuels 1952; Pearlman, Cerceo and Thomas 1953). Evidence for the important role of the placenta in progesterone production came from experiments on *in situ* placenta. The observation that pregnanediol excretion continued to rise for several weeks after a child had been removed from the womb, whilst the placenta was left *in situ* , strongly suggested that this endocrine tissue and not the fetus was the origin of this compound (Allen 1953). In 1959 Cassmer confirmed this with the finding that pregnanediol levels remained elevated when fetal death had been induced, but the placenta had been left *in situ*, and continued to be elevated until the placenta was removed.

In 1945 the precursor for progesterone was shown to be cholesterol (Bloch 1945); however the placenta was found to have very limited capacity to produce cholesterol *de novo*. These findings indicated that the precursor for progesterone must be produced elsewhere and later in 1970 maternal plasma cholesterol was shown to be the principal precursor for progesterone production by the placenta (Hellig, Gattereau, Lefebvre and Bolté 1970).

Allen and Doisy (1923) found that an extract of fluid from Graafian follicles was capable of inducing oestrous behaviour in ovariectomised animals and the finding that human pregnancy urine contained large quantities of this substance (Ascheim and Zondek 1927) stimulated research which led to the isolation and characterization of the oestrogens.

Oestrone was first isolated in human pregnancy urine by Doisy and colleagues in 1929 (Doisy, Veller and Thayer 1929). Oestriol was also isolated around this time (Marrian 1929 and 1930) but it was a further five years before oestradiol was isolated from the follicular fluid of sows' ovaries (MacCorquodale, Thayer and Doisy 1935) and it was identified in human pregnancy urine in 1940 (Huffman, MacCorquodale, Thayer, Doisy, Smith and Smith 1940).

From the mid 1930s to the late 1950s the experimental techniques available indicated that there was a steady increase in urinary excretion of the oestrogens oestrone, oestradiol and oestriol with that of oestriol being quantitatively greater than the other oestrogens, rising by up to 1000 fold compared to non pregnant women (Cohen, Marrian and Watson 1935; Stimmel 1946; Bradshaw and Jessop 1953; Brown 1956). This increase was not due to maternal ovarian production (see references cited by Diczfalusy and Troen 1953). It was also shown that it was not due to maternal adrenal production as pregnant patients with Addison' s disease had relatively normal pregnancy oestrogen levels (Samuels et al 1943; Knowlton et al 1949). The rapid decline in oestrogens after delivery (Cohen et al 1935; Bradshaw and Jessop 1953; Brown 1956) indicated that the placenta or fetus was involved in the production of these hormones.

At the beginning of the 1960s oestrogens were found to be low in women pregnant with an ancephalic fetus indicating that the fetus played a role in oestrogen formation (Fransden and Stakeman 1961). However, experiments showed that after ligation of the umbilical cord the precipitous drop in oestrogen secretion that would usually occur could be prevented by perfusion of the *in situ* placenta with maternal blood (Cassmer 1959). Women with hydatidiform moles were also shown to produce relatively high levels of oestrogens (Siiteri and MacDonald 1963) suggesting a role for the placenta in oestrogen production during pregnancy.

Although oestrogens had been isolated from placental tissue (Westerfeld, MacCorquodale, Thayer, and Doisy 1938; Huffman, Thayer and Doisy 1940) this was

not conclusive proof that it was the site of oestrogen synthesis. In the 1950s the major C19 steroid circulating in plasma was found to be a conjugate of dehydroepiandrosterone (DHEA) (Migeon and Plager 1954) and was later identified as dehydroepiandrosterone sulphate (DHEAS) (Baulieu 1960). The subsequent findings that DHEAS could be hydrolysed to DHEA by the sulphatase enzyme abundant in placental tisssue but not fetal tissue (Pulkkinen 1961; Warren and Timberlake 1962), and that placental tissue was capable of converting DHEA to oestrogens (Ryan 1959a), was suggestive that this sulphate could serve as a precursor for oestrogen synthesis in the pregnant woman. Further research followed which demonstrated that DHEAS was in fact a precursor for oestrone and oestradiol synthesis but that oestriol was formed through a different pathway. (Siiteri and MacDonald 1963; Baulieu and Dray 1963, Bolté, Mancuso, Eriksson, Wiqvist and Diczfalusy 1964a; Bolté, Mancuso, Eriksson, Wiqvist and Diczfalusy 1964b; Warren and Timberlake 1964; MacDonald and Siiteri 1965). These findings indicated that the placenta obtains steroid precursors from the mother and fetus and converts them to oestrogens.

Thus it was apparent that the production of progesterone and the oestrogens in human pregnancy involved an interplay between mother, fetus and the placenta. The synthesis of progesterone and the oestrogens in the fetal placental unit are shown diagrammatically in figure 1.1.

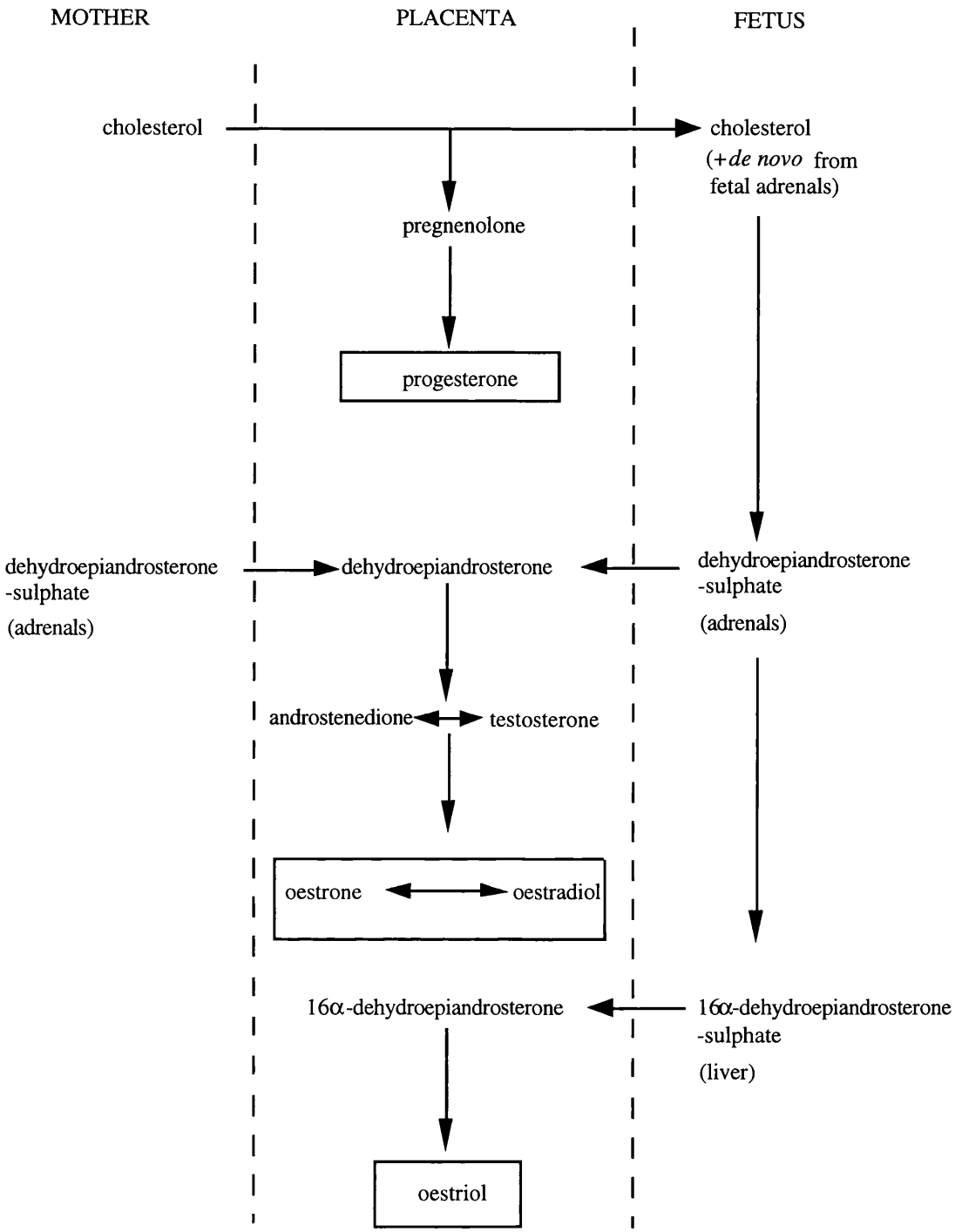


Figure 1.1. Production of the oestrogens and progesterone in the human placenta from fetal and maternal precursors.

1.2. General introduction: the role of steroids in pregnancy and parturition

Numerous complex interactions take place between the mother and fetus during the course of gestation, ultimately resulting in parturition. Steroid hormones are synthesized by the fetus and placenta during pregnancy and are important components both in maintaining uterine quiescence and in the initiation of labour. In the sheep and rat the onset of labour is preceded by a sharp rise in maternal plasma oestrogen levels and a fall in progesterone production which results in an increase in the oestrogen:progesterone ratio (Bassett, Oxborrow, Smith and Thorburn 1969; Csapo and Wiest 1969; Yoshinaga, Hawkins and Stocker 1969; Challis 1971). These changes appear to modulate certain factors such as gap junction formation, oxytocin receptors and prostaglandin synthesis and release which are associated with an increase in uterine activity. The release of oxytocin that occurs after vaginal distension in sheep is inhibited by progesterone but potentiated by oestrogen and an increase in the number of oxytocin receptors during labour has been attributed to stimulation of receptor synthesis by the rising oestrogen levels in rats (Roberts and Share 1969; Alexandrova and Soloff 1980; Soloff, Fernstrom, Periyasamy, Soloff, Baldwin and Wieder 1983). Progesterone withdrawal in sheep enhances prostaglandin production (Liggins, Fairclough, Grieves, Kendall and Knox 1973; Ledger, Webster, Anderson and Turnbull 1985) and oestradiol administration in several species increases uterine prostaglandin synthesis (Caldwell, Tillson, Brock and Speroff 1972; Liggins et al 1973; Ryan, Clark, Van Orden, Farley, Edvinsson, Sjoberg, Van Orden and Brody 1974; Demers, Yoshinaga and Greep 1974; Ham, Cirillo, Zanetti and Kuehl 1975; Castracane and Jordan 1975).

Co-ordinated uterine muscle activity is essential for delivery of the fetus and appears to be dependant upon the formation of gap junctions which facilitate the spread of electrical activity throughout the uterus (Garfield, Sims and Daniel 1977; Garfield, Sims, Kannan and Daniel 1978). These myometrial gap junctions have been subsequently shown to be formed of clusters of a protein, connexin-43 (Beyer, Kistler, Paul, Goodenough 1989; Lang, Beyer, Schwartz and Gitlin 1991; Risek, Guthrie, Kumar and Gilula 1991). Rising oestrogens and decreasing progesterone levels increase

the number and size of gap junctions in the myometrium of rats and sheep (Garfield, Rabideau, Challis and Daniel 1979a; Garfield, Kannan and Daniel 1980; Mackenzie and Garfield 1985). They are also present in the myometrium of women during late pregnancy and those undergoing labour and have been found to correlate with the degree of cervical dilation (Garfield, Rabideau, Challis and Daniel 1979b; Garfield and Hayashi 1981; Balducci, Risek, Gilula, Hand, Egan, and Vintzileos 1993). The messenger ribonucleic acid (mRNA) encoding the human myometrial gene for connexin-43 is elevated during late pregnancy and labour (Chow and Lye 1994). This is suggestive of a role for the oestrogens and progesterone in their synthesis and experiments have been performed which support this. Progesterone has been shown to decrease whilst oestradiol stimulates the transcription of connexin-43 in rat myometrium (Lye, Nicholson, Mascarenhas, MacKenzie and Petrocelli 1993; Petrocelli and Lye 1993). Connexin-43 gap junctions have also been shown to form spontaneously in primary myometrial cell cultures when oestradiol is present (Anderson, Grine, Eng, Zhao, Barbieri, Chumas and Brink 1993).

Free calcium is also important in the mechanism of muscular excitation-contraction. Contractions result from the phosphorylation of muscle myosin causing it to interact with actin. As the myosin and actin filaments slide past one another cross-bridges form between the two proteins which generate the contractile force of labour. The enzyme responsible for the phosphorylation of myosin requires the protein calmodulin which in turn is activated by calcium. The uncoupling of excitation and contraction in the rabbit uterus has been shown to be prevented by the presence of progesterone, and it is thought that this steroid modulates the intracellular calcium levels, thereby maintaining the uterus in a quiescent state prior to parturition (Currie and Jeremy 1979).

Human pregnancy is also characterised by rising levels of uterine prostaglandins and receptors for oxytocin in the myometrium and decidua which rise progressively in concentration throughout pregnancy (Fuchs, Fuchs, Husslein and Soloff 1984). However in humans the oestradiol:progesterone and oestrone:progesterone ratio do not

rise very much towards term (Tulchinsky, Hobel, Yaeger and Marshall 1972; Mathur, Landgrebe and Williamson 1980; Anderson, Hancock and Oakey 1985), but it has been shown that the onset of labour is proceeded by a rise in the oestriol:progesterone ratio (McGarrigle and Lachelin 1984; Darne, McGarrigle and Lachelin 1987). The effects of the oestrogens and progesterone on myometrial activity, prostaglandin and oxytocin synthesis in other species suggest that the ratio of oestriol:progesterone at term in human pregnancy may be of some importance in determining the onset of labour.

Factors which may control the enzyme systems responsible for synthesising oestrogens and progesterone are thus important in evaluating how the onset of human labour is governed.

1.3. The biosynthesis of progesterone by the placenta.

The steroid hormones have a basic strucutre of three cyclohexane rings and one cylcopentane ring which can be seen in figure 1.3.1. The four rings are designated by letters whilst carbon atoms on the steroid ring are numbered. Any groups substitued on the ring are identified by the number of the carbon atom to which they are attached. If there is a double bond present it is identified by the lower of the carbon number of the atoms sharing the bond.

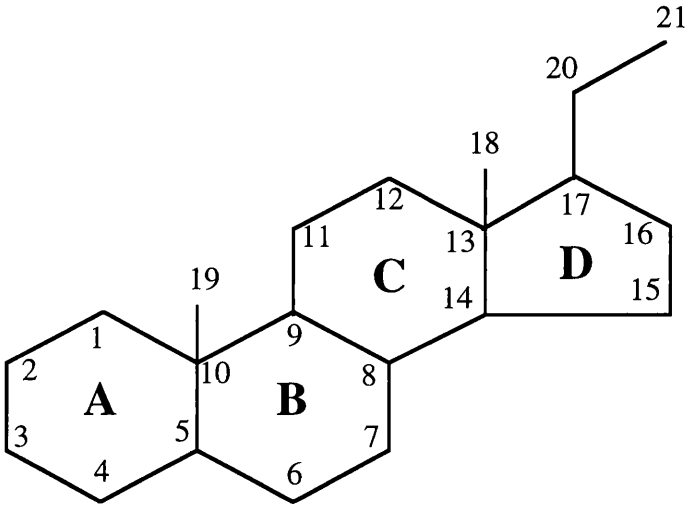


Figure 1.3.1. The basic structure of a steroid with the accepted numbering of carbon atoms and labelling of rings.

Synthesis of all steroid hormones commences with the precursor molecule cholesterol (Bloch 1945). The placenta has only a limited ability to produce cholesterol *de novo* and mainly obtains it from the maternal circulation (Woolever, Goldfien and Page 1961; van Leusden and Villee 1965; Zelewski and Villee 1966; Hellig et al 1970). The principal source of cholesterol utilised by the placenta is thought to be low density lipoprotein (LDL) (Winkel, Synder, MacDonald and Simpson 1980). Its uptake is mediated by specific high affinity receptor sites on the cytotrophoblast cell surface (Winkel, Gilmore, MacDonald and Simpson 1981), the number of which have been found to be regulated by these cells in primary culture (Winkel, MacDonald, Hemsell and Simpson 1981). Receptors for LDL are detectable as early as 6 weeks gestation in microvillus membranes (Alsat, Bouali, Goldstein, Malassiné, Berthelier, Mondon and Cedard 1984).

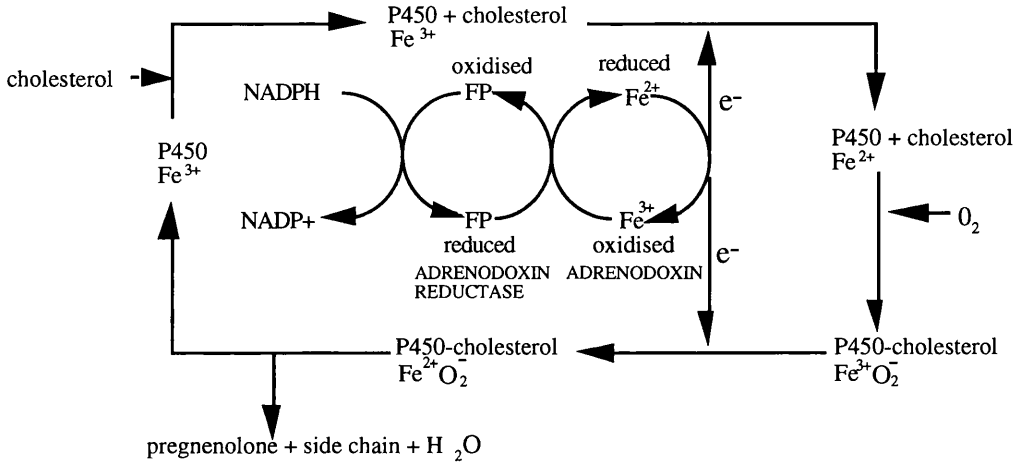
Cholesterol is converted first to pregnenolone (Saba, Hechter and Stone 1954; Staple, Lynn and Gurin 1956) through a series of reactions known as cholesterol side chain cleavage (CSCC). An enzyme system capable of cleaving cholesterol was first isolated in mammalian tissue in 1954 (Lynn, Staple and Gurin 1954) and later localized to the mitochondria in this tissue (Halkerston, Eichorn and Hechter 1959; Halkerston, Eichorn and Hechter 1961; Constantopoulos and Tchen 1961a, Constantopoulos and Tchen 1961b). Further studies on porcine adrenals located it to the inner mitochondrial membrane (Yago and Ichii 1969; Yago, Kobayashi, Sekiyama, Kurokawa, Iwai, Suzuki and Ichii 1970).

Experiments on bovine adrenal mitochondrial extracts have suggested that there are several intermediates in the conversion of cholesterol to pregnenolone, 22R-hydroxycholesterol, 20α-hydroxycholesterol, and 20α, 22R-dihydroxycholesterol (Roberts, Bandy and Lieberman 1969; Burstein, Byon, Kimball and Gut 1976; Burstein and Gut 1976; Hume and Boyd 1978; Hume, Kelly, Taylor and Boyd 1984). Subsequent studies on human placental mitochondria indicate that these intermediates are also formed in this tissue (Tuckey 1992).

Studies on other steroid transforming enzymes have helped in the understanding of the nature of the enzyme system involved in CSCC. Initial work in the 1950s indicated that 21 hydroxylation in adrenal microsomes is reversibly inhibited by carbon monoxide (Ryan and Engel 1957). The finding that a pigment of unknown function in liver microsomes reversibly binds carbon monoxide (CO) with the resulting complex having an absorption at 450 nanometers (nm) initiated subsequent studies on 21β-hydroxylase and 11β-hydroxylase (Garfinkel 1957, Klingenberg 1958). Both these enzyme systems were subsequently found to have this characteristic absorption at 450 nm (Estabrook, Cooper and Rosenthal 1963; Harding, Wong, Nelson 1964; Harding, Wilson, Wong and Nelson 1965). As the pigment involved in binding carbon monoxide in these enzymes was found to be a haemoprotein it was called cytochrome P-450 (Omura and Sato 1962). Purification of the 11β-hydroxylase enzyme system in mitochondria showed that it was composed of three fractions, a flavoprotein (adrenodoxin reductase), a non iron-haem protein (adrenodoxin) and a fraction consisting of cytochrome P-450 (Omura, Sato, Coope, Rosenthal and Estabrook 1965). These researchers suggested that electron pairs could pass through an electron transport chain comprised of the flavoprotein and non-haem iron protein to cytochrome P-450. Studies on bovine adrenal cortex implicated the involvement of P-450 in CSCC (Simpson and Boyd 1966; Simpson and Boyd 1967). The enzyme requires both molecular oxygen and reduced diphosphopyridine nucleotide (NADPH) (Halkerston et al 1959; Constantopoulos and Tchen 1960; Constantopoulos and Tchen 1961; Halkerston et al 1961), with three molecules of oxygen and NADPH being consumed in the production of pregnenolone from cholesterol (Shikita and Hall 1974). Kinetic and electrophoretic studies indicate that the enzyme system involves one catalytic site (Duque, Morisaki and Ikekawa 1978).

A role for this cytochrome P-450 (P-450cscc) in mitochondrial fractions in human term placental tissue has been confirmed (Meigs and Ryan 1967; Meigs and Ryan 1968; Mason and Boyd 1971), and the gene structure encoding the enzyme has been isolated

(Morohashi, Sogawa, Omura and Fujii-Kuriyama 1987). Figure 1.3.2 illustrates mitochondrial electron flow to cytochrome P-450cscc.



FP-flavoprotein

Figure 1.3.2. Mitochondrial electron flow to cytochrome P-450cscc. The cytochrome interacts with cholesterol and then reduction of cytochrome P-450 (Fe^{3+}) to cytochrome P-450 (Fe^{2+}) occurs. Molecular oxygen then interacts with this reduced form of cytochrome resulting in hydroxylation of cholesterol and subsequent cleavage of the side chain.

The end product of CSCC is pregnenolone and its conversion to progesterone (figure 1.3.3) has been demonstrated in studies with the human placenta (Pearlman et al 1953; Sobrevilla, Hagerman and Villee 1964). Pregnenolone is a delta 5-3β-hydroxysteroid and in order for it to be converted to the delta 4-3 ketosteroid progesterone the C3 hydroxyl group must be oxidised to form a ketone. The enzyme responsible for this was first demonstrated in various endocrine tissues, including rat placenta, in the early 1950s (Samuels, Helmreich, Lasater and Reich 1951). It requires diphosphopyridine nucleotide (NAD+) to accept hydrogen produced by oxidation (Samuels et al 1951). Conversion of the hydroxysteroid to its respective ketone also involves the migration of a double bond from C5 to C4. The overall conversion thus involves oxidation and isomerization. The enzyme complex is called 3β-hydroxysteroid deydrogenase isomerase (3β-HSD). It was first localized in bovine adrenal cells, and later in the human placenta, in the microsomes and mitochondria (Beyer and Samuels 1956; Koide and Torres 1965). It is present in both cytotrophoblast cells and syncytiotrophoblast cells of the human placenta (Mason, Ushijima, Doody, Nagai, Naville, Head, Milewich,

Rainey and Ralph 1993). The placenta expresses a type of 3β-HSD (type I) that is different from the type expressed in the adrenals and gonads (type II) (Rhéaume, Lachance, Zhao, Breton, Dumont, de Launoit, Trudel, Luu-The, Simard and Labrie 1991).

Purification, kinetic studies of the dehydrogenase and isomerase substrates and molecular analysis of the amino acid sequencing in human placenta have determined that 3β-HSD comprises a single protein which can metabolise all the main substrates (Thomas, Berko, Faustino, Myers and Strickler 1988; Luu-The, Lachance, Labrie, Leblanc, Thomas, Strickler and Labrie 1989; Luu-The, Takahashi and Labrie 1990; Lorence Murry, Trant, Mason 1990). This is also the case in other mammalian tissue (Ishii-Ohba, Inano and Tamaoki 1987; Rutherford, Chen and Shivley 1991) unlike that of *Pseudomonas* bacteria in which the dehydrogenase and isomerase activities are carried out by separate enzymes (Talalay and Wang 1955). The enzyme in the microsomes and mitochondria appears to be identical (Thomas, Myers and Strickler 1989). Although some researchers using affinity labeling experiments suggest that both the activity of 3β-hydroxy-5-ene steroid dehydrogenase and the isomerase are expressed at a single catalytic site in human placental microsomes (Thomas et al 1988; Strickler and Thomas 1993), others have suggested that the isomerase reaction occurs at a separate site (Luu-The, Takahashi, de Launoit, Dumont, Lachance and Labrie 1991).

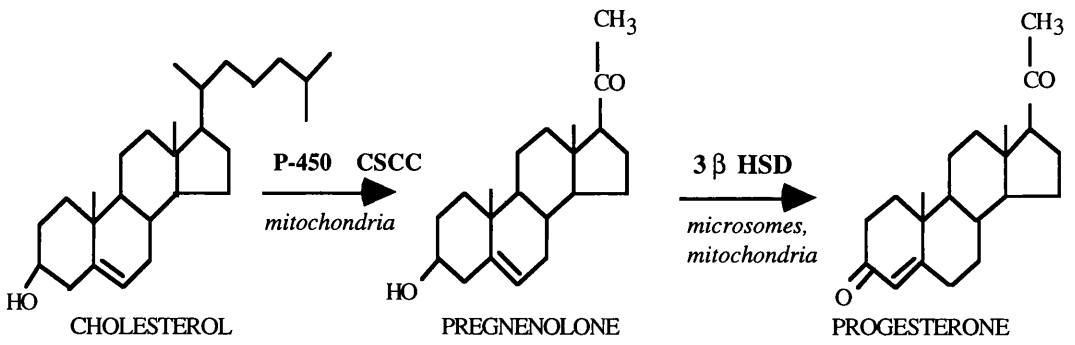


Figure 1.3.3. Conversion of cholesterol to progesterone.

1.4. The biosynthesis of oestrogens by the placenta

The production of progesterone by the placenta does not require any fetal involvement but this is not the case with the synthesis of the oestrogens. In the late fifties and early sixties it was recognized that although the placenta was involved in oestrogen formation the levels were lower in women pregnant with anencephalic fetuses which led to the conclusion that the fetus also played an important role in oestrogen formation (Fransden and Stakeman 1961).

The initial steps in the transformation of pregnenolone to oestrogens are its sulphation and 17α-hydroxylation by 17α-hydroxylase. There appears to be no human placental 17α-hydroxylase activity and these reactions are carried out in the fetal adrenals (Sobrevilla et al 1964; Solomon 1966; Jaffe, Pérez-Palacios and Lamont 1968). A microsomal 17,20 lysase enzyme catalyses the conversion of 17α-hydroxypregnenolone sulphate to dehydro-epiandosteronesulphate (DHEAS) which has been shown to be the principal precursor for oestrone and oestradiol synthesis by the placenta (Siiteri and MacDonald 1963; Baulieu and Dray 1963; Bolté et al 1964a; Warren and Timberlake 1964; MacDonald and Siiteri 1965; Siiteri and MacDonald 1966). The conversion of pregnenolone to DHEAS is illustrated in figure 1.4.1.

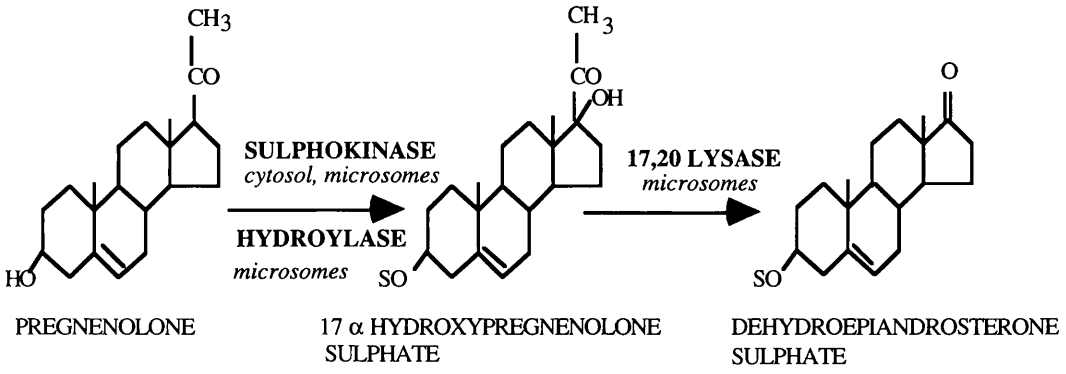


Figure 1.4.1. The conversion of pregnenolone to DHEAS.

In order that the placenta can utilise DHEAS, the sulphate moiety must be removed by the sulphatase enzyme which is abundant in placental tissue but absent in fetal tissue

(Pulkkinen 1961; Warren and Timberlake 1962). Immunohistochemical studies have localized sulphatase to the endoplasmic recticulum of the syncytiotrophoblast of the placenta (Salido, Yen, Barajas and Shapiro 1990). Dehydroepiandrosterone is then converted to androstenedione, an obligatory intermediate in oestrone and oestradiol production (Ryan 1959a) although oestradiol may also be produced to a lesser extent directly through testosterone (Anderson and Lieberman 1980) (figure 1.4.2.). In the human approximately half of the oestrone and oestradiol produced by the placenta at term is derived from maternal adrenal DHEAS and the rest from fetal DHEAS (MacDonald and Siiteri 1966).

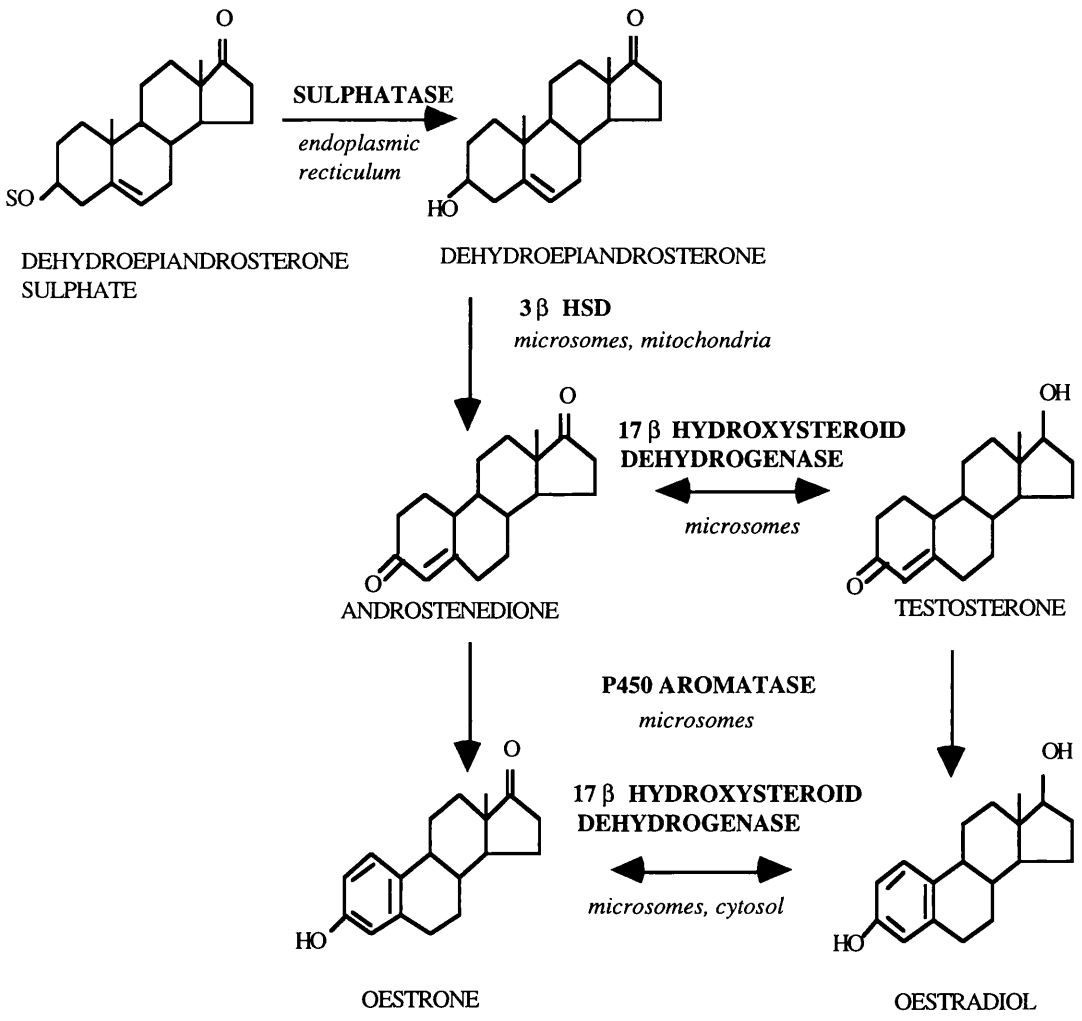


Figure 1.4.2. The conversion of DHEAS to oestrone and oestradiol.

Research in the 1960s established that oestriol synthesis occurs via a separate pathway to that of the other oestrogens (Baulieu and Dray 1963; Siiteri et al 1963; Bolte et al 1964a; Bolte et al 1964b). The main pathway for oestriol production in the placenta is

through 16α-hydroxyDHEAS formed in the fetal liver (MacDonald and Siiteri 1965; MacDonald and Siiteri 1966; Kirschner, Wiqvist and Diczfalusy 1966; Easterling, Simmer, Dignam, Frankland and Naftolin 1966; Siiteri and MacDonald 1966). The sulphate moiety is removed by the placenta where it is converted mainly to 16α-hydroxyandrostenedione and 16α-hydroxyoestrone (Ryan 1959b; Magendantz and Ryan 1964; Numazawa, Osada and Osawa 1985; Yoshida and Osawa 1991) before finally being converted to oestriol (figure 1.4.3).

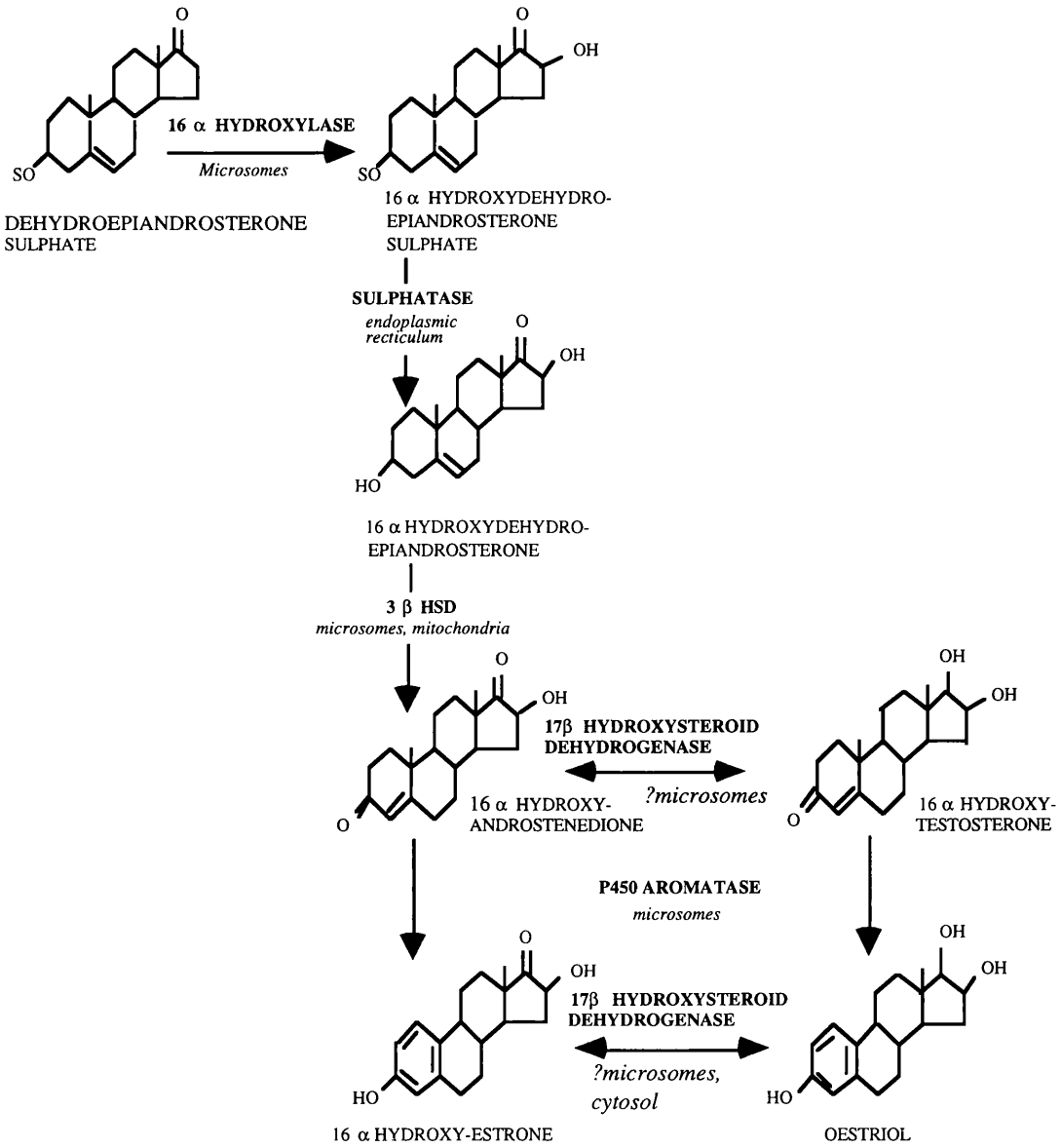


Figure 1.4.3. The conversion of DHEAS to oestriol.

The final conversion of the precursors to the oestrogens is called aromatization. In the late fifties Ryan (1958) first described the ability of placental microsomal fractions to aromatize androstenedione to oestrone with the utilization of NADPH and oxygen and it was suggested that a cytochrome was involved. Further studies on placental microsomes showed that the aromatization of androstenedione was insensitive to carbon monoxide, whilst other research suggested that the aromatization of 16α–hydroxytestosterone was reversibly inhibited by CO, casting some doubt on whether the enzyme system involved a cytochrome or whether more than one enzyme system existed (Meigs and Ryan 1968; Meigs and Ryan 1971; Thompson and Siiteri 1974a; Thompson and Siiteri 1974b; Canick and Ryan 1975; Canick and Ryan 1976). However experiments using inhibitors against cytochrome P-450, an antibody raised against porcine hepatic NADPH P-450 reductase and carbon monoxide inhibition have confirmed that the enzyme complex is a cytochrome (P-450arom) with the electron transport chain consisting of a NADPH reductase (Thompson and Sitteri 1973; Thompson and Siiteri 1974a; Siiteri and Thompson 1975; Thompson and Siiteri 1976).

Three moles of oxygen and three moles of NADPH are required for each mole of oestrogen that is formed indicating that three hydroxylations are required to convert androstenedione to oestrone (Meigs and Ryan 1971; Thompson and Siiteri 1974a; Thompson and Siiteri 1975). The first hydroxylation occurs at the C19 position producing 19-hydroxyandrostenedione (Meyer 1955) which in turn is sequentially hydroxylated to produce 19-dihydroxyandrostenedione or its dehydrated form 19-oxoandrostenedione (Akhtar and Skinner 1968; Skinner and Akhtar 1969). The last step appears to be non-enzymatic with the intermediate being unstable (Townsley and Brodie 1968; Hosoda and Fishman 1974; Cole and Robinson 1988).

In 1975 Canick and Ryan postulated that separate sites or even distinct species of cytochrome P-450 aromatase are involved in the synthesis of oestradiol and oestriol in the human placenta. These researchers concluded from further studies that there are two populations of aromatase present in placental microsomes, one capable of binding

androstenedione only and the other both 16α-hydroxytestosterone and androstenedione (Canick and Ryan 1976). Two placental aromatase populations, a major form capable of binding androstendione only and a minor (less abundant form) capable of binding both androstendione and 16α-hydroxyandrostenedione have since been isolated (Osawa, Tochigi, Higashiyama, Yarborough, Nakamura and Yamamoto 1982). A unique, new form of aromatase present within placental microsomes has more recently been reported by Harada (1988) which is different to those previously purified and described (Osawa et al 1982; Mendelson, Wright, Evans, Porter and Simpson 1985; Nakajin, Shinoda and Hall 1986; Tan and Muto 1986). Other researchers have investigated the kinetics of the aromatization of androstenedione, testosterone, 15α- and 16α-hydroxylated androgens and concluded that 16α-hydroxylated androgens are metabolised by a seperate aromatase (Cantineau, Kremers, De Graeve, Giellen and Lambotte 1982). A purified form of aromatase that failed to aromatise 16α-hydroxyandrostenedione or 16α-hydroxytestosterone but was capable of catalysing both androstenedione and testosterone has been reported, suggesting that multiple enzymes are involved in aromatization (Hagerman 1987). A molecular study suggested the existence of two genes for human placental aromatase but the reserchers did not ascertain whether both were actually expressed (Chen, Besman, Sparkes, Zollman, Klisak, Mohandas, Hall and Shively 1988). Other investigators, however, have concluded that a single enzyme is responsible for all the aromatase reactions (Kelly, Judd and Stolee 1977; Kellis and Vickery 1987; Vickery and Kellis 1987). A number of reports suggest that aromatase is encoded by a single gene and a single polypeptide chain capable of aromatizing androstenedione, testosterone and 16α-hydroxyandrostenedione has been isolated (Corbin, Graham-Lorence, McPhaul, Mason, Mendelson and Simpson 1988; Means, Mahendroo, Corbin, Mathis, Powell, Mendelson and Simpson 1989; Harada, Yamada, Saito, Kibe, Dohmae and Takagi 1990).

In addition to the lack of consensus regarding the number enzymes involved in aromatization there is disagreement as to whether the hydroxylations of androstenedione take place at a single catalytic site or at separate sites on one enzyme

(Kelly et al 1977; Fishman and Goto 1981). The aromatization of 16α–hydroxy androgens has been suggested to take place at a different catalytic site to that of androstenedione but more recent research indicates that although the sites are separate they are interactively linked (Canick and Ryan 1976; Purohit and Oakey 1989).

Although most research has been on microsomal aromatase the enzyme system has also been located to the mitochondria (Shaw, Dalziel and O'Donnell 1969; Renwick and Oliver 1973). Immunocytochemical studies have localized microsomal aromatase to the endolplasmic recticulum in fractions of human placentae, in particular to the syncytiotrophoblast, but have failed to localise mitochondrial aromatase suggesting that this might be due to contamination from the microsomes (Fournet-Dulguerov, MacLusky, Leranth, Todd, Mendleson, Simpson and Naftolin 1987; Kitawaki, Inoue, Tamura, Yamamoto, Noguchi, Osawa and Okada 1992).

The inter-conversion of oestrone-oestradiol and androstenedione-testosterone is carried out by 17β-hydroxysteroid deydrogenase (17β-HSD). An enzyme system capable of this reaction was first described in human tissue (including placental tissue) by Ryan and Engel (1953). It was later partially purified and characterised in human placental tissue by Langer and Engel (1958). To date a number of enzymes have been cloned for this system (Andersson, Geissler, Patel and Wu 1995). Kinetic and molecular studies indicated that four of these forms of 17β-HSD exist in human placental tissue (Blomquist, Linderman and Hakanson 1985; Blomquist, Linderman and Hakanson 1987; Adamski, Normand, Iffinders, Monté, Begue, Stehelin, Jungslut, de Launoit 1995; Luu-The et al unpublished observations cited by Labrie, Luu-The, Lin, Labrie, Simard, Breton, Bélange 1997). Most research has centered on two types 17β-HSD located in the cytosol and microsomes. Purification of a cytosolic 17β-HSD showed that two mRNAs coded for this enzyme which originated from one gene (Luu-The, Labrie, Zhao, Couët, Lachance, Simard, Leblanc, Côte, Bérubé, Gagné and Labrie 1989; Luu-The, Labrie, Simard, Lachance, Zhao, Couët, Leblanc and Labrie 1990) whilst more recently a gene encoding a microsomal 17β-HSD has been cloned (Wu, Einstein,

Geissler, Chan, Elliston and Anderson 1993). These two forms of 17β-HSD have been designated type I and type II respectively. Type I is present mainly in the cytosol and is highly reactive with C18 steroids such as the oestrogens, oestrone and oestradiol, whilst type II, present in microsomes, is highly reactive with both oestradiol and the androgen testosterone (Wu et al 1993; Geissler, Davis, Wu, Bradshaw, Patel, Mendonca, Elliston, Wilson, Russell and Andersson 1994; Beaudoin, Blomquist and Tremblay 1995; Blomquist, Bealka, Hensleigh and Tagatz 1994). Type II 17β-HSD also has 20α-HSD activity (Blomquist et al 1985; Wu et al 1993; Andersson et al 1995). The human placental type I enzyme utilizes both NAD(H) and NADP(H) whilst type II activity appears to be preferentially oxidative and utilises NAD^+ as the cofactor (Warren and Crist 1967; Wu et al 1993; Andersson et al 1995). Although two other forms of 17β-HSD, nominated type IV and type V, have been found to be present in the human placenta, their importance in the production of the oestrogens in this tissue is unknown, with relatively little type IV being found in comparison to the type I and type II enzymes. The abundance of the type V enzyme in human placental tissue has not yet been reported (Adamski et al 1995). It is thought that the type IV enzyme may convert E2 to E1 as its amino acid structure is similar to that of the porcine type IV 17β-HSD enzyme which preferentially catalyses this reaction in the oxidative direction (Leenders, Adamski, Husen, Thole and Jungblut 1994; Adamski et al 1995). The type V enzyme however, has a higher affinity for andostendione, catalysing the reaction in the reductive direction towards testosterone (Labrie et al 1997).

Although placental tissue has been shown to have high levels of both type I and II 17β-HSD activity (Martel, Rhéaume, Takahashi, Trudel, Couet, Luu-The, Simard and Labrie 1992; Wu et al 1993) type II has been shown to decrease over time in placental cell cultures (Beaudoin et al 1995) suggesting that the mechanisms regulating their expression are different.

The microsomal type II enzyme has been located to the syncytiotrophoblast in first trimester and term placentae and although it was also found to be transiently expressed

by cytotrophoblast cells from placentae of 10-13 weeks gestation it was not located to these particular cell types at term (Fournet-Dulguerov et al 1987; Dupont, Labrie, Luu-The and Pelletier 1991). More recently mRNA for the type I enzyme has been isolated in both freshly isolated cytotrophoblast cells and syncytiotrophoblast cells, but very little type II activity has been found to be present in isolated cytotrophoblast cells and it was undetectable in syncytiotrophoblast cells cultured from term placentae (Beaudoin et al 1995).

1.5. The regulation of steroid synthesis

1.5.1. Corticotrophin releasing factor and adrenocorticotrophic hormone and placental steroid synthesis

While it has been established that mother, fetus and placenta act co-operatively to produce steroid hormones it is still uncertain how parturition is regulated in the human. Corticotrophin releasing factor (CRF) has been suggested to be important in the timing of parturition. It is a neuropeptide produced by the hypothalamus and is widely recognised to modulate the production of adrenocorticotrophic hormone (ACTH) from the anterior pituitary. It was at first detected only during the third trimester of pregnant women (Sasaki, Liotta, Luckey, Margioris, Suda , Krieger 1984) but with the advent of more sensitive assays has since been found to rise in maternal serum throughout human gestation, being at its highest in the weeks preceeding labour (Goland, Wardlow, Stark, Brown and Frantz 1986; Sasaski, Shinkawa, Margioris, Liotta, Sato, Murakami, Go, Shimizu, Hanew and Yoshinaga 1987; Laatikainen, Virtanen, Räisänen and Salminen 1987; Campbell, Linton, Wolfe, Scraggs, Jones and Lowry 1987). It has also been found to be elevated several weeks before delivery in pregnancies associated with preterm labour (Campbell et al 1987; Warren, Patrick and Goland 1992). Levels of CRF decrease within a few hours of delivery suggesting a placental origin for this hormone (Sasaski et al 1984; Goland et al 1986; Sasaski et al 1987; Laatikainen, et al 1987; Okamoto, Takagi, Makino, Sata, Iwata, Nishino, Mitsuda, Sugita, Otsuki and Tanizawa 1989).

Immunoreactive and bioactive CRF have been shown to be present in human placentae, as well as mRNA for CRF, which has been shown to increase more than twenty fold five weeks before the onset of labour (Shibasaki, Odagiri, Shizume and Ling 1982; Petraglia, Sawchenko, Rivier and Vale 1987; Sasaki et al 1987; Grino, Chrousos and Margioris 1987; Sasaki, Tempst, Liotta, Margioris, Hood, Kent, Sato, Shinkawa, Yoshinaga and Krieger 1988; Frim, Emanuel, Robinson, Smas, Adler and Majzoub

1988; Saijonmaa, Laatikainen and Wahlström 1988; Riley, Walton, Herlick and Challis 1991; Cooper, Brooks, Miller and Greer 1994).

The immunohistochemical localization of a CRF like peptide in human placentae first reported it to be present in the cytotrophoblast cells of tissue obtained at term but more recent studies have localized it mainly to the syncytiotrophoblast of term placentae (Petraglia et al 1987; Riley et al 1991; Cooper et al 1994; Warren and Silverman 1995).

This peptide has also been reported to stimulate the production of ACTH from placental tissue (Petraglia et al 1987). The bioactivity of CRF is regulated by a CRF binding protein (Orth and Mount 1987). Messenger RNA for this binding protein has been isolated in human placental tissue and has been located in the syncytiotrophoblast (Potter, Behan, Fischer, Linton, Lowry and Vale 1991; Petraglia, Potter, Cameron, Sutton, Behan, Woods, Sawchenko, Lowry and Vale 1993). It has been suggested that the levels of this binding protein are sufficient to inactivate most of the CRF present in pregnancy which would account for the fact that maternal ACTH levels are only slightly raised above those of non pregnant women (Rees, Burke, Chard, Evans and Letchworth 1975). However recent studies indicate that CRF binding protein levels fall a few weeks before term, thereby increasing the amount of bioavailable CRF prior to delivery (Linton, Perkins, Woods, Eken, Wolfe, Behan, Potter, Vale and Lowry 1993; McLean, Bisits, Davis, Woods, Lowry and Smith 1995). There is some evidence that CRF has a role to play in parturition. The contractile response of the myometrium to oxytocin can be primed and potentiated by CRF (Quatero and Fry 1989). The synthesis of prostaglandins has also been shown to be enhanced by CRF (Jones and Challis 1989; Jones and Challis 1990) and whilst CRF mRNA transcription is stimulated by glucocorticoids in placental primary cell culture (Robinson, Emanuel, Frim and Majzoub 1988), immunoreactive CRF has been shown to be inhibited by progesterone in mixed placental cell preparations (Jones, Brooks and Challis 1989). Thus it is thought that local withdrawal of progesterone might increase CRF, which could affect placental ACTH production and fetal adrenal ACTH production.

Adrenocorticotrophic hormone is synthesised in the human pituitary as part of a large precursor pro-opiomelanocortin (POMC) of approximately 35,000 daltons (Miller, Johnson, Baxter and Roberts 1980) which is post translationally processed into smaller peptides one of which is ACTH. Adrenocorticotrophic hormone like compounds in human placental tissue were reported as early as the 1950s and it was suggested and later confirmed that the site of production was the placenta itself (Jailer and Knowlton 1950; Assali and Hamermesz 1954; Allen, Cook, Kendall and McGilvra 1973; Miyakawa and Maeyama 1974). Further studies show that both immunassayable and bioassayable ACTH are present in placental tissue (Genazzani, Fraioli, Hurlimann, Fioretti and Felber 1975; Rees et al 1975; Liotta, Osathanondh, Ryan and Krieger 1977). Bioactive ACTH has been shown to be released by perifused human placenta from both early and late gestation (Waddell and Burton 1993) and it has been immunohistochemically localized to the syncytiotrophoblasts and intermediate trophoblast cells (Al-Timimi and Fox 1986). The peptides produced by the placenta from the processing of placental POMC include a melanocyte stimulating hormone and α and β lipotropins and β endorphins (Nakai, Nakao, Oki and Imura 1978; Odigari, Sherrell, Mount, Nicholoson and Orth 1979; Liotta and Krieger 1980; Laatikainen, Saijonmaa, Salminen and Whalström 1987; Margioris, Grino, Protos, Gold and Chrousos 1988).

For some time it was recognised that ACTH modulated adrenal steroid synthesis at the step involving the conversion of cholesterol to progesterone (Stone and Hechter 1954) and later the site of action of ACTH was restricted to that part of the pathway involving the conversion of cholesterol to pregnenolone (Halkerston, Eichhorn and Hechter 1961, Karaboyas and Koritz 1965). Various sources of cholesterol exist for pregnenolone synthesis. Within the cell itself there is thought to be a small pool of metabolically active cholesterol which is rapidly turning over. Input into this pool comes from three sources, endogenous synthesis of cholesterol, hydrolysis of cholesterol esters and uptake by the cell of LDL. In order to assert its stimulatory effects and increase pregnenolone synthesis, ACTH theoretically could effect any or all of these to increase

the availability of free cholesterol to the enzyme system present within the mitochondria.

Kinetic studies, light absorption and electron paramagnetic resonance spectroscopy show that the effect of ACTH is to stimulate an increase in the amount of substrate bound to P-450cscc and that it is the rate limiting step in the reaction. This acute effect of ACTH occurs within seconds or minutes and increases the amount of cholesterol available to the mitochondria (Koritz and Kumar 1970; Simpson, Jefcoate, Brownie and Boyd 1972; Brownie, Simpson, Jefcoate, Boyd, Orme-Johnson and Beinert 1972; Brownie, Alfano, Jefcoate, Orme-Johnson, Beinert and Simpson 1973; Alfano, Brownie, Orme-Johnson and Beinert 1973; Jefcoate, Simpson and Boyd 1974; Jefcoate and Orme-Johnson 1975; Williams-Smith, Simpson, Barlow and Morrison 1976; Koritz and Moustafa 1976; Simpson, McCarthy and Peterson 1978).

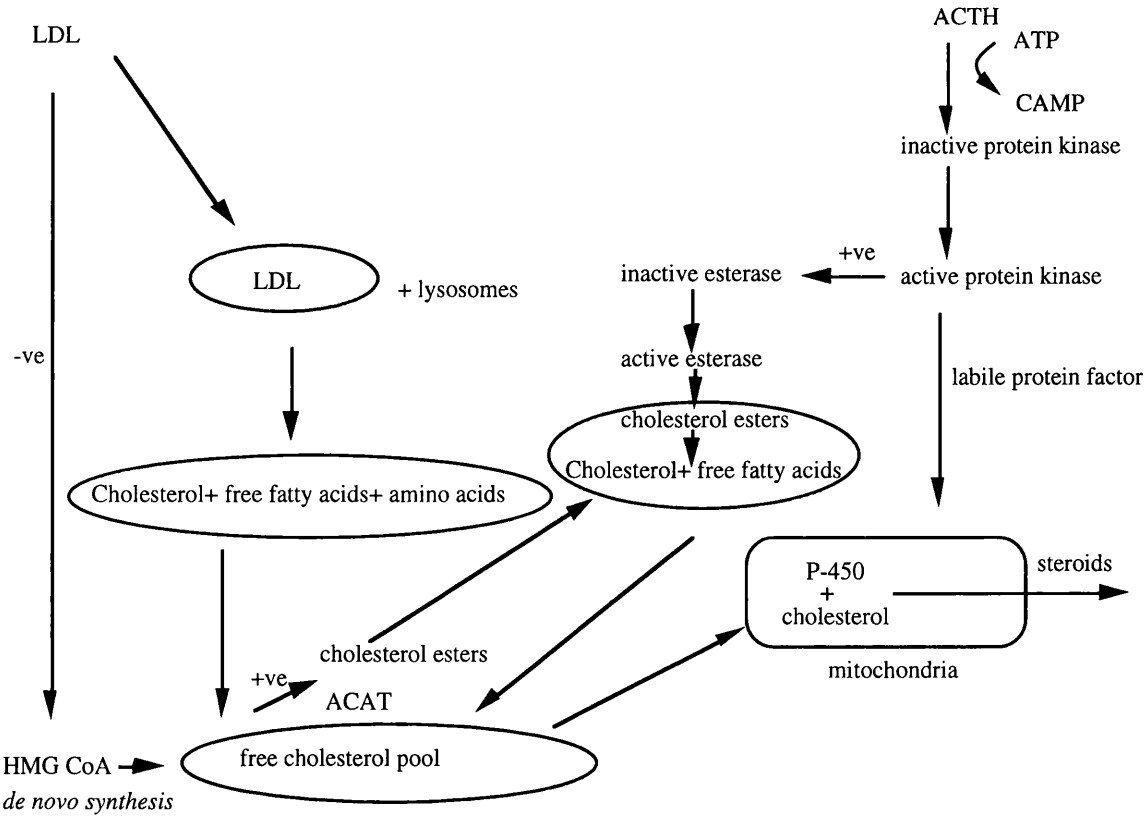
The finding that the administration of ACTH to rats resulted in cholesterol ester stores in the adrenals being depleted, suggested that ACTH was stimulating the conversion of esterified stores into free cholesterol (Davis and Garren 1966). It had already been shown that incubation of beef adrenal slices with ACTH resulted in an increase in the levels of cyclic 3', 5'-adenosine monophosphate (cAMP) in adrenal cells (Haynes 1958) which suggested a role for this compound in the ACTH stimulation of pregnenolone synthesis. It is now known that binding of ACTH to specific receptors on the plasma membrane stimulates cAMP which then activates protein kinase (Gill and Garren 1969; Gill and Garren 1971). Cholesterol ester hydrolase exists in an inactive dephosphorylated form and protein kinase phosphorylates it to the active form thereby increasing production of free cholesterol (Trzeciak and Boyd 1973; Naghshineh, Treadwell, Gallo and Vahouny 1974; Beckett and Boyd 1977; Naghshineh, Treadwell, Gallo and Vahouny 1978). Thus in the adrenal the initial response to ACTH is utilization of cholesterol from a readily available pool which in turn is replenished by hydrolysis of cholesterol ester stores.

The stimulatory action of ACTH *in vivo* was found to be blocked by the protein inhibitors puromycin and cycloheximide (Garren, Ney and Davis 1965). This and a later study which showed that when the protein inhibitor cycloheximide was injected into hypophysectomized rats it prevented an increase in pregnenolone synthesis despite an increase in the conversion of esterified cholesterol to free cholesterol suggested that this protein facilitates the availability of cholesterol to the enzyme system (Davis and Garren 1966). Further studies showed that a protein was involved with the actual association or binding of cholesterol to the enzyme system. When ACTH was injected with cycloheximide into rats the mitochondria obtained from these animals contained a greater amount of cholesterol than those of controls but the actual synthesis of pregnenolone was similar to controls (Arthur, Mason and Boyd 1976; Simpson et al 1978). More recent experiments indicate that a protein is involved in the actual transfer of cholesterol from the outer to the inner mitcochondrial membrane (Privalle, Crivallo and Jefcoate 1983). A protein has now been characterized in the adrenals which is involved in the mobilization of cholesterol from lipid stores to P-450cscc and is known as the steroidogenic acute regulatory protein (StAR protein) (Clark, Wells, King and Stocco 1994; Stocco and Clark 1997). However, this StAR protein does not appear to be present in the human placenta (Sugawara, Holt, Driscoll, Strauss, Lin, Miller, Patterson, Clancy, Hart, Clark and Stocco 1995).

In addition to the hydrolysis of esterified cholesterol this pool of cholesterol may also be supplied to the pool via endogenous synthesis of cholesterol and cellular uptake of serum LDL. Experiments on human fibroblasts showed that the enzyme activities responsible for cholesterol ester formation (acylCoA:cholesterol acyltransferase (ACAT)) and that of *de novo* cholesterol synthesis (3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase)) are regulated in a reciprocal manner with the uptake of LDL suppressing *de novo* synthesis of cholesterol but stimulating cholesterol acyltransferase activity (Brown, Dana and Goldstein 1973; Brown, Dana and Goldstein 1974; Goldstein, Dana and Brown 1974; Brown, Faust and Goldstein 1975; Brown, Dana and Goldstein 1975). This suppression of HMG CoA and

stimulation of ACAT also occurs in bovine adrenals and mouse adrenals (Faust, Goldstein and Brown 1977; Kovanen, Faust, Brown and Joseph 1979). Lipoprotein endocytosis into cells is stimulated by ACTH which increases the number of lipoprotein receptors on the cell surface (Kovanen et al 1979). It has been proposed that if ACTH stimulus is prolonged in the adrenal that cholesterol ester hydrolysis ceases, *de novo* synthesis declines and the output of cholesterol from the pool is balanced by an uptake of plasma lipoproteins due to an increase in the number of LDL receptors. If the supply of LDL to the cell is normal and the number of LDL receptors adequate then the cellular content of cholesterol esters is dependent upon the steroid output. If the stimulus ceases then the lipoprotein uptake would exceed steroid output and the number of LDL receptors would be suppressed (Brown, Kovanen and Goldstein 1979).

The production of pregnenolone and the action of ACTH in the adrenal is shown in figure 1.5.1.

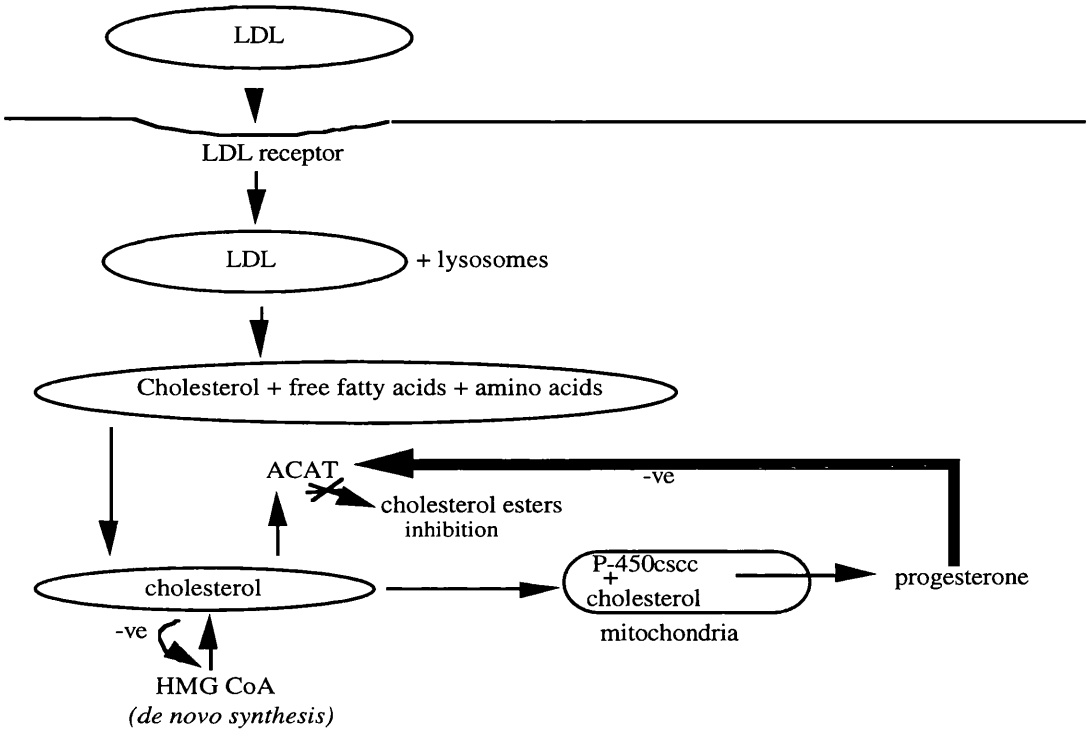


HMG CoA, 3-hydoxy-3-methylglutaryl Co A: ACAT, acyl-CoA-cholesterol acyltransferase.

Figure 1.5.1. The regulation of cholesterol metabolism in the adrenal.

The acute action of ACTH on pregnenolone production however, does not appear to take place in the placenta. Uptake and utilization of LDL by human choriocarcinoma cells occurs in a similar manner to that of fibroblast cells (Simpson, Porter, Milewich, Bilheimer and MacDonald 1978; Simpson, Bilheimer, MacDonald and Porter 1979). The *de novo* production of cholesterol in the placenta however is low, which has been postulated to be due to suppression of HMG CoA reductase by LDL and the concentration of progesterone present within trophoblast cells also appears to inhibit ACAT activity preventing the sequestration of cholesterol in its storage form as esters (Simpson, Porter et al 1978; Simpson and Burkhart 1980a; Simpson and Burkhart 1980b; Winkel et al 1981). The main source of substrate for cytochrome P-450cscc is thought to be in the the form of a continuous supply of maternal LDL (Simpson, Porter et al 1978; Simpson, Bilheimer et al 1979; Winkel, Snyder et al 1980). The rate of LDL uptake and hence progesterone synthesis is thought to be determined by the number of LDL receptors on the cell surface and the number of trophoblast cells (Simpson, Bilheimer et al 1979; Simpson and Burkhart 1980b; Winkel, Gilmore et al 1980; Winkel et al 1981; Simpson and MacDonald 1981).

The production of pregnenolone and its regulation by progesterone in the human trophoblast can be seen in figure 1.5.2.



HMG CoA, 3-hydoxy-3-methylglutaryl CoA: ACAT, acyl-CoA-cholesterol acyltransferase.

Figure 1.5.2. The regulation of cholesterol metabolism in the placental trophoblast.

The acute response to ACTH in the adrenal is followed by a long term stimulatory effect. The initial findings that the stimulatory effect of ACTH correlated with an increase in the cytochrome concentration of the mitochondria indicated that the long term stimulatory effect of ACTH might be due to increased synthesis of the enzymes involved in steroid production (Kimura 1969; Purvis, Canick, Mason, Estabrook and McCarthy 1973). Further studies support this hypothesis with ACTH stimulation resulting in an increase in these steroid producing enzymes including cytochrome P-450cscc and 3β-HSD (Dubois, Simpson, Kramer and Waterman 1981; Funkenstein, McCarthy, Dus, Simpson and Waterman 1983; Kramer, Rainey, Funkenstein, Dee, Simpson and Waterman, 1984; Zuber, Simpson, Hall and Waterman 1985; Mason, Ushijima, Doody, Nagai, Naville, Head, Milewich, Rainey and Ralph 1993). Molecular analysis suggests that the binding of ACTH to its receptor initiates an increase in transcriptional activity in the nucleus providing further evidence that the chronic effect of ACTH on the P-450cscc is to increase the quantity of enzyme available to transform

cholesterol (Dubois et al 1981; John, John, Ashley, MacDonald, Simpson and Waterman 1984; John, John, Boggaram, Simpson and Waterman 1986).

Most studies on the action of ACTH on pregnenolone formation have been on rat and bovine adrenals. The role of ACTH in the human placenta remains uncertain. Although it has been proposed that an increase in ACTH is not essential for the initiation of labour in humans (Winters, Oliver, Colston, MacDonald and Porter 1974) it has been shown to stimulate production of oestradiol and progesterone in human placental minces suggesting that it may have a modulatory role (Barnea, Lavy, Fakih and Decherney 1986).

1.5.2. Insulin like growth factors and placental steroid synthesis

During the last trimester of pregnancy normal women show insulin resistance with enhanced insulin production (Lind, Billewicz and Brown 1973) and this increased production of insulin parallels the rising levels of plasma progesterone and oestradiol (Johansson 1969; De Hertogh, Thomas, Bietlot, Vanderheyden and Ferin 1975; Frienkel 1980), suggesting a possible relationship between steroid synthesis in pregnancy and insulin production (Friekel 1980). Compounds that mimicked the biological actions of insulin, with growth promoting activites, but which failed to cross react with an antibody to insulin were extracted from human blood in 1963 (Froesch, Burgi, Ramseier and Bally and Labhart 1963). Subsequently two polypeptides that have a structural homology to insulin were isolated and named insulin-like growth factors I and II (IGF-I and IGF-II), (Rinderknecht and Humbel 1976a; Rinderknecht and Humbel 1976b; Rinderknecht and Humbel 1978a; Rinderknecht and Humbel 1978b).

Levels of IGF-I increase in maternal serum during pregnancy and are highest during the third trimester (Furlanetto, Underwood, Van Wyk and Handwerger 1978; Wilson, Bennett, Adamson, Nagashima, DeNatale, Hintz and Rosenfeld 1982; Gargosky,

Moyse, Walton, Owens, Wallace, Robinson and Owens 1990; Caufriez, Frankenne, Englert, Golstein, Cantraine, Hennen and Copinischi 1990). As levels of both IGF-I and IGF-II decline rapidly after delivery there is some indication that the placenta produces these peptides. (Furlanetto et al 1978; Wilson et al 1982; Hall, Enberg, Hellem, Lundin, Ottosson-Seeberger, Sara, Trygstad and Öfverholm 1984). Both these insulin like growth factors have been found to be synthesized in the placenta along with specific high affinity binding proteins to which they usually form a complex before being cleared from the circulation (Mills, D'Ercole, Underwood and Ilan 1986; Shen, Wang, Nelson, Jansen and Ilan 1986; Shen, Daimon, Wang, Jansen and Illan 1988; Fant, Munro and Moses 1986; Voutilainen and Miller 1987; Fant, Munro and Moses 1986; Zhou and Bondy 1992; Giudice, Dsupin, Jin, Vu and Hoffman 1993; Han, Bassett, Walton and Challis 1996). Two types of receptors for these insulin-like growth factors have also been localized in placental tissue (Marshall, Underwood, Voina, Foushee and Van Wyk 1974; Bhaumick, Bala and Hollenberg 1981; Massague and Czech 1982; Bhaumick and Bala 1984; Fant et al 1986; Casella, Han, D'Ercole Svoboda and Van-Wyk 1986; LeBon, Jacobs, Cuatrecasas, Kathuria and Fujita-Yamaguchi 1986; Fujita-Yamaguchi, LeBon, Tsubokawa, Henzel, Kathuria, Koyal and Ramachandran 1986; Giudice et al 1993).

Various studies indicate that IGF-I can act to enhance steroidogenic activity. Incubating cultured swine granulosa cells with IGF-I stimulates synthesis of P-450cscc (Veldhuis, Rodgers, Dee and Simpson 1986). The conversion of DHEAS to oestradiol was shown to be inhibited by IGF-I in human choriocarcinoma cells (Ritvos 1988), whilst aromatase activity has been shown to be stimulated by IGF-I in human granulosa and human granulosa luteal cells (Erickson, Garzo and Magoffin 1989). The stimulatory action of ACTH on steroidogenesis also appears to be be potentiated by IGF-I. The stimulation of cortisol by ACTH is enhanced by IGF-I in cultured bovine adrenal cells (Penhoat, Jaillard and Saez 1989) and ACTH stimulation of 17α, 21α and 11β hydroxylase activities were augmented by pretreatment with IGF-I in human adrenocortical cells (Pham-Huu-Trung, Villette, Bogyo, Duclos, Fiet and Binoux 1991).

This potentiating action on ACTH has also been observed with insulin-like growth factor II which has been shown to increase the abundance of ACTH stimulated mRNAs for P-450cscc and 3β-HSD and 17α hydroxylase/17, 20 lysase, in human fetal adrenal cell cultures (Mesiano and Jaffe 1993). A role for these growth factors in human placental steroid synthesis has also been suggested. Experiments on purified human term placental cytotrophoblasts indicate that aromatase activity is inhibited and that cytochrome P-450cscc and 3β-HSD are stimulated by insulin, IGF-I and IGF-II (Nestler and Williams 1987; Nestler 1989; Nestler 1990).

1.6. Reasons for undertaking the present study

There is a scarcity of information regarding CRF and any modulatory role that it may have in placental steroidogenesis and although ACTH has a role in adrenal steroidogenesis little is known of its possible role in placental steroid production. Other reports have suggested that IGF-I and IGF-II have a role to play in steroid regulation in numerous endocrine tissues including the human placenta. In view of these findings and the lack of information concerning CRF and ACTH action on human placental tissue it was decided to design a series of experiments to explore any effects that CRF, ACTH and the insulin-like growth factors might have on steroid production by the human placenta.

The studies so far on placental tissue explants or purified cells have concentrated on progesterone, oestradiol or "oestrogens" (total oestrone and oestradiol) but there appear to be no reports of the effects of various peptides on the synthesis of oestriol. In view of the rise in the oestriol:progesterone ratio in women during the last few weeks of pregnancy it was decided that factors modulating oestriol production should also be included in this study.

1.7. Design of the present study

The study is divided into two parts. The initial work involved looking at possible effects of CRF, ACTH IGF-I on the production of oestrone, oestradiol, oestriol and progesterone in placental tissue minces. The aim was to study the pattern of production at timed intervals during the first three hours of incubation and then at twenty four hours, to see whether any of these peptides had an effect on the production of these steroids. In this way it would be possible to determine whether or not any short term effects are apparent in the first few hours of incubation and if so whether they were still apparent at 24 hours.

The second part of the study was designed so that for purposes of comparison, placental tissue explants and isolated purified placental cytotrophoblast cells were utilized from the same placenta providing two separate models for studying any effects that CRF, ACTH, IGF-I and IGF-II might have on oestrone, oestradiol, oestriol and progesterone production.

It was hoped that these two experimental models would further the understanding of how progesterone and oestrogen production is modulated in the human placenta so providing a clearer understanding of the regulation of these hormones and their possible roles in the process of parturition.

Materials and Methods

2.1. Materials

2.1.1. Chemicals, reagents and buffers

Adrenocorticotrophic hormone (ACTH), 1-39 from porcine pituitary, Sigma

16α-hydroxyandrostenedione (16α-OHA), (4-Androstene-16α-ol-3, 17-dione), Sigma

16α-hydroxytestosterone (16α-OHT), (4-Androstene-16α, 17β-diol-3-one), Sigma

16α-hydroxyoestrone (16α-OHE1), (3,16α-Dihydroxy-1, 3, 5 (10)-estratrien-17-one), Sigma

16α-hydroxydehydroepiandrosterone (16α-OHDHEA), (5-Androstene-3β, 16α-diol-17-one), Sigma

Androstenedione (A), (4-Androstene-3, 17-dione), Sigma

Bovine calf serum, Sigma

Cholesterol, (5-cholesten-3β-ol), Sigma

25-hydroxycholesterol (25-OHcholesterol), (5-cholesten3-3β, 25-diol), Sigma

Corticotrophin releasing factor (CRF), human and rat ; synthetic, Sigma

Dehydroepiandrosterone (DHEA), (5-Androsten-3β-ol-17-one), Sigma

Dehydroepiandrosterone-3-sulphate (DHEAS), (5-Androsten-3β-ol-17-one-sulphate), Sigma

Deoxyribonuclease (DNase), type 1V, Sigma

Dulbecco's modified Eagles medium without L-glutamine, with sodium pyruvate, (DMEM), Sigma

Eosin Y, Sigma

Gentamycin sulphate, Sigma

Hanks balanced salt solutions, without magnesium and calcium (HBSS), Sigma

Harris's haematoxylin, BDH Laboratory Supplies

Hydrochloric acid, BDH Laboratory Supplies

Hepes buffer (pH 7.2-7.4), Sigma

L-glutamine, Imperial Laboratories Europe Ltd.

Low density lipoprotein (LDL), Sigma

β-Nicotinamide adenine dinucleotide tetrasodium salt (NADPH), Grade 3, Sigma

Percoll, Sigma

Pregnenolone (5-pregnen-3β-ol-20-one), Sigma

Recombinant insulin-like growth factor-I (IGF-I), human (N-met$^-$), Bachem

Recombinant insulin-like growth factor-II (IGF-II), human (N-met$^-$), Bachem

2.5% Trypsin in HBBS without phenol red, Imperial Laboratories

Sodium hydrogen carbonate ($NaHCO_3$), BDH Laboratory Supplies

Trypan blue solution (0.4%), Sigma

2.1.2. Laboratory equipment

Baffled flasks, polycarbonate (250 ml), BDH Laboratory Supplies

CO_2 incubator

Flasks, flat bottomed (25 ml)

Gelair flow cabinet

Meat grinder

Metal sieve

12 ml sterile centrifuge tubes

24 well tissue culture plates, 16 mm diameter, Costar

Polypropylene centrifugation tubes (50 ml), Greiner

Polypropylene tubes, 12 by 75 mm

2.1.3. Kit for protein estimation

Protein assay kit (Sigma) containing the following reagents;

Aqueous solution of sodium deoxycholate (DOC), 1.5 mg/ml

Aqueous solution of trichloroacetic acid (TCA), 72% w/v

Bovine serum albumin, Fraction V

Folin and Ciocalteu's phenol reagent

Modified Lowry reagent

2.1.4. Assay kit for human chorionic gonadotrophin (HCG) determination

HCG production was determined in medium collected from isolated placental cell cultures using a radioisotopic assay kit containing avidin coated beads, iodinated HCG standards, controls and wash solution. The kit was purchased from Nichols Institute Diagnostics Ltd.

2.1.5. Assay kit for human placental lactogen (HPL) determination

HPL was assayed in medium collected from isolated placental cell cultures using a kit purchased from Diagnostics Products Corporation containing HPL antibody coated tubes, iodinated HPL, standards and wash solution.

2.1.6. Assay kit for ACTH determination

ACTH was assayed in medium collected from isolated cell preparations using a kit containing avidin coated beads, iodinated ACTH, standards, controls and wash solution. The kit was purchased from Nichols Institute Diagnostics Ltd.

2.1.7. Steroid assays.

Progesterone (P)

Oestrone (E1)

Oestradiol (E2)

Oestriol (E3)

The above steroids were obtained from Steraloids Ltd.

1 mg/ml stock solutions of each steroid in ethanol were diluted 1 in 1000 to obtain solutions of 1 μg/ml and further diluted 1 in 1000 to give 1 ng/ml.

2.1.8. Solvents

Ethanol, AnalaR Grade, BDH Laboratory Supplies.

Diethyl ether, AnalaR Grade, BDH Laboratory Supplies.

2.1.9. Solutions

Assay Buffer

Di-sodium hydrogen orthophosphate (Na_2HPO_4), BDH Laboratory Supplies	8.74 g
Sodium dihydrogen orthophosphate ($Na_2HPO4.2H_2O$), Fisons	6.00 g
Sodium chloride (NaCl), BDH Laboratory Supplies	9.00 g
Gelatine, Fisons	1.00 g
Sodium azide (NaN_3), BDH Laboratory Supplies	1.00 g

All were dissolved in 1 litre of deionized water.

Carbonate Solution

Sodium carbonate, anhydrous (Na_2CO_3)	152.0 g
Sodium hydrogen carbonate ($NaHCO_3$)	245.0 g

Dissolved in 1 litre of deionized water.

2.1.10. Radioactivity

1, 2, 6,-^{3}H progesterone, Amersham.

2, 4, 6,7 -^{3}H oestrone, Amersham.

2, 4, 6, 7 -^{3}H oestradiol, Amersham.

2, 4, 6, 9 -^{3}H oestriol, Amersham and DuPont NEN, DuPont (U.K.) Ltd.

Oestriol iodinated 125, Amerlex-m-Lifescreen Ltd.

2.1.11. Antisera

Sheep anti progesterone antibody, Bioclin

Sheep anti oestrone antibody, Steranti Research Ltd.

Sheep anti 17β–oestradiol, Bioclin

Anti-oestriol-6(cmo)-bovine serum albumin-antiseru, Steranti

Theses were all supplied freeze dried and reconstituted in assay buffer in 100 µl aliquots and stored at -40°C until required.

2.1.12. Dextran coated charcoal solution

Activated charcoal, BDH Ltd.	0.50 g
Dextran grade C, BDH Ltd.	0.05 g

Suspended in 100 ml of assay buffer.

2.2.13. Scintillation counting

Ultima Gold Liquid Scintillation cocktail, Packard.

Packard Tri-Carb 4000 series Liquid Scintillation counter.

2.1.14. Glassware

Extraction tubes 1 by 16 cm.

Reaction tubes 1 by 12 cm.

All glassware was rinsed and soaked in Decon 75 for at least 12 hours. It was then rinsed and left to soak in 1% hydrochloric acid for at least 1 hour before being rinsed 6 times in deionized water and dried for a minimum of 2 hours at 300°C.

2.1.15. Pipettes

Finn pipettes with disposable plastic tips

Glass pipettes

Sterile 3 ml plastic Pasteur pipettes

2.1.16. Addresses of product suppliers

Amersham, Little Chalfont, Buckinghamshire, England

Amerlex-m-Lifescreen Ltd., Watford, England

Bachem, Saxon Biochemicals, California

Bioclin, Cardiff, Wales

BDH Laboratory Supplies, Poole, England

Costar, Cambridge, England

Diagnostics Products Corporation, Glyn Rhonwy, Caernarfon, Gwynedd, Wales

DuPont NEN, DuPont (U.K.) Ltd., Stevenage, Hertfordshire, England

Fisons, Loughborough, Leicestershire, England

Imperial Laboratories Europe Ltd, West Portway, Andover, Hampshire, England

Nichols Institute Diagnostics Ltd., Newport, Essex, England

Packard, 9371 Groningen, Holland

Steranti Research Ltd., St Albans, Hertforshire, England

Steroloids, Croydon, Surrey, England

Methods

2.2. Preparation of placental tissue and isolation of cytotrophoblast cells

2.2.1. Collection and processing of placental tissue

Ethical permission for this study was granted by the joint UCH/UCL committee on the ethics of clinical investigations.

Placentae were collected immediately after delivery from women who had gone into spontaneous labour.

2.2.2. Incubation of placental tissue explants

Cotyledons were dissected and rinsed in 0.5 l ice cold saline before being minced and collected in a fine metal sieve. The tissue was then rinsed thoroughly in 1.5 l of ice cold saline, weighed and re suspended in ice-cold DMEM. Several aliquots were taken to obtain baseline values. Approximately 300 mg of tissue was transferred to each flask which contained 3 ml of DMEM with one of the following reagents.

CRF (100 nmol/l)

ACTH (10 nmol/l)

IGF-I (20 ng/ml)

IGF-I (400 ng/ml)

IGF-II (20 ng/ml)

IGF-II (400 ng/ml)

NADPH (1 mmol/l)

A total of six replicate flasks were prepared for each experiment with each reagent. As preliminary experiments showed that NADPH enhanced production of some of the steroids a series of experiments containing the above reagents with/without NADPH was conducted.

The flasks were incubated at 37°C in 5% carbon dioxide for a total period of 24 hours. At fixed time intervals 110 µl aliquots were taken and frozen at -40°C until the respective radioimmunoassays were carried out.

At the end of the experiment the tissue was centrifuged 1200 RCF for 10 minutes and any supernatant aspirated off. This procedure was repeated twice more with the addition of 10 ml of saline in order to remove any remaining culture medium. The pellet was then frozen at -40°C until protein estimation could be performed.

2.2.3. Isolation of cytotrophoblast cells

The isolation of cytotrophoblast cells was performed using the method of Kliman, Nestler, Sermasi, Sanger and Strauss (1986).

Fragments of cotyledons were dissected from the maternal side of the placenta and rinsed in 500 ml of ice cold saline. The tissue was then placed in a sieve, cut into small pieces with scissors and then rinsed in 1 l of ice cold saline. Between 60 g and 80 g of villus tissue was collected in this way, divided and transferred to two 250 ml baffled flasks containing 150 ml HBBS, 0.125% trypsin solution, and 30 mg DNase (1600-1700 Kunitz units/mg solid. One Kunitz is defined as that amount of enzyme which produces a change in absorbancy at a wavelength of 260 nm of 0.001 per min , per ml, at pH 5.0 and at 25°C, using DNA as a substrate) which had previously been warmed at 37°C. The flasks were then placed in a preheated water bath at 37°C and shaken at 180 strokes per minute for 30 minutes. Each flask was then placed at an

angle in the flow cabinet so that the tissue could settle and the supernatant (approximately 100 ml) could be collected. A further 100 ml HBSS, 0.125% trypsin solution and 20 mg DNase were then added to each flask and incubated for a further 30 minutes. After the incubatory period was complete the supernatant (approximately 100 ml) was once again removed and 75 ml warmed HBSS, 0.125% trypsin solution and 15 mg DNase were added to the tissue. The flasks were then incubated for a final 30 minute period. At the end of the final incubation as much supernatant as possible was removed without taking up any of the tissue.

After each successive incubatory period the supernatant which had been removed was layered over 1.5 ml bovine calf serum in four 50 ml centrifuge tubes and spun at 1000 RCF for ten minutes. The supernatant was discarded and the pellets were resuspended in 1ml DMEM containing 25 mM Hepes by sucking up and down with a sterile plastic Pasteur pipette. All pellets were pooled in a 50 ml tube. Once all the incubations were complete and the cells resuspended they were divided between two 50 ml centrifuge tubes which were filled with DMEM and spun at 1000 RCF for 10 minutes. The supernatant was discarded and the pellets were each resuspended in 6 ml DMEM. Approximately 3 ml of cell suspension was then layered onto four respective gradients. The gradients were made by layering the stock solutions given in table 2.2.3.

Percoll concentration (%)	Percoll (ml)	Water (ml)	10x HBSS (ml)	Volume (ml) layered in each tube.
70	13.125	3.750	1.875	2.00
60	6.000	3.000	1.000	2.00
55	5.500	3.500	1.000	4.00
50	5.000	4.000	1.000	2.00
45	4.500	4.500	1.000	2.00
40	4.000	5.000	1.000	2.00
35	3.500	3.500	1.000	2.00
30	6.000	12.000	2.000	2.00
20	2.000	7.000	1.000	2.00
10	1.000	8.000	1.000	3.00

Table 2.2.3. The volumes of reagents taken to obtain the Percoll gradient used to isolate cytotrophoblast cells.

The tubes were then centrifuged at 1200 RCF for 30 minutes. The solution above the 35% band was removed before the bands containing the cytotrophoblast cells (35% - 55%) were pipetted off and transferred to two 50 ml tubes. The tubes were filled with DMEM and shaken vigorously before spinning at 1000 RCF for 10 minutes. The resulting pellets were each resuspended in 1 ml of DMEM and pooled.

2.2.4. Morphology and the estimation of count and viability of isolated cells

A small volume of cell suspension was removed from the cell pool and transferred to several slides so that smears could be made and then allowed to air dry. The cells were stained using the following procedure.

The slides were;

a) immersed in Harris's haematoxylin for one minute and rinsed with deionized water,
b) immersed in acid alcohol (containing 30 parts 99.5% ethanol, 10 parts distilled water and 2 parts concentrated hydrochloric acid) for 15 seconds and rinsed with deionized water,

c) immersed in 2% sodium bicarbonate and rinsed with deionized water,

d) stained in 0.5% eosin Y solution for 10 seconds and rinsed in deionized water.

The slides were allowed to air dry before examination under high power oil immersion.

In order to determine the count and viablity an equal volume of cell suspension was added to an equal volume of trypan blue, vortexed and counted on a haemocytometer so that the percentage viability of the cell suspension was recorded at the same time as the count.

Estimation of contamination with granulocyes, using the method of Yam, Li and Crosby (1971), was kindly performed by Dr Ian Addison.

Cell preparations were incubated as described in section 2.2.6. and isolated cells were also incubated with 20% calf serum at 37°C and 5% CO_2 for up to 96 hours to assess steroid production, as well as the production of HCG, HPL and ACTH, of these cell preparations in two placentae.

2.2.5. Precursors

Preliminary experiments indicated that the cytotrophoblast cells produced very little steroids unless appropriate substrate precursors were present. Cell preparations from two placentae were incubated for 24 hours with several precursors. Isolated cells were incubated with either DHEAS, DHEA, A and T at a concentration of 1 μmol/l to assess E1 and E2 production. In order to assess E3 production cells were incubated with either 16α-OHDHEA, 16α-OHA, 16α-OHT or 16α-OHE1 at a concentration of 1 μmol/l. The production of P by isolated cytotrophoblast cells incubated with either cholesterol (5μmol/l), 25-OHcholesterol (5μmol/l), LDL (1.5 mg/ml) or pregnenolone

(1 μmol/l) was also measured. The precursors chosen to assess aromatase activity in cytotrophoblast cells were A and 16α-OHA. Due to the low levels of P production by cell preparations incubated with either cholesterol, 25-OHcholesterol or LDL, pregnenolone was chosen as the precursor for P formation.

Preliminary pilot studies were performed to assess the effects of the different precursors on each steroid. All precursors were added individually and in combination to isolated cell preparations from each placenta. On the basis of these preliminary experiments it was decided to add the precursors separately rather than in combination.

2.2.6. Cell and tissue incubates

Cell preparations obtained from each placenta were plated into 1 ml of DMEM containing 4 mmol/l glutamine, 50 μg/ml gentamycin sulphate and the appropriate steroid precursor with or without one of the following:

CRF (100 nmol/l)
ACTH (10 nmol/l)
IGF-I (20 ng/ml)
IGF-I (400 ng/ml)
IGF-II (20 ng/ml)
IGF-II (400 ng/ml).

A minimum of 100,000 viable cells were plated per well. Baseline levels were obtained by plating out 3 wells which were immediately placed in the freezer at -40°C, for each separate experiment. A further 6 wells containing cell preparations from each individual placenta were used for each separate experiment. For purposes of comparison tissue minces were also prepared from three of the placentae from

which the cytotrophoblast cells were also isolated. Approximately 200 mg portions of these minces were transferred to wells containing 2 ml of the same buffer solutions used for the cell incubates. The culture dishes were placed in a humidified incubator at 37°C and 5% CO_2 for 24 hours. At the end of this period the culture dishes containing the cell preparations were frozen at -40°C. An aliquot was taken from any wells containing tissue which was also frozen at -40°C. Tissue from each well was then transferred to a labelled 12 ml conical centrifuge tube and the well was rinsed twice with saline. These saline rinses were added to the tube and the tissue was then washed in exactly the same way as described in section 2.2.2 and frozen at -40°C until the protein content was analysed.

2.3. Protein estimation

The kit used for the estimation of protein content was purchased from Sigma and was based on a procedure of Lowry modified by Peterson (1977). Lowry reagent solution and Folin and Ciocalteu's working solution were made up with deionized water in accordance with the dilution information provided with the kit.

Duplicate standards were prepared by diluting BSA with deionized water to a volume of 1ml so that the final concentrations were 50, 100, 200, 300 and 400 µg/ml. Blank tubes contained 1 ml water. All placental pellets and controls were diluted with 1 ml water before 100 µl of DOC solution was added to every assay tube. The tubes were vortexed and left to stand at room temperature prior to the addition of 100 µl TCA solution then vortexed once again before centrifugation at 1000 RCF for 10 minutes. The supernatant was aspirated off and 1ml Lowry reagent solution was added to the blanks and standards. To tubes containing plasma or placental protein a total of 10 ml Lowry reagent solution was added. The tubes were then placed on a rotary mixer and the protein was allowed to dissolve over 48 hours.

After all the protein was visibly dissolved an appropriate volume of the placental protein solution (50 µl - 200 µl) was added to duplicate tubes and made up to 1 ml with Lowry reagent solution. The tubes were then separated into appropriately sized batches containing a blank, duplicate standards, controls and samples.

All assay tubes were then diluted with 1 ml water, vortexed and allowed to stand at room temperature for 20 minutes. Each tube was then vortexed and 500 µl of Folin and Ciocalteu's working solution was added whilst the solutions were being mixed. The colour was allowed to develop for 30 minutes. The blank and a standard or sample were then transferred to two respective plastic 3 ml cuvettes and the absorbance was measured at 750 nm on a spectrophotometer. All readings were completed within twenty minutes.

A calibration curve was then plotted of absorbance of the standards versus their concentrations and the unknown protein concentrations determined from this.

The intra-assay variability was determined by calculating the variation between duplicates of 50 different samples taken from 20 different assays using the formula described in section 2.6.5 and was found to be 4.7%. The inter-assay variation was calculated from the control values obtained from 20 different assays and was 6.1%.

2.4. Assays for steroids produced by tissue incubates

P, E2 and E3 levels were measured in all placental preparations. Due to the small volume taken at each time point it was not always possible to measure E1 levels.

All assays contained two controls. The samples from one time period were always assayed on the same day with equal numbers of the different conditions being assayed in the same batch.

2.4.1. Assay for P produced by tissue incubates

A standard curve containing triplicate concentrations of 0, 31.8, 63.6, 127.2, 254.4, 508.7, and 1017.5 pmol/l P was prepared by pipetting out the required volumes of stock solution into glass test tubes and drying down at 40°C with an air pump. After all the ethanol had evaporated 400 µl of assay buffer was pipetted into each tube and the tubes were vortexed for 5 minutes.

An appropriate volume of the frozen aliquot was pipetted into glass test tubes and the total volume made up to 500 µl with assay buffer. The tubes were vortexed and duplicate aliquots transferred to labelled test tubes and the volume made up to 400 µl with assay buffer. Four tubes containing only buffer solution (and precursors when present) were also included in the assay along with two tubes to show non-specific binding. Tritiated P (100 µl containing 10,000 counts/minute (cpm)) and 100 µl antiserum were pipetted into all tubes except those designated to non-specific binding where 100 µl assay buffer was substituted for the antiserum. The tubes were vortexed for a few seconds and incubated in a water bath at 37°C for one hour. The tubes were then incubated overnight at 4°C.

The following day the tubes were placed in ice-water at 0°C for one hour. A solution of charcoal coated dextran was prepared and mixed continually with a magnetic stirrer in an ice-water bath at 0°C for 30 minutes before use. The charcoal suspension was then added (200 µl) to batches of 16 tubes which were quickly vortexed. This procedure was repeated until all the tubes contained charcoal. To reduce assay drift charcoal was added to the standard curve when approximately half the tubes in the assay had had charcoal added to them. The assay was then left at 0°C for twenty minutes before centrifuging at 2000 RCF for 10 minutes at 0°C. After centrifugation the tubes were transferred back to the iced water and 500 µl of the supernatant was pipetted into scintillation vials containing 1.75 ml scintillation cocktail in the same

order that the charcoal had been added. The vials were then shaken vigorously and were each counted for 5 minutes on a liquid scintillation counter.

The amount of steroid present was then computed by logit/log transformation. The steroid concentration present in each well was then expressed as the amount secreted per mg protein present in the flask. As aliquots were taken off at various time intervals and the same volume replaced with fresh medium the results were adjusted to take into account the dilution effect of replacing the medium in the flasks.

2.4.2. Assay for E1 produced by tissue incubates

A standard curve containing triplicate concentrations of 0, 18.5, 37.0, 74.0, 147.9, 295.9, 591.7 and 1183.4 pmol/l E1 was prepared as previously described for the P assay in section 2.4.1.

The E1 assay was carried out in the same way as the P assay except that appropriate duplicate aliquots were pipetted into appropriately labelled test tubes and the volume made up to 400 µl before addition of tritiated E1 (100 µl containing 10,000 cpm) and 100 µl E1 antiserum.

2.4.3. Assay for E2 produced by tissue incubates

A standard curve containing triplicate concentrations of 0, 18.2, 36.4, 72.9, 145.8, 291.6, 583.1 and 1166.2 pmol/l E2 was prepared as previously described for the P assay in section 2.4.1.

The E2 assay was carried out in the same way as the P assay except that appropriate duplicate aliquots were pipetted into appropriately labelled test tubes and the volume

made up to 400 µl before addition of tritiated E2 (100 µl containing 10,000 cpm) and 100 µl E2 antiserum.

2.4.4. Assay for E3 produced by tissue incubates

A standard curve containing triplicate concentrations of 0, 17.3, 34.7, 69.4, 138.7, 277.4, 554.8 and 1109.6 pmol/l E3 was prepared as previously described for the P assay in section 2.4.1.

The E3 assay was carried out in the same way as the P assay but using tritiated E3 (100 µl containing 10,000 cpm) and 100 µl E3 antiserum. During the course of this study tritiated E3 no longer became available and iodinated E3 was used in its place. The assays were performed in an identical manner with the exception that 100 µl of iodinated E3 contained 7,000 disintegrations/minute and after addition of the charcoal solution the aliquots were transferred to labelled plastic tubes and not scintillation vials. These tubes were then counted for 3 minutes on a RIAStar gamma counter, and the results were computed by logit/log transformation.

2.5. Assays for steroids produced by isolated cytotrophoblast cells

2.5.1. Assay for P produced by isolated cytotrophoblast cell incubates

Triplicate tubes were prepared for a standard curve containing 0, 31.8, 63.6, 127.2, 254.4, 508.7, and 1017.5 pmol/l P and dried down at 40°C using a stream of air produced by a pump.

Appropriate volumes were taken from the culture wells and pipetted into glass extraction tubes with the addition of a suitable volume of carbonate buffer (1:1 carbonate buffer to sample) and diethyl ether was then added to the tubes such that the ratio of diethyl ether to sample was at least 20:1. A series of tubes was prepared which contained only the carbonate buffer solution and diethyl ether. All tubes were vortexed for 10 minutes and frozen at -20°C for 20 minutes. The supernatant was then carefully decanted into an appropriately labelled test tube and dried down at 40°C with an air pump. The supernatants of those tubes containing only carbonate buffer and diethyl ether were decanted into blank tubes. Five hundred µl assay buffer was added to all tubes before vortexing for 5 minutes. Appropriate aliquots were taken from each sample tube. The same volume was transferred from the blank tubes to those containing the standards. The volumes in all the tubes were then standardized to 400 µl with assay buffer. The tubes containing the standards were then vortexed for 5 minutes and the radioactivity and antiserum were added to all the tubes as described previously in section 2.4.1.

2.5.2. Assay for E1 produced by isolated cytotrophoblast cell incubates

Duplicate volumes were taken from the wells and transferred to labelled glass extraction tubes and diethyl ether was added in a ratio of at least 20:1 (diethyl ether to sample). A number of tubes containing just diethyl ether were also included. All tubes were vortexed for 10 minutes and frozen at -20°C for 20 minutes. The supernatant was then decanted into labelled glass reaction tubes. Those tubes containing diethyl ether alone were decanted into tubes containing the E1 standards, in triplicate, at concentrations of 18.5, 37.0, 74.0, 147.9, 295.9, 591.7, 1183.4 pmol/l. After drying down at 40°C 400 µl buffer was added and the tubes were vortexed for 5 minutes. The assay procedure was then carried out as previously described in section 2.4.2.

2.5.3. Assay for E2 produced by isolated cytotrophoblast cell incubates

Duplicate volumes of the samples were pipetted into labelled extraction tubes and carbonate buffer was added in a ratio of 2:1 (buffer to sample). Diethyl ether was added in a ratio of at least 20:1 (diethyl ether to sample). The tubes were vortexed for 10 minutes, frozen at -20°C for 20 minutes and then the supernatants were decanted into the respective tubes. The tubes containing only carbonate buffer and diethyl ether were decanted into the tubes containing the E2 standards, in triplicate, at concentrations of 0, 18.2, 36.4, 72.9, 145.8, 291.6, 583.1 and 1166.2 pmol/l. All the tubes were dried down and 400 µl assay buffer added. All the tubes were vortexed for 5 minutes and the assay procedure was identical to that described for the tissue incubates in section 2.4.3.

2.5.4. Assay for E3 produced by isolated cytotrophoblast cell incubates

Appropriate volumes of samples were pipetted into labelled glass extraction tubes and carbonate buffer was added in a ratio of at least 2:1 (buffer to sample). Certain tubes to be decanted into the tubes containing the standards contained only carbonate buffer. Diethyl ether was added in ratio of at least 20:1 (diethyl ether to sample) and the tubes were vortexed for 10 minutes before being frozen at -20°C for 20 minutes. The supernatants were decanted into the respective tubes. The tubes containing only carbonate buffer and diethyl ether were decanted into the tubes containing E3 standards, in triplicate, at concentrations of 0, 17.3, 34.7, 69.4, 138.7, 277.4, 554.8 and 1109.6 pmol/l. All the tubes were dried down and 400 µl assay buffer added. The supernatants were decanted, dried down and 400 µl assay buffer was added. All the tubes were vortexed for 5 minutes and the assay procedure was then identical to that described for the tissue incubates in section 2.4.4.

2.5.5. Assay for HCG produced by isolated cytotrophoblast cells

The assay was performed in accordance with the information provided with the kit and all reagents were made up accordingly. All standards, controls and samples were assayed in duplicate.

The standard curve (concentrations 0, 5, 10, 25, 100 and 300 mIU/ml) was prepared by pipetting 50 µl of the standards provided to the bottom of polypropylene tubes. Two controls provided with the kit were included in each assay. Appropriate aliquots were taken from the medium in which the cells had been incubated and pipetted into labelled polypropylene tubes. The volume was then made up to 50 µl with DMEM. Iodinated antiboby solution (200 µl) was added to each standard, sample and control. The tubes were vortexed and an avidin coated bead was added to each. The tubes were then incubated at room temperature for one hour. During the incubatory period the tubes were shaken by hand 100 times every 15 minutes. After incubation the beads were washed twice in a solution provided with the kit and the liquid aspirated from each tube. The tubes were counted on a gamma counter for one minute and the amount of HCG was computed by logit/log transformation.

2.5.6. Assay for HPL produced by isolated cytotrophoblast cells

The assay was performed in accordance with the information provided with the kit and all reagents were made up accordingly. All standards and samples were assayed in duplicate.

A standard curve was prepared by pipetting 50 µl of the standards provided (concentrations 0.0, 0.025, 0.1, 0.25, 0.5 and 1.0 µg/ml) to the bottom of duplicate polypropylene tubes. Aliquots (50 µl) of the medium in which the cells had been incubated were pipetted into appropriately labelled tubes. Iodinated HPL (1 ml) was

added to each standard and sample. The tubes were vortexed and incubated at 37°C for three hours. The liquid from each tube was decanted simultaneously. Each tube was counted on a gamma counter for one minute and the amount of HPL was computed by logit/log transformation.

2.5.7. Assay for ACTH produced by isolated cytotrophoblast cells

The assay was performed in accordance with the information provided with the kit and all reagents were made up accordingly. All standards, samples and controls were assayed in duplicate.

A standard curve was prepared by pipetting (200 µl) of the standards provided (concentrations 0, 5, 14, 50, 140, 465 and 1400 pg/ml) to the bottom of labelled polypropylene tubes. Two controls provided with the kit were also pipetted in the same manner. Aliquots (200 µl) were taken from the medium in which the cells had been incubated and pipetted into labelled polypropylene tubes. Iodinated ACTH antiboby solution (100 µl) was added to each standard, sample and control. The tubes were vortexed and an avidin coated bead was added to each. The tubes were then incubated at room temperature for twenty hours. After incubation the beads were washed twice in a solution provided with the kit and the liquid aspirated from each tube. The tubes were counted on a gamma counter for one minute and the amount of ACTH was computed by logit/log transformation.

2.6. Validation of the hormone assays

2.6.1. Recoveries for assays involving extraction procedures

The percentage recovery for each steroid was calculated by adding 10,000 cpm of the dissolved tritiated steroid in 100 ml DMEM to twenty glass extraction tubes. The mixture was then extracted and assayed in the same way as for the sample aliqouts taken for assay as previously described. The following values are the percentage recovery obtained for each steroid. All results were adjusted to allow for the percentage recovery.

P 94%

E1 90%

E2 95%

E3 90%

2.6.2. Blank values for the steroid assays

There were no significant differences between the values measured in tubes containing DMEM alone and the zero tubes of the standard curve.

2.6.3 Sensitivities of the steroid assays

The lower limits of sensitivity of each of the assays per tube have already been established previously in the laboratory and are as follows:

E1 10 fmol.

E2 10 fmol

E3 12 fmol

P 14 fmol

2.6.4. Precision of the steroid assays

Precision profiles are used to asses the performance of an assay throughout a concentration range and determine the working range for the assay. The acceptable range for this study was that the % CV should be less than 10%.

A precision profile was determined for E2. A series of tubes was prepared containing different concentrations of the steroid in 50 µl buffer and 100 µl carbonate buffer. The number of tubes for each concentration was 5 and the known concentrations were 9.1, 18.2, 36.4, 72.9, 145.8, 291.6, 583.1, 801.8, 1020.4 and 1166.2 pmol/l for E2. An additional number of tubes containing the assay buffer and carbonate buffer were also prepared for the standard curve. A total of 4.4 ml of diethyl ether was added to each tube. All tubes were vortexed for 10 minutes. The tubes were then placed at -20°C for 20 minutes until the aqueous layer had frozen.The supernatant was then carefully decanted into an appropriately labelled test tube. Those tubes prepared for the standard curve were decanted into tubes containing known amounts of E2 dissolved in ethanol which consisted of the following duplicate concentrations; 0, 31.8, 63.6, 127.2, 254.4, 508.7, 1017.5 pmol/l. All the tubes were dried down at 40°C using an air stream supplied by an air pump.

After the tubes had dried 400 µl of assay buffer was pipetted into each tube and the tubes were vortexed for two minutes. Tritiated E2 (10,000 cpm) and antiserum (100 µl) were added to each tube with the exception of two tubes into which assay buffer (100 µl) was added in place of the antiserum. These two tubes were used to determine the non-specific binding of the assay. All the tubes were vortexed and placed in a water bath at 37°C for 1 hour. The assay was then kept at 4°C overnight and placed in

iced water the following morning for 1 hour. A charcoal solution was kept mixing in an ice-water bath during this period and 200 µl of this mixture was added to each tube. The tubes were vortexed and left in ice-water for 20 minutes after which time they were centrifuged at 2000 RCF for 10 minutes. 500 µl of the supernatant was transferred from each tube into a scintillation vial containing 1.75 ml scintillation fluid and the vials were shaken thoroughly and then counted on a scintillation counter for 5 minutes each.

The actual values of the replicates were then computed from the standard curve by logit/log transformation. The mean, SD and the % CV were then calculated for each set of 5 replicates and a precision profile curve was plotted as shown in figure 2.6.4. The working range as determined by the precision profile was 150 to 1100 pmol/l.

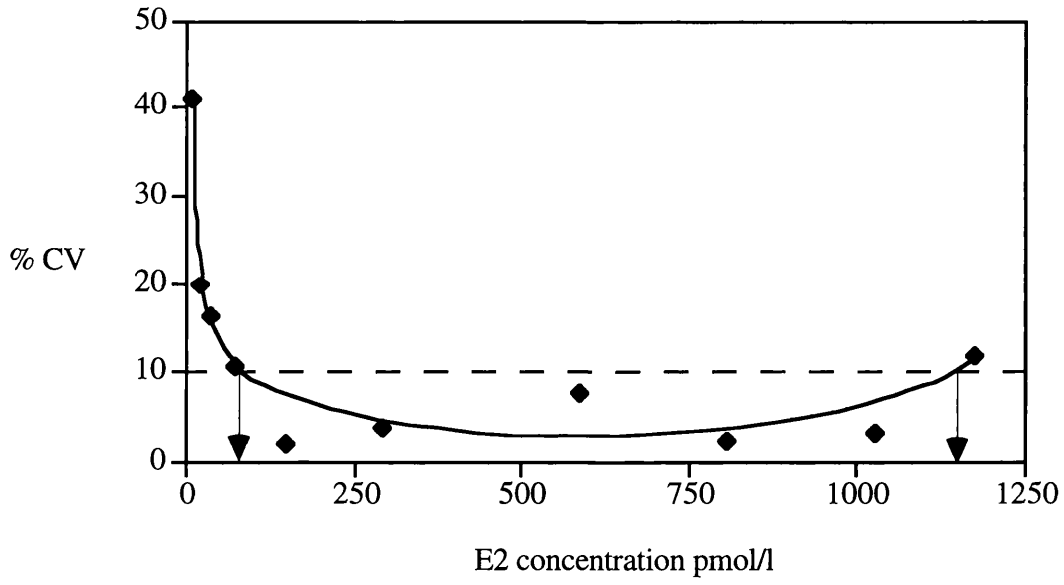


Figure 2.6.4. The precision profile curve for E2. The dashed line indicates the acceptable precision and defines the working range for the assay. The arrows indicate the working range of the assay.

A similar procedure was undertaken for P with the exception that the volume of assay buffer and carbonate buffer added to each tube was 50 µl and the volume of diethyl ether used for extraction was 2.2 ml. The standard curve for P consisted of the following concentrations; 0, 31.8, 63.6, 127.2, 254.4, 508.7, 1017.5 pmol/l and the known concentrations were 15.9, 31.8, 63.6, 127.2, 254.4, 508.7, 699.5, 890.3 and

1017.5 pmol/l. The working range as determined by the precision profile was 60 to 700 pmol/l.

These profiles were very similar to precision profiles previously determined in the laboratory, and on this basis the previously determined working ranges for the E1 and E3 assays were used, which were 140 to 1000 pmol/l and 150 to 900 pmol/l.

The volume of the aliquots taken from the incubation media for the tissue and isolated cytotrophoblast cell experiments were chosen so that the values would fall within the working range of each standard curve.

2.6.5. The intra-assay and inter-assay coefficient of variation for the steroid assays

Intra-assay determination was calculated by one of two ways.

Method 1

Intra-assay variability can be assessed by measuring the variation between duplicate samples covering the whole range of concentrations obtained from different assays. Fifty such duplicates were chosen at random from different assays for each steroid and the resulting CV was calculated using the formula

$$CV = \sqrt{\frac{\Sigma d^2}{2n}}$$

where d = the percentage difference between duplicates and n = the number of pairs of duplicate determinations. Using this method the % CV for each steroid is listed below in table 2.6.5a.

	Method 1
E1	7.2%
E2	5.5%
E3 (tritiated)	7.8%
E3 (iodinated)	8.0%
P	8.7%

Table 2.6.5.a The % CV of steroid assays in the present study determined by using method 1.

Method 2

Three pools were prepared by mixing the excess DMEM taken from a number of tissue explant experiments which contained low, medium and high concentrations of each of the steroids. The volumes used for assay were chosen to fall within the range defined by the precision profile. Twenty replicates were assayed for each pool and the coefficient of variation for each pool was calculated. The values for the % CV are given in table 2.6.5b.

		Method 2		
		Low pool	**Medium pool**	**High pool**
E1	mean	165.8	508.7	805.0
	sd	13.4	63.6	80.5
	% CV	12.7%	8.0%	10.0%
E2	mean	170.5	622.0	853.4
	sd	17.9	73.2	123.7
	% CV	9.5%	8.5%	6.9%
E3 (tritiated)	mean	161.8	447.3	805.6
	sd	16.5	55.9	115.1
	% CV	9.8%	8.0%	7.0%
E3 (iodinated)	mean	165.8	454.4	798.9
	sd	19.5	59.8	84.9
	% CV	8.5%	7.6%	9.4
P	mean	137.7	276.0	565.7
	sd	12.3	33.3	43.2
	% CV	11.2%	8.3%	13.1%

Table 2.6.5.b The % CV of steroid assays in the present study determined by using method 2. The number of replicates per pool was 20. The mean values given are in fmoles/tube.

The inter-assay variability was determined from medium controls (n = 20) used in different assays and the % CV obtained for each steroid is listed below in table 2.6.5.c.

assay	medium control fmol/tube	SD	% CV
E1	525	72.9	7.2%
E2	617	59.3	10.4%
E3 (tritiated)	452	61.1	7.4%
E3 (iodinated)	457	57.1	8.0%
P	272	18.9	14.4%

Table 2.6.5.c The inter-assay variability for each steroid assay.

2.6.6. Cross reactivities of the steroids assayed

Table 2.6.6 gives the percentage cross reaction of various steroids with the four antisera used in this study. As the table shows the cross reactivities of the precursors used in this study were negligible with the exceptions of those experiments in which pregnenolone was used. There was measurable cross reactivity of the progesterone antiserum with pregnenolone in these experiments. All assays contained tubes with the appropriate concentration of precursor in DMEM. The values for P levels in these cases were adjusted by subtracting the levels obtained in these tubes from all other tubes in the assay.

	Anti-E1 antiserum	Anti-E2 antiserum	Anti-E3 antiserum	Anti-P antiserum
E1	100	2.2	0.02	nt
E2	0.5	100	1.3	nt
E3	0.1	1.1	100	nt
P	<0.01	<0.01	<0.01	100
A	0.02	<0.01	<0.01	nt
DHEA	0.01	0.10	0.10	nt
pregnenolone 1 μmol	<0.01	<0.01	<0.01	2.3%
pregnenolone 10 μmol	<0.01	<0.01	<0.01	5.4%
pregnenolone 20 μmol	<0.01	<0.01	<0.01	7.9%
17-OHP	<0.01	<0.01	<0.01	0.3
16α-OHA	nt	nt	<0.2	nt
16α-hydroxyoestrone	nt	nt	<1.0	nt
16α-hydroxytestosterone	nt	nt	<0.2	nt
cortisol	<0.01	<0.01	<1.0	0.1
corticosterone	nt	nt	nt	0.8
testosterone	<0.01	<0.01	<0.01	<0.01
11-deoxycorticosterone	nt	nt	nt	0.9
11-deoxycortisol	nt	nt	nt	<<0.01

Table 2.6.6. The percentage cross reactivities of E1, E2, E3 and P antisera with other steroids (nt = not tested).

2.6.7. Recoveries of HCG, HPL and ACTH assays

The recoveries for the HCG, HPL and ACTH assays are as described in the information provided with the appropriate kits. In all kits sera were spiked with known amounts of the respective hormone. The recovery was determined by dividing the recovered or observed values by those levels which were expected.

The recovery for HCG ranged from 95.5-107.7% in three spiked serum samples.

The recovery for HPL ranged from 100-110% in six serum samples spiked with 10 μg/ml HPL.

The recovery for ACTH ranged from 89-109% in plasma samples with endogenous ACTH <1 pg/ml spiked with 18-3 pg/ml ACTH and was 92-100% in samples with endogenous levels of 8 pg/ml spiked with 25-50 pg/ml ACTH.

2.6.8. Sensitivities of HCG, HPL and ACTH assays

The sensitivity for each assay is that as described in the information provided with the respective kit.

The sensitivity of the HCG assay is defined as the smallest single value which can be distingushed from zero at the 95% confidence limit and is 0.5 mIU/ml.

The sensitivity of the HPL assay is defined as the apparent concentration two standard deviations below the counts at maximum binding and is 0.02 μg/ml.

The sensitivity of the ACTH assay is defined as the smallest single value which can be distingushed from zero at the 95% confidence limit and is 1.0 pg/ml.

2.6.9. Precision of HCG, HPL and ACTH assays

The intra-assay variance and inter-assay variance of the HCG, HPL and ACTH assays were as defined in the information provided with the kits and are given below.

The intra-assay variance for the HCG assay was calculated from replicate determinations on each of two human serum pools (n=20) in a single assay. The intra-assay % CV for the two serum pools with mean values of 22.4 and 45.6 mIU/ml was 4.3% and 4.8% respectively. Inter-assay variance was calculated from data obtained from different assays on two human serum pools with mean values of 23.8 (n=39) and 44.6 mIU/ml (n=25), and was 7.6% and 7.5% respectively.

The intra-assay % CV for the HPL assay was calculated for 20 pairs of tubes in a single assay whilst the inter-assay % CV was calculated for each replicate tubes in 20 different assays. The intra-assay % CV for two serum pools with means of 140 ng/ml and 870 ng/ml was 4.7% and 3.3% respectively. The inter-assay % CV for two serum pools with means of 160 ng/ml and 940 ng/ml was 8.6% and 6.7% respectively.

The intra-assay % CV for the ACTH assay was calculated from replicates of 20 pairs of controls in a single assay whilst the inter-assay % CV was calculated from two controls assayed 62 times. The intra-assay % CV for a pool with a mean of 35 pg/ml was 3.0%. The inter-assay % CV for a serum pool with a mean of 36 pg/ml was 7.8%.

2.6.10. Cross reactivities of HCG, HPL and ACTH assays

The information provided with the kits details the cross reactivities of HCG, HPL and HCG. There was no measurable cross reactivties between any of the hormones HPL, HCG or ACTH.

2.7. Statistical analysis of data

Parametric tests are used for statistical analyses when data is normally distributed whereas non-parametric tests make no assumption as to the form of data distribution. Due to the small numbers used in each experiment in this study it was difficult to determine whether the results were normally distributed. On this basis the Mann Whitney U test, a non parametric test, was chosen for use in the statstical analysis. When comparing means, an independent t test may be used for normally distributed data, whereas the Mann Whitney U test may be used as an equivalent non parametric

test that analyses data according to ranks rather than means. All data were analysed using the Quick Statistica 3.0a package for the Apple Macintosh.

Results

3.1. Preliminary experiments on the validation of the isolated cytotrophoblast cell type and the production of hormones by these isolated cells

3.1.1. Morphology, viability and production of hormones by isolated cell preparations

The cells isolated in this study when plated were small, round and mononuclear as seen under phase contrast microscopy and after staining with haematoxylin and eosin. When cultured without bovine calf serum very few of the cells aggregated and they appeared similar in appearance at 24 hours to those plated at the start of the experiment. Cells cultured in 20% bovine serum had begun to aggregate by 24 hours and by 96 hours the majority of cells had formed syncytia; these cells produced HCG and HPL which had risen after incubation for 96 hours (see table 3.1.1). ACTH was also measured and was approximately 1-2 pg/100,000 viable cells at both 24 and 96 hours. These ACTH levels were not affected by addition of CRF (100 nmol/l and 1 μmol/l) to the culture medium.

	HCG (mIU/100,000 viable cells)		**HPL (ng/100,000 viable cells)**	
	Experiment 1	**Experiment 2**	**Experiment 1**	**Experiment 2**
24 hours	20.44 ± 1.00	7.35 ± 0.38	381.0 ± 17.52	~
96 hours	6227.8 ± 740.1	271.7 ± 60.22	1053.6 ± 33.08	79.46 ± 1.93

Table 3.1.1. Mean ± SE values for HPL and HCG levels produced by cytotrophoblast cells cultured for 96 hours in 20% bovine calf serum (~ levels below sensitivity of assay).

Viability of the cytotrophoblast cells obtained from each placenta in this study was determined with trypan blue and was found to be more than 90%. Contamination with granulocytes of the purified cytotrophoblast cells obtained from three placentae was less than 10%.

3.1.2. Choice of precursors for E1, E2, E3 and P production

Although tissue explants incubated in DMEM alone produced measurable levels of E1, E2, E3 and P, isolated cytotrophoblast cell preparations did not produce detectable levels of these steroids without the addition of appropriate steroid precursors. A number of precursors, DHEA, DHEAS, A and T were added to the cells to assess the production of both E1 and E2. The production of E1 and E2 did not generally appear to be altered by the precursor used in the experiment. The precursor chosen for further experiments was A as this allows the activity of both aromatase and 17β-HSD to be observed. The mean (± SE) values for the levels of E1 and E2 produced by the cells are given in table 3.1.2a

	Experiment 1		Experiment 2	
Precursor	**E1**	**E2**	**E1**	**E2**
DHEA	161.6 ± 6.96	376.9 ± 19.99	44.16 ± 5.10	163.46 ± 12.37
DHEAS	199.8 ± 30.19	416.8 ± 38.19	41.44 ± 3.24	150.34 ± 8.06
A	203.5 ± 14.02	532.7 ± 22.98	41.06 ± 6.36	146.59 ± 15.89
T	181.6 ± 26.26	454.4 ± 25.45	30.72 ± 1.64	127.85 ± 7.90

Table 3.1.2a. Mean ± SE values for E1 and E2 levels (pmol/100,000 viable cells/24 hours) produced by cytotrophoblast cells isolated from two placentae and incubated for 24 hours with the respective precursor at a concentration of 1 μmol/l (number of wells per experiment for each precursor = 4).

Several precursors, 16α-OHDHEA, 16α-OHA, 16α-OHT and 16α-OHE1 were chosen to assess the production of E3 by cytotrophoblast cells isolated from two placentae and incubated for 24 hours. There were no significant differences when 16α-OHA was used as a precursor compared to those incubated with 16α-OHDHEA. However E3 levels produced by cytotrophoblast cells isolated from both placentae were significantly lower when 16α-OHT was used as a precursor compared to cells incubated with 16α-OHA ($p < 0.03$). The levels of E3 produced by isolated cytotrophoblast cells from both placentae were significantly higher when 16α-OHE1 was used as a precursor compared to those cells incubated with 16α-OHA ($p < 0.03$). Table 3.1.2b gives the mean values for E3 levels produced by isolated cytotrophoblast cells incubated with the various precursors for 24 hours.

Precursor	Experiment 1	Experiment 2
16α-OHDHEA	353.6 ± 19.52	55.54 ± 2.88
16α-OHA	262.8 ± 43.39	53.28 ± 3.58
16α-OHDT	117.0 ± 4.21	32.71 ± 3.54
16α-OHE1	972.3 ± 16.12	1288.4 ± 59.60

Table 3.1.2b. Mean ± SE values for E3 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells obtained from two placentae and incubated with respective precursors at a concentration of 1 μmol/l for 24 hours (number of wells per experiment for each precursor = 4).

One precursor originally chosen to assess P production in placental cells was 25-hydroxycholesterol as this was chosen by Nestler and Williams (1987) and Kliman (1986) in their studies on cytotrophoblast cells. The initial studies however indicated that addition of 25-hydroxycholesterol (5 μmol/l) had little effect on P output by the cells as there was no significant increase in P levels beween those cells incubated with, and those cells incubated without 25-hydroxycholesterol (mean values from two placentae are given in table 3.1.2c). Further studies indicated that increasing the concentration of 25-hydroxycholesterol to 80 μmol/l had no effect on P production. Cholesterol and LDL were also used as potential precursors, but no measurable levels of P were obtained from cells cultured with cholesterol alone, and although P levels measured after incubating with 1.5 mg/ml LDL were higher than those values obtained from incubating cells alone, they were only approximately 30% of those measured in cell preparations incubated with 1μmol/l pregnenolone and only double those of cells incubated without precursors (mean values are given in table 3.1.2d). It was decided therefore to use pregnenolone as the substrate for P production.

Precursor	Experiment 1 (n=6)	Experiment 2 (n=5)
cells only	176.5 ± 67.92	509.6 ± 87.96
25-hydroxycholesterol (5 μmol/l)	125.3 ± 15.07	593.8 ± 64.82

Table 3.1.2.c. Mean ± SE values for P levels (pmol/100,000 viable cells/24 hours) produced by cytotrophoblast cells incubated with/without 25-hydroxycholesterol (5 μmol/l) for 24 hours (n= number of wells used in each experiment with each precursor).

Precursor	P
cells only	0.82 ± 0.10
LDL	1.42 ± 0.09
Pregnenolone	4.02 ± 0.25

Table 3.1.2.d. Mean ± SE values for P levels (pmol/100,000 viable cells/24 hours) produced by cytotrophoblast cells isolated from one placentae, incubated with/without LDL (5mg/ml) or pregnenolone (1μmol/l) for 24 hours. Number of wells in each case = 5.

Several experiments were conducted on cytotrophoblast cells to assess the effects of substrate concentrations of the chosen precursors on steroid production. Cell preparations from two placentae, containing 100,000 viable cells per well were plated in triplicate in 1 ml of DMEM containing the relevant precursor and incubated for 24 hours. Cell preparations from the same two placentae were used for all the experiments conducted in sections 3.1.3 to 3.1.5.

3.1.3. Choice of precursor concentration for E1 and E2 production

The aromatase enzyme system appeared to reach saturation at concentrations greater than 600 nmol/l (figures 3.1.2a and 3.1.2b) with the experimental conditions used, although there appeared to be a peak of activity in cell preparations obtained from each placenta for both E1 and E2 production at concentrations of 400 nmol/l (placenta 1) and 200 nmol/l (placenta 2). A concentration of approximately 700 nmol/l A was chosen for all experiments as approximately 1%-2% of A was converted to these oestrogens at this concentration in 24 hours and the enzyme system appeared to be saturated. Mean ± SE E1 and E2 levels produced by these cell preparations are given in tables 3.1.3a and 3.1.3b respectively. The same concentration of A was chosen for use in those tissue explant preparations incubated at the same time as the isolated cells.

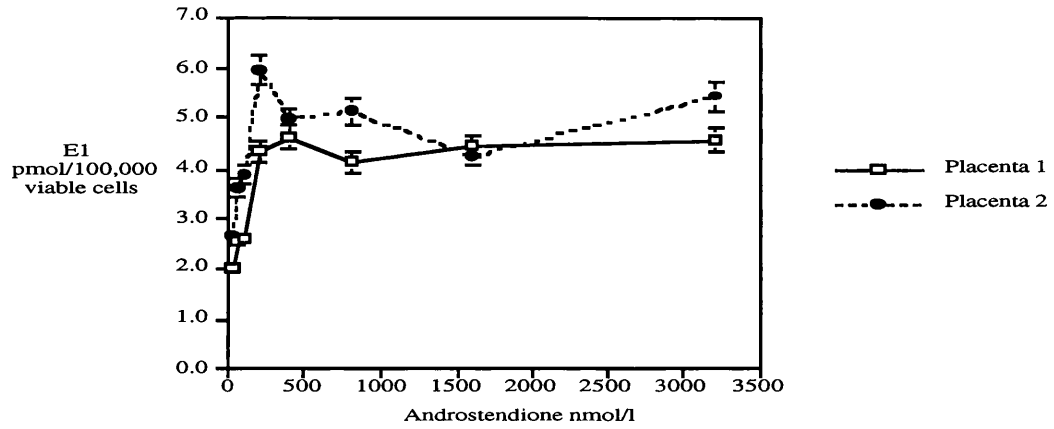


Figure 3.1.3a. Production of E1 by cytotrophoblast cells isolated from two placentae and incubated with various concentrations of A for 24 hours.

	E1	
A nmol/l	**Placenta 1**	**Placenta 2**
25	2.00 ± 0.09	2.66 ± 0.41
50	2.53 ± 0.25	3.60± 0.091
100	2.58 ± 0.57	3.88 ± 0.40
200	4.33 ± 0.69	5.97 ± 0.68
400	4.64 ± 0.10	4.97 ± 0.25
800	4.13 ± 0.45	5.14 ± 0.11
1600	4.44 ± 0.64	4.25 ± 0.59
3200	4.56 ± 0.35	5.44 ± 0.64

Table 3.1.3a. Mean ± SE values for E1 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various concentrations of A.

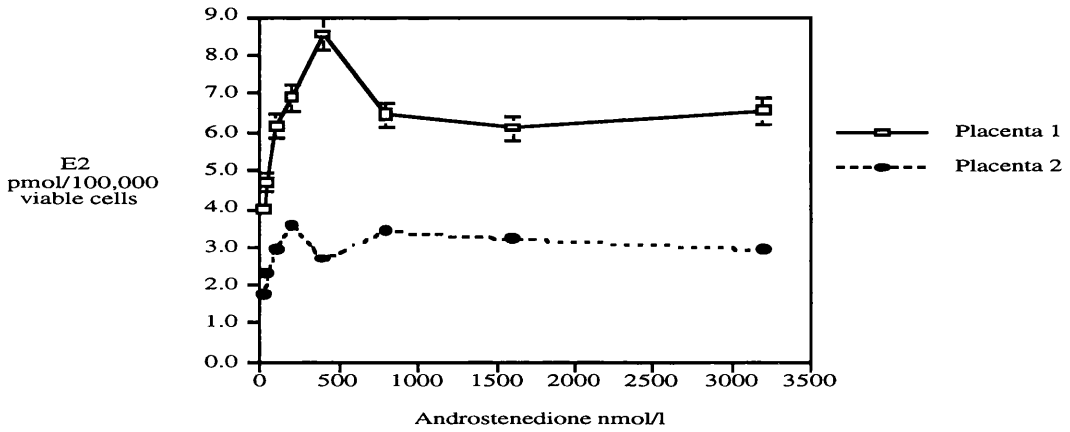


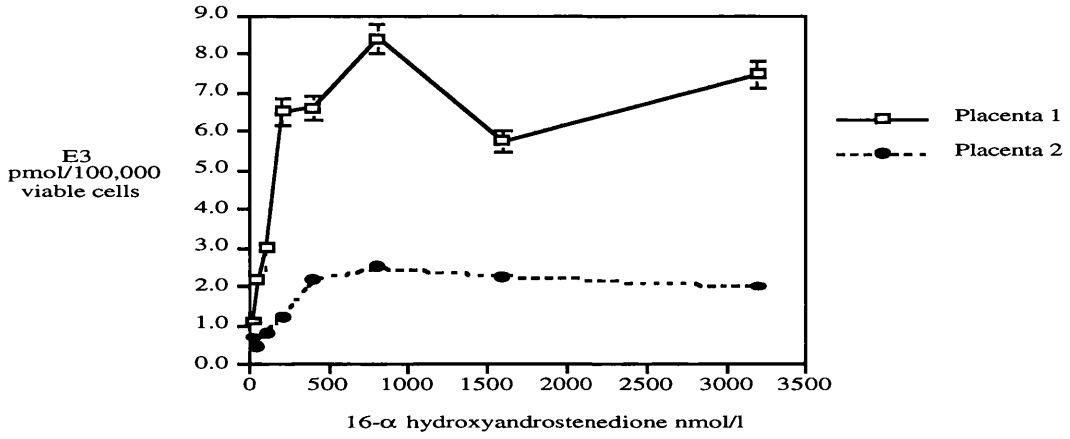
Figure 3.1.3b. Production of E2 by cytotrophoblast cells isolated from two placentae and incubated with various concentrations of A for 24 hours.

	E2	
A nmol/l	**Placenta 1**	**Placenta 2**
25	4.03 ± 0.08	1.74 ± 0.15
50	4.72 ± 0.42	2.31 ± 0.13
100	6.18 ± 0.54	2.96 ± 0.17
200	6.87 ± 0.86	3.61 ± 0.28
400	8.54 ± 0.69	2.68 ± 0.17
800	6.46 ± 0.86	3.42 ± 0.86
1600	6.12 ± 0.37	3.20 ± 0.20
3200	6.55 ± 0.12	2.90 ± 0.06

Table 3.1.3b. Mean ± SE values for E2 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various concentrations of A.

3.1.4. Choice of precursor concentration for E3 production

The aromatase enzyme appeared to be saturated at around 800 nmol/l (figure 3.1.3). A precursor concentration of 1000 nmol/l was chosen for all experiments as there was between 0.3-1% conversion of 16α-OHA to E3 at this concentration and the enzyme system appeared to be saturated. The mean ± SE values for E3 levels produced by these cell preparations are shown in table 3.1.4. The same concentration of 16α-OHA was chosen to be used in the tissue explant preparations incubated at the same time as the isolated cells.



3.1.4. Production of E3 by cytotrophoblast cells isolated from two placentae and incubated with various concentrations of 16α-OHA for 24 hours.

	E3	
16α–OHA nmol/l	**Placenta 1**	**Placenta 2**
25	1.12 ± 0.72	0.72 ± 0.19
50	2.11 ± 0.31	0.49 ± 0.03
100	2.97 ± 0.90	0.78 ± 0.15
200	6.50 ± 0.36	1.22 ± 0.14
400	6.60 ± 0.01	2.16 ± 0.15
800	8.40 ± 6.88	2.49 ± 0.46
1600	5.73 ± 0.97	2.26 ± 0.62
3200	7.55 ± 0.79	2.00 ± 0.21

Table 3.1.4. Mean ± SE values for E3 levels (pmol//100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various concentrations of 16α-OHA.

3.1.5. Choice of precursor concentration for P production

In placenta 1 the 3β–HSD enzyme system appeared to become saturated at a concentration of pregnenolone of 20 μmol/l but saturation did not occur in placenta 2, with production still increasing at concentrations of pregnenolone of 80 μmol/l (see figure 3.1.4). However even at concentrations as low as 5 μmol only around 10% of the precursor was being transformed to P with the percentage dropping to about <5% at concentrations of 10 μmol/l and 20 μmol/l, which is in agreement with the results of Nestler (1989). The mean ± SE values for levels of P produced by these cell preparations are shown in table 3.1.5. It was decided to use 3 concentrations of pregnenolone precursors for further experiments (1 μmol/l, 10 μmol/l and 20 μmol/l). The lower concentration was used for experiments involving tissue minces as this was thought to approximate to more physiological levels.

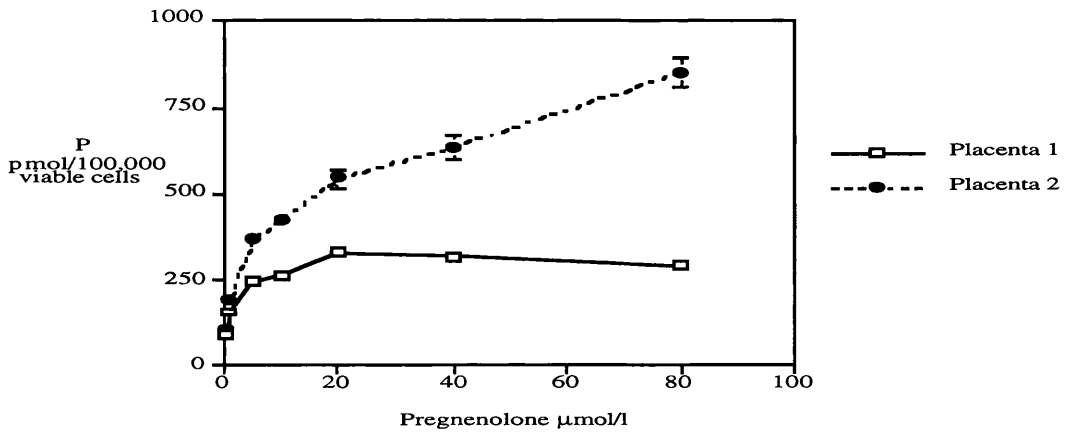


Figure 3.1.5. Production of P by cytotrophoblast cells isolated from two placentae incubated with various concentrations of pregnenolone for 24 hours.

	P	
Pregnenolone µmol/l	**Placenta 1**	**Placenta 2**
0.5	89.9 ± 2.7	106.8 ± 9.0
1.0	155.8 ± 32.9	193.8 ± 14.0
5.0	244.8 ± 24.5	365.5 ± 16.4
10.0	262.4 ± 58.1	424.0 ± 16.4
20.0	329.7 ± 14.3	542.8 ± 39.2
40.0	318.1 ± 27.6	631.6 ± 35.4
80.0	292.5 ± 7.4	848.4 ± 177.3

Table 3.1.5. Mean ± SE values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various concentrations of pregnenolone.

3.1.6. Steroid hormone production by isolated cells incubated for 96 hours

The 3β-HSD and aromatase activity were assesed at 24 and 96 hours using the chosen precursor concentrations, 20-µmol/ pregnenolone, 700 nmol/l A and 1000 nmol/l 16α-OHA. The levels of E1, E2, E3 and P were generally higher at 96 hours than those measured at 24 hours with the exception of E1 levels measured in experiment 2 which were not significantly different. The levels of steroids produced by placentae incubated for 96 hours are shown in table 3.1.6.

	E1 (pmol)	E2 (pmol)	E3 (pmol)	P (nmol)
24 hours				
experiment 1	9.31 ± 1.52	16.37 ± 1.20	78.41 ± 20.62	1.12 ± 0.77
experiment 2	270.42 ± 25.66	449.37 ± 13.00	318.25 ± 23.22	8.65 ± 0.11
experiment 3	35.93 ± 2.13	154.82 ± 4.17	59.32 ± 3.22	4.35 ± 0.53
96 hours				
experiment 1	17.29 ± 4.41	60.70 ± 6.37	146.79 ± 31.12	2.71 ± 0.21
experiment 2	242.18 ± 21.87	462.55 ± 16.47	417.78 ± 37.55	15.07 ± 0.51
experiment 3	126.68 ± 19.90	355.30 ± 29.32	226.57 ± 20.98	10.47 ± 0.28

Table 3.1.6. The mean ± SE values for E1, E2, E3 and P levels (/100,000 viable cells/24 hours) produced by trophoblast cells incubated with 20% bovine calf serum and a respective precursor.

3.2. Preliminary note on the graphical representation of data

Each placenta in this study has been assigned an individual character and number, for example placenta A1 shown in figure 3.3.2 is the same placenta A1 as that shown in figure 3.6.2.

Due to the large variation between levels of steroids produced by individual placentae all the results (see raw data in the appendix) have been expressed in graphic form as percentage differences between the means of the steroid levels obtained from those tissue explants or cells incubated with either CRF/ACTH/IGF-I/IGF-II or NADPH, and the mean values obtained from the control (100%) for each individual placenta. In the case of the experiments involving tissue minces the controls consisted of explants incubated with DMEM alone, whereas the controls for the isolated cell experiments were those cells incubated in DMEM and the appropriate steroid precursor.

Statistically significant differences obtained between controls and tissue explants/ isolated cytotrophoblast cells incubated under the relevant experimental conditions are represented in the graphs as ; * significant difference $p \leq 0.05$, ** significant difference $p \leq 0.01$.

3.3. The effects of CRF and ACTH on E2 production by tissue explants

In a study on 3 placentae incubated in DMEM alone (controls) or with the medium containing CRF there was a significant increase in E2 in one placenta A2, at 40 minutes ($p < 0.05$, see figure 3.3.1), compared to the control for this placenta. However in placenta A4 there was a significant decrease in E2 levels produced at 20 minutes ($p < 0.05$) when compared to the control. These effects were not consistent throughout the experiment and overall there was no effect of CRF on E2 levels from placental tissue minces at 24 hours of incubation. The mean ± SE and median levels for E2 from the tissue minces incubated as controls or with CRF are given in table 3.3.1 .

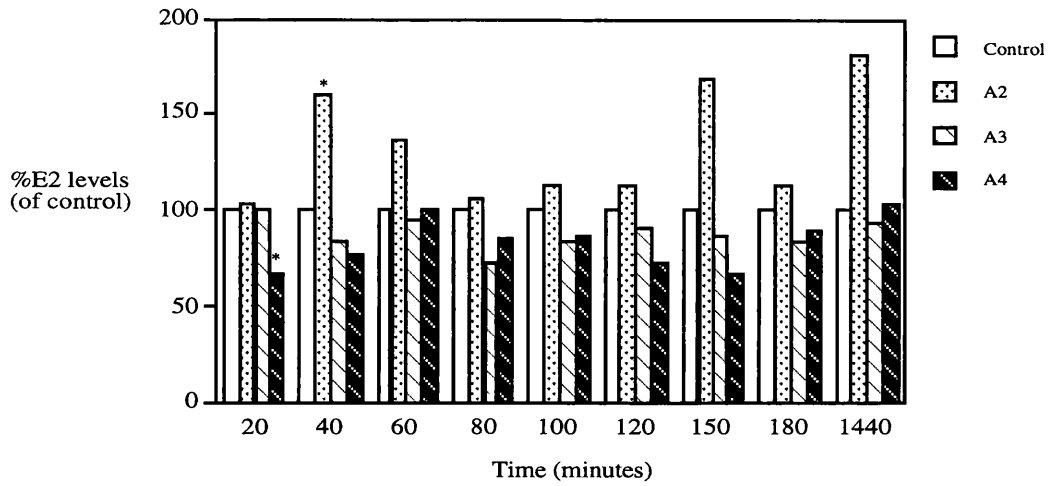


Figure 3.3.1. The effects of CRF (100 nmol/l) on E2 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

There was no effect of ACTH on tissue explants in comparison to the controls for each of the three placentae studied with the exception of placenta A1 in which there was significant increase observed at a single time point (150 minutes, $p < 0.05$). Figure 3.3.2 illustrates the changes in levels of E2 throughout the 24 hour incubatory period. The mean ± SE and median values for E2 levels produced by the tissue minces incubated as controls or with ACTH are given in table 3.3.2.

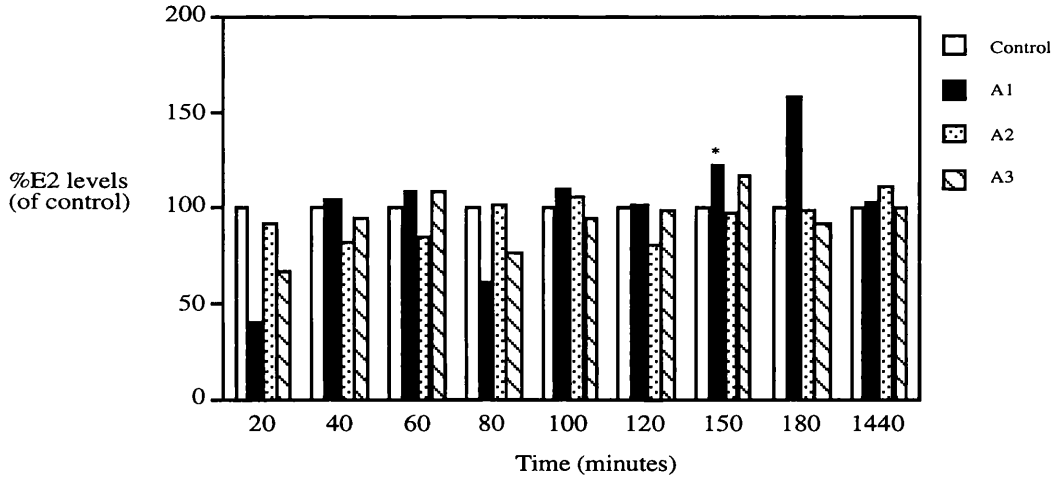


Figure 3.3.2. The effect of ACTH (10 nmol/l) on E2 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

Time (minutes)		Placenta A2 Control	Placenta A2 CRF	Placenta A3 Control	Placenta A3 CRF	Placenta A4 Control	Placenta A4 CRF
20	mean ± SE	282.1 ± 40.6	291.9 ± 46.0	1114.8 ± 126.7	1146.7 ± 221.54	920.8 ± 91.9	620.3 ± 102.0
	median	280.7	265.1	1140.3	1193.8	909.1	546.6
40	mean ± SE	263.9 ± 40.6	424.4 ± 55.5	582.0 ± 47.8	485.5 ± 85.04	837.8 ± 146.3	650.8 ± 96.0
	median	239.7	428.4	571.7	462.1	681.0	563.3
60	mean ± SE	299.0 ± 19.0	410.3 ± 56.29	561.3 ± 24.4	531.6 ± 59.3	786.5 ± 137.9	788.3 ± 93.5
	median	292.8	388.2	561.2	544.4	705.4	764.9
80	mean ± SE	252.1 ± 15.4	266.3 ± 24.3	743.2 ± 131.8	547.3 ± 60.4	826.9± 84.9	702.5 ± 89.7
	median	244.5	246.9	652.7	482.6	757.7	628.8
100	mean ± SE	284.5 ± 17.6	323.8 ± 32.6	592.8 ± 34.2	495.8 ± 66.8	866.5 ± 142.7	743.8 ± 87.0
	median	278.5	329.8	574.7	479.1	776.1	695.3
120	mean ± SE	380.8 ± 53.5	432.1 ± 78.1	645.3 ± 23.2	588.7 ± 80.6	1155.9 ± 142.9	853.4 ± 108.0
	median	337.0	333.9	644.2	549.7	1116.3	762.3
150	mean ± SE	314.3 ± 16.6	530.9 ± 194.3	575.2 ± 17.8	503.3 ± 46..3	1139.1 ± 238.6	768.2 ± 126.9
	median	305.4	326.2	578.2	463.6	1001.8	674.0
180	mean ± SE	306.3 ± 16.6	345.4 ± 28.4	564.2 ± 33.7	472.5 ± 44.9	759.5 ± 92.8	674.6 ± 77.4
	median	300.5	326.2	534.6	463.6	708.5	674.0
1440	mean ± SE	323.6 ± 16.0	590.9 ± 226.0	820.1 ± 42.3	773.6 ± 54.40	950.5 ± 74.9	982.2 ± 112.7
	median	334.3	377.4	828.2	748.7	962.7	110.7

Table 3.3.1. Mean ± SE and median values for E2 levels (fmol/mg protein) produced by placental explants incubated with/without CRF (100 nmol/l) for 24 hours.

Time (minutes)		Placenta A1 Control	Placenta A1 ACTH	Placenta A2 Control	Placenta A2 ACTH	Placenta A3 Control	Placenta A3 ACTH
20	mean ± SE	1424.8± 441.0	579.2 ± 78.5	282.1 ± 40.6	260.4 ± 14.0	1144.8 ± 126.7	774.9 ± 246.8
	median	1224.1	587.2	266.0	229.6	1140.3	517.7
40	mean ± SE	725.7 ± 139.7	759.1 ± 133.9	263.9 ± 40.1	217.0 ± 7.93	582.0 ± 47.8	552.2 ± 97.9
	median	593.4	741.0	239.8	223.5	571.7	462.1
60	mean ± SE	600.4 ± 70.0	655.5 ± 69.4	299.0 ± 19.0	251.9 ± 20.6	561.3 ± 24.4	604.7 ± 95.6
	median	590.9	668.7	292.8	270.4	561.2	544.2
80	mean ± SE	1067.1 ± 423.8	655.9 ± 53.0	252.1 ± 15.4	254.1 ± 23.6	743.2 ± 131.8	566.8 ± 46.6
	median	590.1	588.3	244.5	262.7	652.7	482.6
100	mean ± SE	644.6 ± 86.1	707.3 ± 92.5	284.6 ± 17.6	298.2 ± 15.5	592.8 ± 34.2	565.2 ± 57.1
	median	551.2	649.6	278.3	284.7	574.7	479.1
120	mean ± SE	623.9 ± 48.5	628.7 ± 96.0	380.8 ± 53.5	309.6 ± 11.9	645.3 ± 23.2	632.0 ± 50.6
	median	611.2	547.9	337.0	302.3	644.2	549.7
150	mean ± SE	727.5 ± 71.0	892.7 ± 64.9	314.3 ± 16.6	304.3 ± 22.3	575.2 ± 17.8	675.7 ± 106.9
	median	670.9	823.4	305.4	315.6	578.2	463.6
180	mean ± SE	728.5 ± 70.3	1153.5 ± 237.2	306.3 ± 16.6	300.00 ± 14.2	564.2 ± 33.7	519.0 ± 31.6
	median	730.9	1159.9	300.5	300.7	534.6	456.1
1440	mean ± SE	726.8 ± 95.0	750.6 ± 117.4	323.7 ± 16.0	360.5 ± 32.6	820.1 ± 42.3	824.5 ± 36.2
	median	684.8	614.34	334.2	358.8	828.2	748.7

Table 3.3.2. Mean ± SE and median values for E2 levels (fmol/mg protein) produced by placental explants incubated with/without ACTH (10 nmol/l) for 24 hours.

3.4. The effects of CRF and ACTH on E1 and E2 production by isolated cytotrophoblast cell incubates

The effects of CRF on E1 production by cells prepared from seven placentae are shown in figure 3.4.1 and the mean ± SE and median values for E1 levels from those cell preparations incubated with A with/without CRF are given in table 3.4a. There was no effect on E1 levels produced by cell preparations isolated from five placentae compared to controls, but E1 production was significantly inhibited by CRF in cell preparations from two placentae (B2 $p < 0.01$, B3 $p < 0.01$).

Figure 3.4.2 shows the effects of ACTH on E1 production by cytotrophoblast cells. There was no effect of ACTH on E1 production from cells in four placentae as compared to controls but in three placentae there was significant inhibition (B2 $p < 0.05$, B3 $p < 0.01$, B4 $p < 0.01$). The mean ± SE and median values for E1 levels from those cell preparations incubated with/without ACTH are given in table 3.4a.

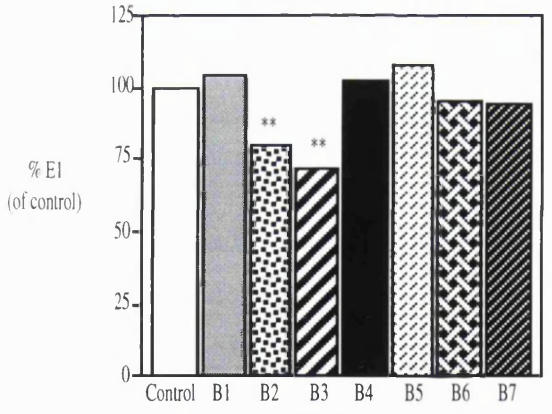


Figure 3.4.1

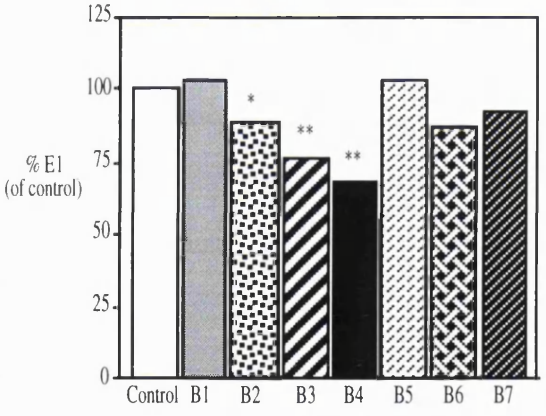


Figure 3.4.2.

Figures 3.4.1 and 3.4.2. The effect of CRF (100 nmol/l) and ACTH (10 nmol/l) on E1 production by isolated cytotrophoblast cells obtained from seven placentae.

The effects of CRF on E2 production by cytotrophoblast cells compared to those incubated with A alone (controls) is illustrated in figure 3.4.3. In comparison to the controls there were no significant differences in E2 production in four of the placentae studied. However E2 levels were significantly higher than those of the controls in placental cell preparations from three placentae (B2 $p < 0.01$, B3 $p < 0.01$, B6, $p < 0.05$).

E2 production was not significantly affected in cell preparations from five of the placentae studied when incubated with ACTH compared to those incubated with A alone, but was significantly increased in two placentae (B2 $p < 0.05$, B3 $p < 0.01$).

The mean ± SE and median levels for E2 from those cell preparations incubated with/without CRF or ACTH are given in table 3.4a.

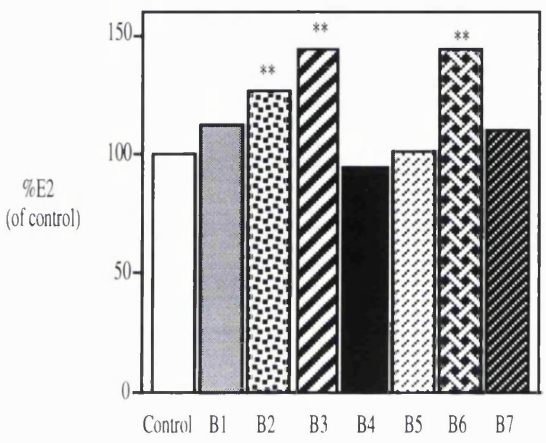


Figure 3.4.3.

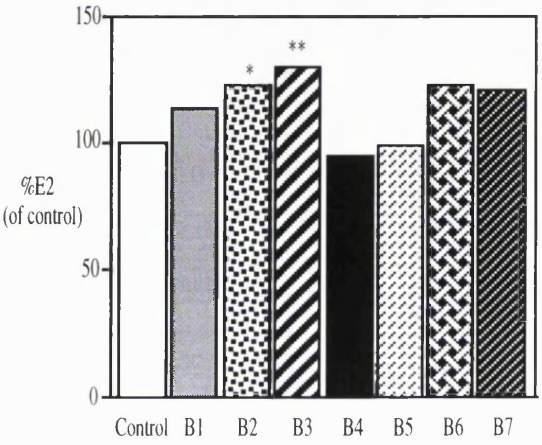
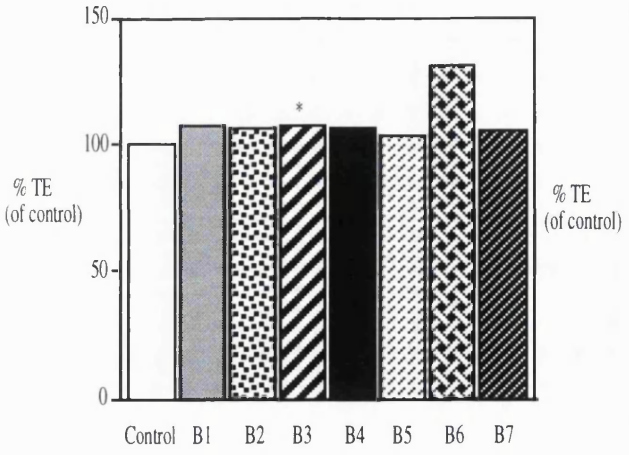


Figure 3.4.4.

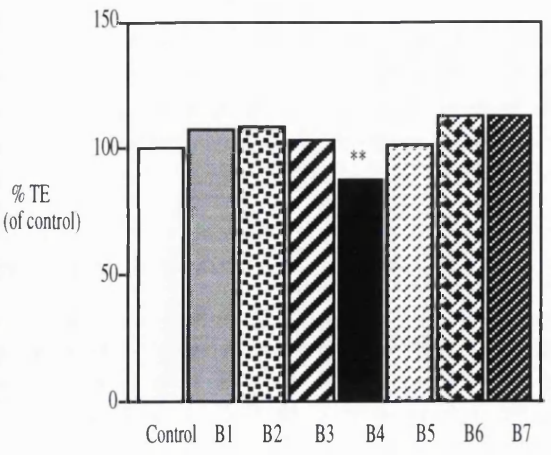
Figures 3.4.3 and 3.4.4. The effects of CRF (100 nmol/l) and ACTH (10 nmol/l) respectively on E2 production by isolated cytotrophoblast cells obtained from seven placentae.

The values of the levels of E1 and E2 produced in each well were combined and this value the total oestrone and oestradiol (TE) was calculated for each placenta. Figures 3.4.5 and 3.4.6 show the effects of CRF and ACTH on TE production and the mean ± SE and median levels for TE from those cell preparations incubated with/without CRF or ACTH are given in table 3.4b. In only one of the seven placentae was there a significant increase in TE (B3 $p < 0.05$) produced by cells incubated with CRF in comparison to the controls. Of the seven placentae incubated with ACTH, TE levels were significantly reduced in only one placentae (B4 $p < 0.01$) compared with controls .



CRF

Figure 3.4.5.



ACTH

Figure 3.4.6.

Figures 3.4.5 and 3.4.6. The effects of CRF (100 nmol/l) and ACTH (10 nmol/l) on TE production by isolated cytotrophoblast cells obtained from seven placentae.

	E1		E2	
Placenta	**Mean ± SE**	**Median**	**Mean ± SE**	**Median**
B1 Control	20.30 ± 2.04	19.73	11.80 ± 0.73	11.62
CRF	21.21 ± 1.53	21.25	13.18 ± 0.63	13.91
ACTH	20.92 ± 1.67	22.11	13.43 ± 0.65	13.78
B2 Control	2.30 ± 0.07	2.30	3.03 ± 0.11	3.10
CRF	1.83 ± 0.04	1.83	3.83 ± 0.07	3.85
ACTH	2.04 ± 0.07	2.03	3.69 ± 0.19	3.82
B3 Control	3.80 ± 0.08	3.78	3.81 ± 0.10	3.86
CRF	2.70 ± 0.25	2.75	5.50 ± 0.20	5.40
ACTH	2.91 ± 0.09	2.85	4.98 ± 0.15	4.82
B4 Control	2.08 ± 0.06	2.05	5.73 ± 0.11	5.67
CRF	2.14 ± 0.09	2.22	5.44 ± 0.29	5.43
ACTH	1.42 ± 012	1.50	5.43 ± 0.20	5.55
B5 Control	2.64 ± 0.17	2.81	5.25 ± 0.17	5.34
CRF	2.85 ± 0.19	2.77	4.78 ± 0.28	4.95
ACTH	2.73 ± 0.23	2.74	5.20 ± 0.25	5.05
B6 Control	3.58 ± 0.20	3.75	9.23 ± 1.05	9.53
CRF	3.42 ± 0.22	3.34	13.40 ± 1.29	12.37
ACTH	3.11 ± 0.21	3.17	11.29 ± 0.90	10.47
B7 Control	2.75 ± 0.13	2.82	6.68 ± 0.18	7.78
CRF	2.61 ± 0.10	2.60	7.34 ± 0.35	6.93
ACTH	2.55 ± 0.22	2.45	8.03 ± 0.19	8.14

Table 3.4a. Mean ± SE and median values for E1 and E2 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with A alone (control) or with A and CRF (100 nmol/l)/ACTH (10 nmol/l) for 24 hours.

	TE	
Placenta	**Mean ± SE**	**Median**
B1 Control	32.10 ± 2.31	31.98
CRF	34.38 ± 1.66	33.41
ACTH	34.35 ± 1.61	35.55
B2 Control	5.32 ± 0.17	5.46
CRF	5.66 ± 0.09	5.70
ACTH	5.73 ± 0.14	5.88
B3 Control	7.61 ± 0.13	7.70
CRF	8.20 ± 0.25	7.99
ACTH	7.89 ± 0.19	7.87
B4 Control	7.81 ± 0.07	7.78
CRF	7.58 ± 0.09	7.75
ACTH	6.85 ± 0.26	7.07
B5 Control	7.89 ± 0.30	8.07
CRF	7.63 ± 0.44	7.77
ACTH	7.93 ± 0.44	7.77
B6 Control	12.81 ± 1.07	13.04
CRF	16.82 ± 1.21	15.66
ACTH	14.40 ± 0.99	13.96
B7 Control	9.43 ± 0.11	10.42
CRF	9.95 ± 0.41	9.70
ACTH	10.58 ± 0.12	10.46

Table 3.4b. Mean ± SE and median values for TE levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with A alone (control) or with A and CRF (100 nmol/l)/ACTH (10 nmol/l) for 24 hours.

3.5. The effects of CRF and ACTH on E1 and E2 production by tissue explants as compared to isolated cytotrophoblast cells from the same placentae

In addition to their effects on isolated placental cells the effects of CRF and ACTH on E1 and E2 were also studied on minced tissue preparations in three of the placentae B1, B2 and B3. The tissue experiments were conducted under similar conditions to the experiments with the cytotrophoblasts. Unlike the cell preparations isolated from the same placentae, there were no significant differences in E1, E2 or TE production in tissue preparations when these were incubated with either CRF or ACTH compared with controls, with the exception of E1 and TE levels produced by tissue obtained from placenta B2 (see figures 3.5.2 and 3.5.6). Oestrone and TE levels produced by tissue explants from this placenta were significantly decreased on incubation with ACTH compared to controls ($p < 0.05$). Figures 3.5.1-3.5.6 show the comparison of cell and tissue incubates.

The mean ± SE and median values for E1, E2 and TE produced by the tissue explants are shown in tables 3.5a and 3.5b .

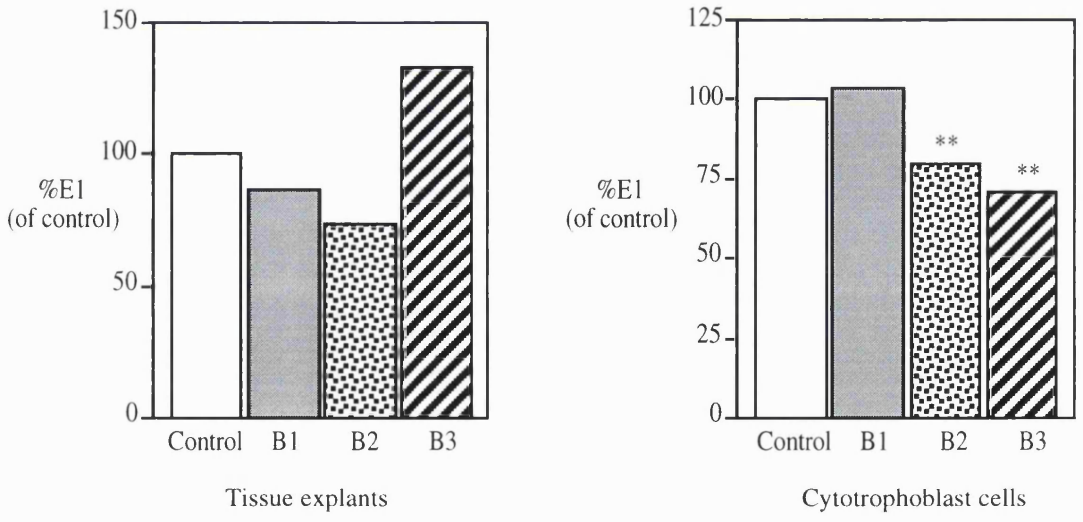


Figure 3.5.1. The effect of CRF (100 nmol/l) on E1 production by both tissue explants and purified cytotrophoblast cells obtained from three placentae.

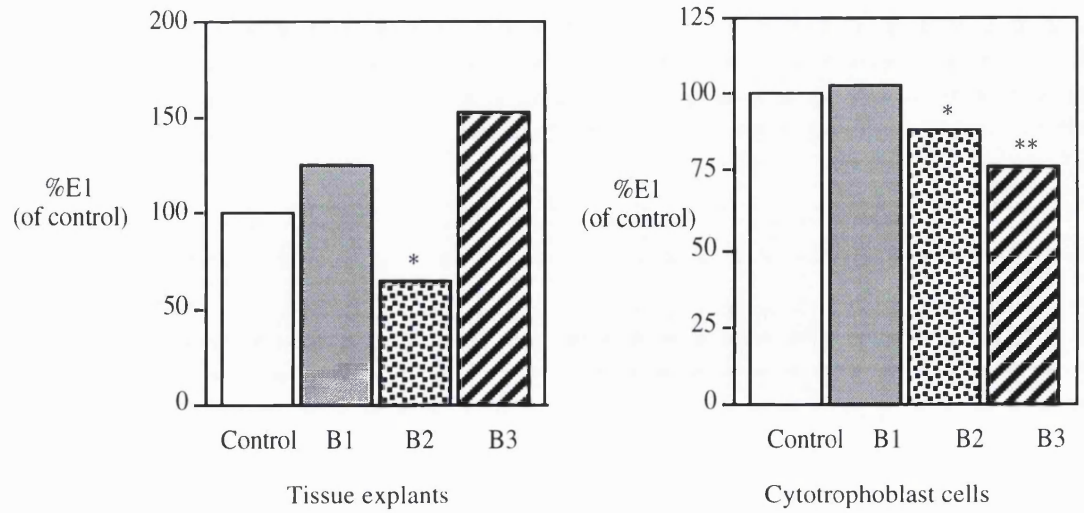


Figure 3.5.2. The effects of ACTH (10 nmol/l) on E1 production by both tissue explants and purified cytotrophoblast cells obtained from three placentae.

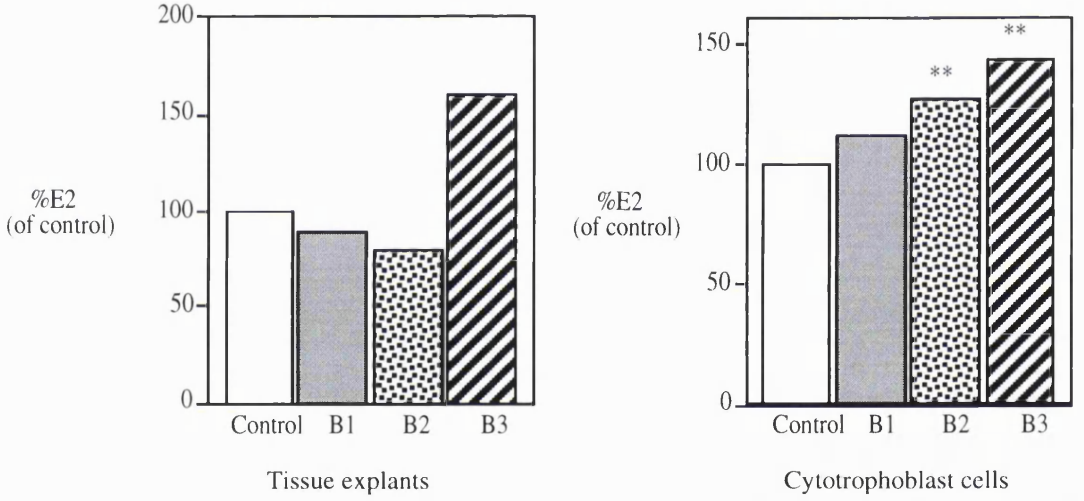


Figure 3.5.3. The effect of CRF (100 nmol/l) on E2 production by both tissue explants and purified cytotrophoblast cells obtained from three placentae.

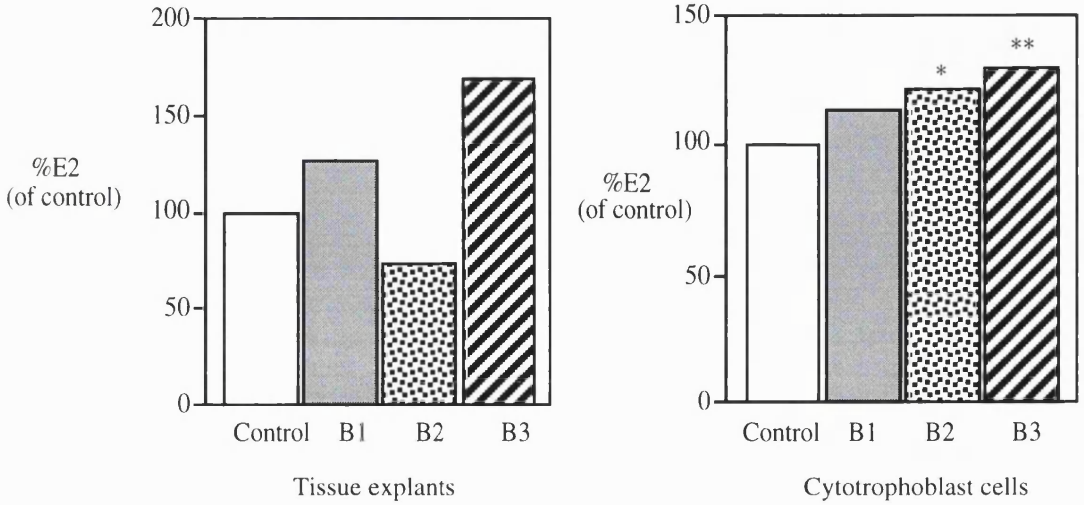


Figure 3.5.4. The effect of ACTH (10 nmol/l) on E2 production by tissue explants and purified cytotrophoblast cells obtained from three placentae.

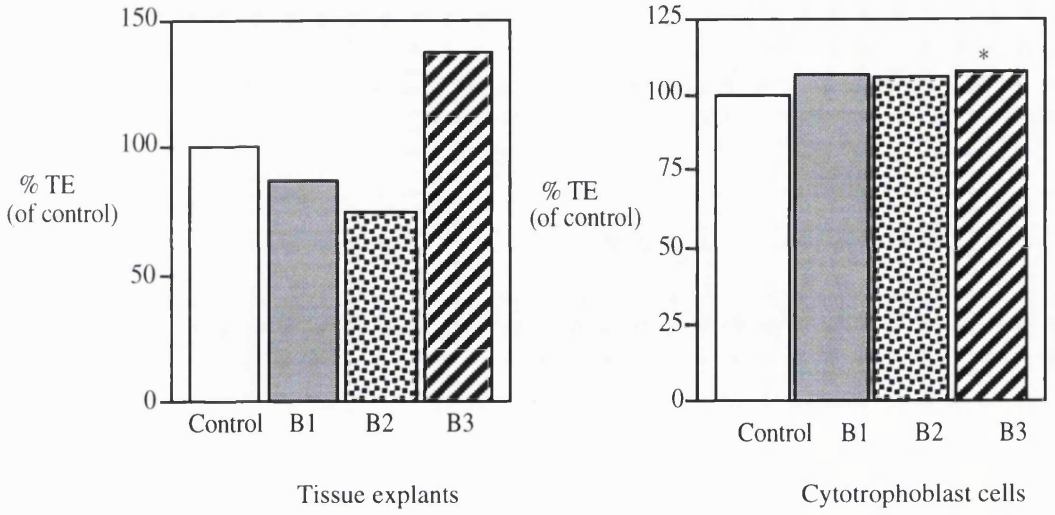


Figure 3.5.5. The effect of CRF (100 nmol/l) on TE production by both tissue explants and purified cytotrophoblast cells obtained from three placentae.

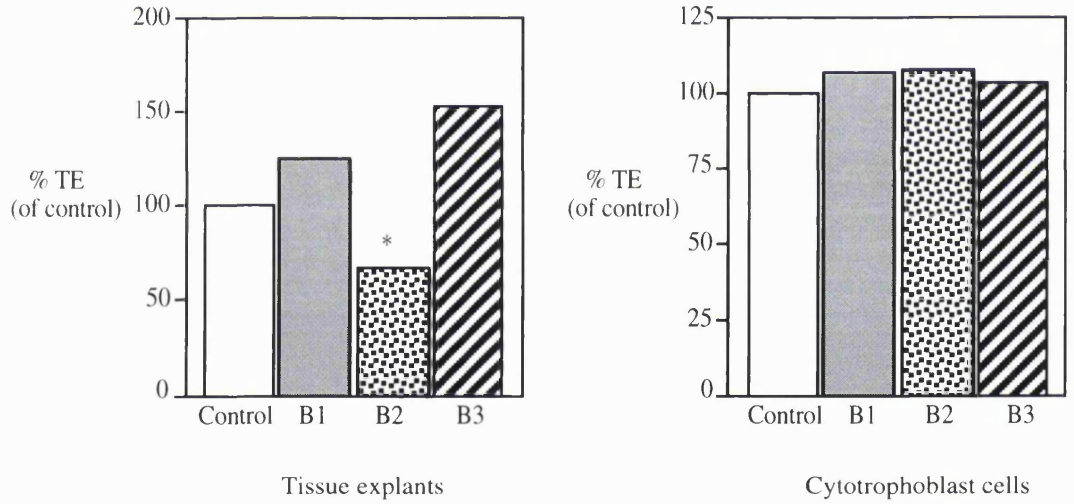


Figure 3.5.6. The effect of ACTH (10 nmol/l) on TE production by tissue explants and purified cytotrophoblast cells obtained from three placentae.

	E1		E2	
Placenta	**Mean ± SE**	**Median**	**Mean ± SE**	**Median**
B1 Control	3.13 ± 0.34	2.73	0.53 ± 0.05	0.54
CRF	2.71 ± 0.35	2.45	0.46 ± 0.03	0.44
ACTH	3.94 ± 0.23	3.85	0.67 ± 0.02	0.66
B2 Control	25.53 ± 2.83	23.91	3.47 ± 0.40	3.43
CRF	18.74 ± 4.33	16.11	2.72 ± 0.58	2.38
ACTH	16.62 ± 1.45	15.39	2.54 ± 0.20	2.40
B3 Control	4.45 ± 0.67	4.23	0.78 ± 0.07	0.72
CRF	5.95 ± 1.04	5.91	1.24 ± 0.03	1.01
ACTH	6.78 ± 1.86	5.29	1.32 ± 0.37	1.00

Table 3.5a. Mean ± SE and median values for E1 and E2 levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with A alone (control) or with A and CRF (100 nmol/l)/ACTH (10 nmol/l)for 24 hours.

	TE levels	
Placenta	**Mean ± SE**	**Median**
B1 Control	3.66 ± 0.37	3.27
CRF	3.17 ± 0.35	2.89
ACTH	4.60 ± 0.25	4.55
B2 Control	29.00 ± 3.01	27.22
CRF	21.46 ± 4.90	18.70
ACTH	19.16 ± 1.61	17.64
B3 Control	5.23 ± 0.73	4.91
CRF	7.19 ± 1.31	6.92
ACTH	8.10 ± 2.23	6.27

Table 3.5b. Mean ± SE and median values for TE levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with A alone (control) or with A and CRF (100 nmol/l)/ACTH (10 nmol/l) for 24 hours.

3.6. The effects of CRF and ACTH on E3 production by placental tissue explants

There was no significant change in E3 levels in tissue incubated with CRF or ACTH when compared to those explants incubated with DMEM only. Figures 3.6.1 and 3.6.2 show the effect of CRF and ACTH on three placental tissue explants as compared to controls.

The mean ± SE and median values for E3 levels produced by placental tissue minces over 24 hours when incubated in DMEM alone or DMEM and CRF or ACTH are shown in tables 3.6.1 and 3.6.2 respectively .

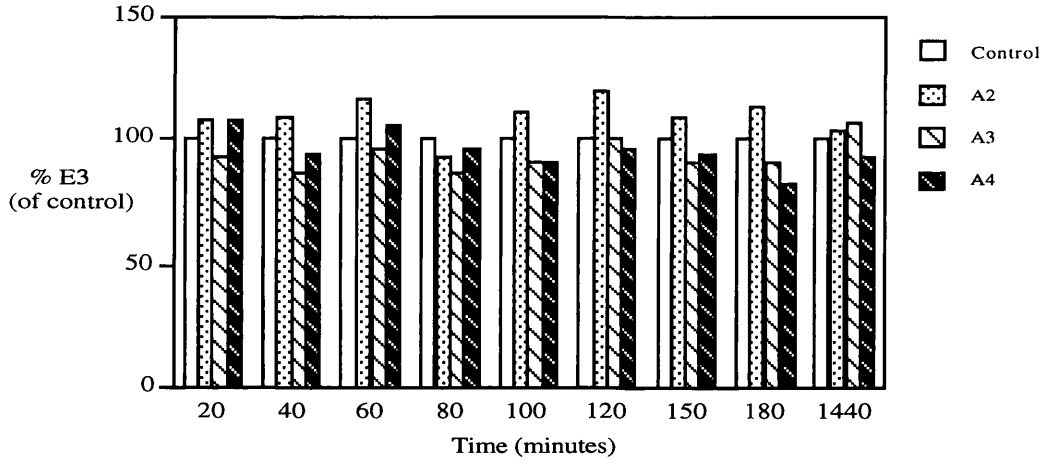


Figure 3.6.1. The effect of CRF (100 nmol/l) on E3 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

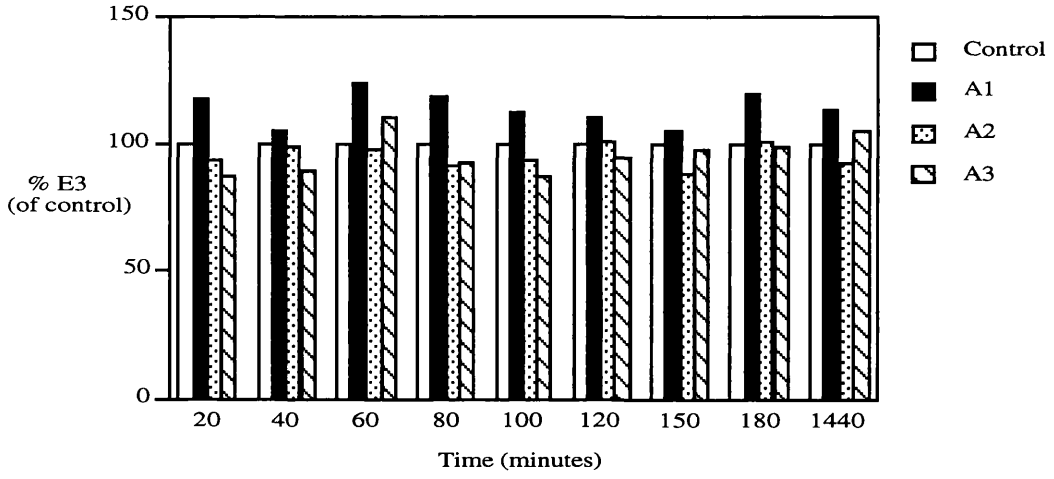


Figure 3.6.2. The effect of ACTH (10 nmol/l) on E3 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

Time (minutes)		Placenta A2 Control	Placenta A2 CRF	Placenta A3 Control	Placenta A3 CRF	Placenta A4 Control	Placenta A4 CRF
20	mean ± SE	2.28 ± 0.07	2.45 ± 0.39	0.72 ± 0.12	0.67 ± 0.10	1.86 ± 0.38	2.02 ± 0.29
	median	2.33	2.01	0.68	0.67	1.84	1.85
40	mean ± SE	2.06 ± 0.20	2.24 ± 0.47	0.89 ± 0.09	0.77 ± 0.07	2.16 ± 0.26	2.03 ± 0.25
	median	2.23	1.84	0.83	0.76	2.04	1.86
60	mean ± SE	2.26 ± 0.15	2.63 ± 0.46	0.92 ± 0.03	0.88 ± 0.07	2.06 ± 0.19	2.17 ± 0.31
	median	2.27	2.28	0.89	0.83	1.99	1.96
80	mean ± SE	2.51.± 0.17	2.33 ± 0.18	0.94 ± 0.05	0.81 ± 0.04	2.12 ± 0.24	2.03 ± 0.22
	median	2.55	2.21	0.94	0.77	1.99	1.88
100	mean ± SE	2.55 ± 0.20	2.84 ± 0.39	0.92 ± 0.07	0.83 ± 0.08	2.54 ± 0.25	2.30 ± 0.37
	median	2.41	2.60	0.91	0.77	2.35	1.93
120	mean ± SE	2.58 ± 0.14	3.08 ± 0.46	0.98 ± 0.05	0.99 ± 0.08	2.49 ± 0.27	2.38 ± 0.29
	median	2.58	2.69	1.0	0.95	2.35	2.10
150	mean ± SE	2.99 ± 0.38	3.28 ± 0.59	0.90 ± 0.04	0.82 ± 0.06	2.28 ± 0.18	2.16 ± 0.31
	median	2.69	2.56	0.90	0.85	2.20	1.97
180	mean ± SE	2.54 ± 0.19	2.86 ± 0.39	1.29 ± 0.05	1.17 ± 0.11	2.66 ± 0.27	2.19 ± 0.13
	median	2.42	2.62	1.25	1.16	2.55	2.19
1440	mean ± SE	3.14 ± 0.34	3.24 ± 0.34	1.51 ± 0.11	1.54 ± 0.20	2.50 ± 0.16	2.40 ± 0.32
	median	3.02	3.34	1.40	1.40	2.57	2.20

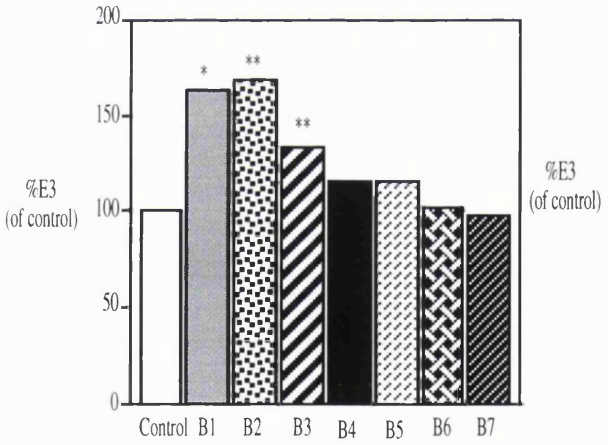
Table 3.6.1. Mean ± SE and median values for E3 levels (pmol/mg protein) produced by placental explants incubated with/without CRF (100 nmol/l) for 24 hours.

Time (minutes)		Placenta A1 Control	Placenta A1 ACTH	Placenta A2 Control	Placenta A2 ACTH	Placenta A3 Control	Placenta A3 ACTH
20	mean ± SE	0.68 ± 0.07	0.80 ± 0.09	2.28 ± 0.07	2.14 ± 0.11	0.72 ± 0.12	0.62 ± 0.09
	median	0.63	0.77	2.33	2.06	0.68	0.68
40	mean ± SE	0.61 ± 0.06	0.64 ± 0.08	2.06 ± 0.20	2.02 ± 0.13	0.89 ± 0.09	0.79 ± 0.06
	median	0.58	0.64	2.23	1.97	0.83	0.76
60	mean ± SE	0.68± 0.07	0.85 ± 0.10	2.26 ± 0.15	2.20 ± 0.15	0.92 ± 0.03	1.01 ± 0.81
	median	0.65	0.79	2.27	2.18	0.89	1.01
80	mean ± SE	0.68 ± 0.05	0.80 ± 0.09	2.51 ± 0.17	2.28 ± 0.15	0.94 ± 0.05	0.87 ± 0.06
	median	0.70	0.73	2.55	2.35	0.94	0.89
100	mean ± SE	0.82 ± 0.05	0.93 ± 0.10	2.55 ± 0.20	2.38 ± 0.27	0.92 ± 0.07	0.81 ± 0.06
	median	0.83	0.91	2.41	2.39	0.91	0.81
120	mean ± SE	0.73 ± 0.07	0.81 ± 0.11	2.59 ± 0.14	2.61 ± 0.11	0.98 ± 0.05	0.92 ± 0.04
	median	0.77	0.82	2.58	2.54	1.00	0.81
150	mean ± SE	0.80 ± 0.05	0.84 ± 0.09	2.99 ± 0.38	2.64 ± 0.98	0.90 ± 0.04	0.88 ± 0.63
	median	0.82	0.81	2.69	2.54	0.90	0.91
180	mean ± SE	0.85 ± 0.09	1.02 ± 0.20	2.54 ± 0.19	2.54 ± 0.09	1.29 ± 0.05	1.28 ± 0.07
	median	0.76	0.90	2.42	2.53	1.25	1.32
1440	mean ± SE	1.02 ± 0.09	1.16 ± 0.20	3.14 ± 0.34	2.90 ± 0.38	1.45 ± 0.11	1.51 ± 0.14
	median	1.00	1.03	3.02	2.67	1.40	1.50

Table 3.6.2. Mean ± SE and values for E3 levels (pmol/mg protein) produced by placental explants incubated for 24 hours with/without ACTH (10 nmol/l).

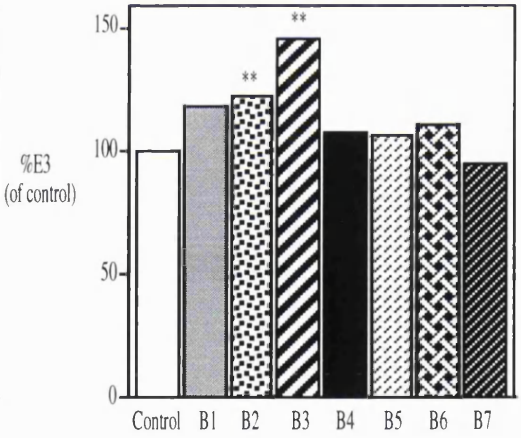
3.7. The effects of CRF and ACTH on E3 production by isolated cytotrophoblast cells

The effect of CRF and ACTH on E3 production in cytotrophoblast cells was studied in cell preparations in seven placentae. The addition of CRF had no effect in four of the placentae but there were significant increases in E3 levels in three of the placentae as compared with controls (B1 $p < 0.05$, B2 $p < 0.01$, B3 $p < 0.01$). These differences are illustrated in figure 3.7.1. In those preparations incubated with ACTH there were no significant changes in E3 production in five placentae in comparison to those of controls whereas there was a significant rise in E3 production in 2 placentae (B2 $p \leq 0.01$, B3 $p < 0.01$) which can be seen in figure 3.7.2. The mean ± SE and median values for E3 levels produced by those cell preparations incubated with/without CRF or ACTH are shown in table 3.7.



CRF

Figure 3.7.1



ACTH

Figure 3.7.2

Figures 3.7.1 and figure 3.7.2. The effects of CRF (100 nmol/l) and ACTH (10 nmol/l) respectively on E3 production by cytotrophoblast cells isolated from seven placentae.

Placenta	Mean ± SE	Median
B1 Control	1.53 ± 0.06	1.51
CRF	2.51 ± 0.04	2.48
ACTH	1.82 ± 0.29	1.55
B2 Control	0.18 ± 0.01	0.18
CRF	0.31 ± 0.01	0.22
ACTH	0.22 ± 0.07	0.30
B3 Control	0.69 ± 0.01	0.69
CRF	0.92 ± 0.02	0.92
ACTH	1.01 ± 0.08	0.89
B4 Control	0.90 ± 0.07	0.89
CRF	1.04 ± 0.01	1.04
ACTH	0.97 ± 0.04	0.97
B5 Control	0.70 ± 0.01	0.70
CRF	0.80 ± 0.06	0.79
ACTH	0.74 ± 0.02	0.75
B6 Control	3.07 ± 0.11	3.17
CRF	3.12 ± 0.15	3.11
ACTH	3.41 ± 0.24	3.18
B7 Control	0.99 ± 0.08	1.07
CRF	0.96 ± 0.05	0.95
ACTH	0.93 ± 0.07	0.91

Table 3.7. Mean ± SE and median E3 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with 16α-OHA alone (control) or 16α-OHA and CRF (100 nmol/l) or ACTH (10 nmol/l) for 24 hours.

3.8. The inhibitory effect of A on E3 production by isolated cytotrophoblast cells

Initial pilot studies were undertaken to assess the effects of various possible precursors on steroid output from cytotrophoblast cells. It was noticed that when the cells were incubated with 25-hydroxycholesterol with A and 16α-OHA in combination there was a marked decrease in E3 production compared to levels produced by cells incubated with 16α-OHA alone. Further studies confirmed that the steroid responsible for this was A. This decrease in synthesis was seen in concentrations as low as 70 nmol/l A in all three

placentae studied (figures 3.8.1a and 3.8.1b). The mean ± SE and median values for E3 levels measured in these experiments are given in tables 3.8.1. The p values are tabulated where appropriate.

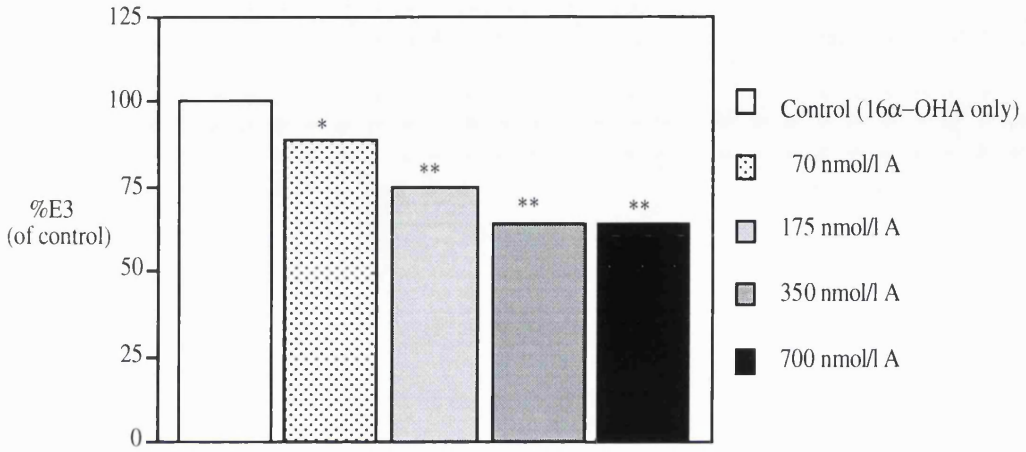


Figure 3.8.1.a The effect of various concentrations of A on E3 production by cytotrophoblast cells obtained from placenta 3 and incubated with 16α-OHA.

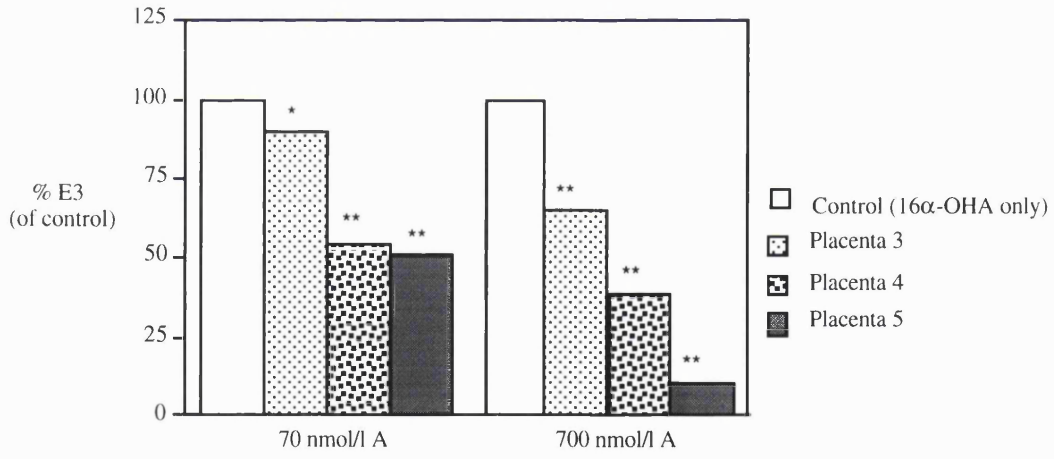


Figure 3.8.1b. The effect of A on E3 production by cytotrophoblast cells obtained from three placentae and incubated with 16α-OHA.

Placenta	Mean	Median	P value
3 Control	83.53 ± 2.25	82.89	
A 70 nmol/l	73.73 ± 2.56	74.64	0.016
A 175 nmol/l	63.15 ± 2.42	62.69	0.004
A 350 nmol/l	53.37 ± 1.71	54.62	0.004
A 700 nmol/l	53.38 ± 3.35	52.62	0.004
4 Control	167.47 ± 8.95	171.33	
A 70 nmol/l	91.81 ± 4.27	91.12	0.004
A 700 nmol/l	64.22 ± 9.13	72.24	0.004
5 Control	336.68 ± 49.81	310.16	
A 70 nmol/l	171.70 ± 17.39	180.88	0.016
A 700 nmol/l	34.49 ± 15.21	22.64	0.004

Table 3.8.1 Mean ± SE and median values for E3 levels (fmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various concentrations of A. The p values are tabulated as appropriate.

In two of these placenta (4 and 5) experiments to test the effects of A on E3 production in placental tissue minces were also undertaken. There was no significant decrease in E3 levels from those minces incubated with 16α-OHA and A compared to controls containing 16α-OHA alone.

Tables with the mean ± SE and median values for these experiments are given in table 3.8.2. The p values are tabulated where appropriate.

Placenta		Mean E3 levels	Median E3 levels
3	Control	13.02 ± 0.94	12.90
	A 700 nmol/l	11.90 ± 0.88	12.01
4	Control	2.89 ± 0.26	2.67
	A 700 nmol/l	2.44 ± 0.27	2.20

Table 3.8.2. Mean ± SE and median values for E3 levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with 16α-OHA and with/without A (700 nmol/l).

3.9. The effects of CRF and ACTH on E3 levels produced by placental tissue explants compared to isolated cytotrophoblast cells from the same placentae

In comparison to the isolated cells obtained from placentae B1, B2 and B3 (discussed in section 3.7), there was no significant rise in E3 production by tissue explants incubated with CRF. On incubation with ACTH E3 levels were not significantly different from controls in two placentae but were significantly lower than the control in one placenta (B1, $p < 0.05$, figure 3.9.4). The mean ± SE and median values for E3 levels produced by tissue minces are given in table 3.9.

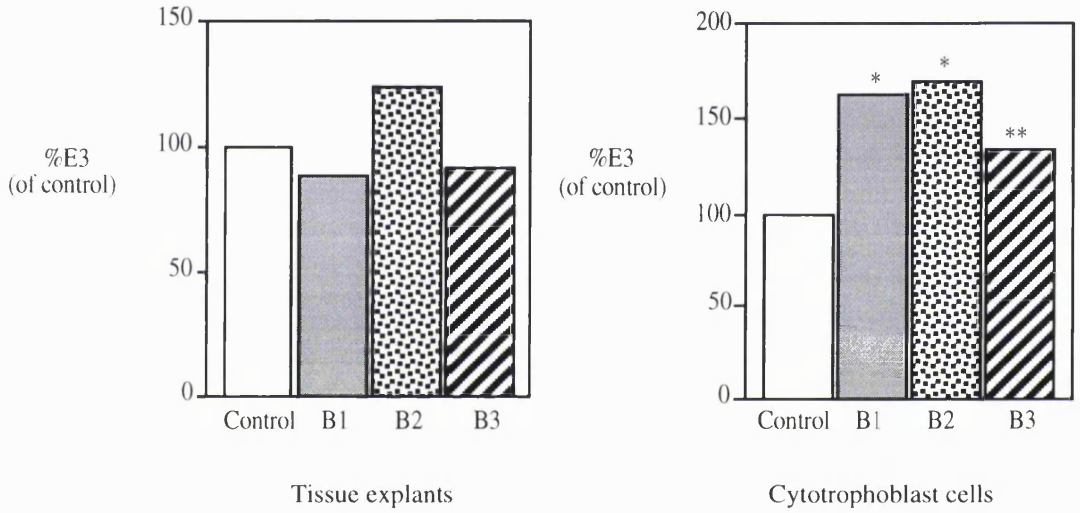


Figure 3.9.1. The effect of CRF (100 nmol/l) on E3 production by both tissue explants and cytotrophoblast cell preparations obtained from three placentae.

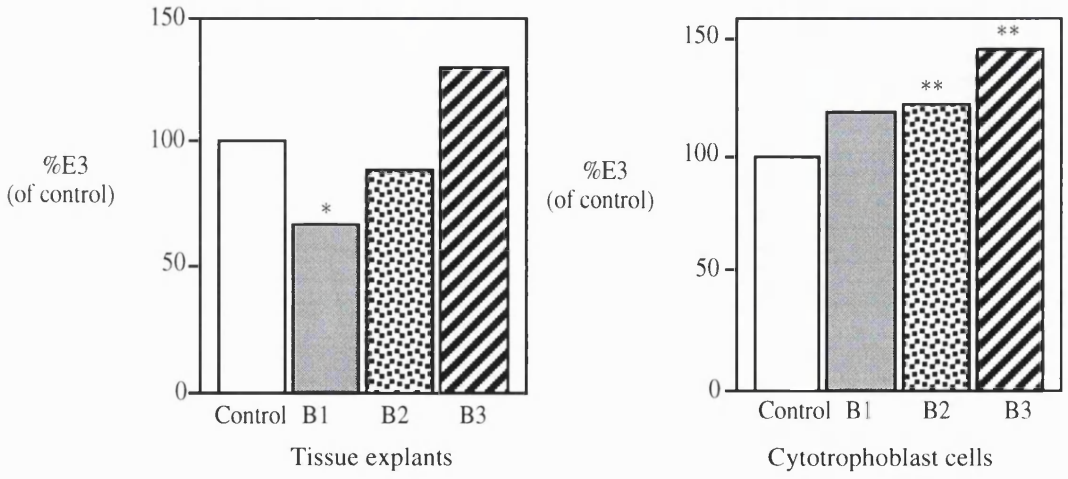


Figure 3.9.2. The effects of ACTH (10 nmol/l) on E3 production by both tissue explants and cytotrophoblast cell preparations obtained from three placentae.

Placenta	Mean ± SE	Median
B1 Control	4.66 ± 0.08	4.02
CRF	4.13 ± 0.05	3.46
ACTH	3.13 ± 0.31	3.09
B2 Control	8.89 ± 0.67	8.86
CRF	11.05 ± 0.17	9.76
ACTH	7.85 ± 0.80	7.79
B3 Control	6.06 ± 0.46	5.92
CRF	5.51 ± 0.43	5.74
ACTH	7.84 ± 0.87	7.24

Table 3.9. The mean ± SE and median values for E3 levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with 16α-OHA or with 16α-OHA and CRF (100 nmol/l) or ACTH (10 nmol/l).

3.10. The effect of CRF and ACTH on P levels produced by placental tissue explants

There were no significant changes in levels of P produced by tissue explants obtained from three individual placentae incubated with CRF or ACTH in comparison to the controls, with the exception of placenta A2 which showed a significant decrease in P production at one time point ($t = 60$ minutes, $p < 0.01$) when incubated with ACTH.

Figures 3.10.1 and 3.10.2 illustrate the pattern of P production over 24 hours in three placentae. The mean ± SE and median levels for those placentae incubated with/without CRF or ACTH are given in tables 3.10.1 and 3.10.2 respectively .

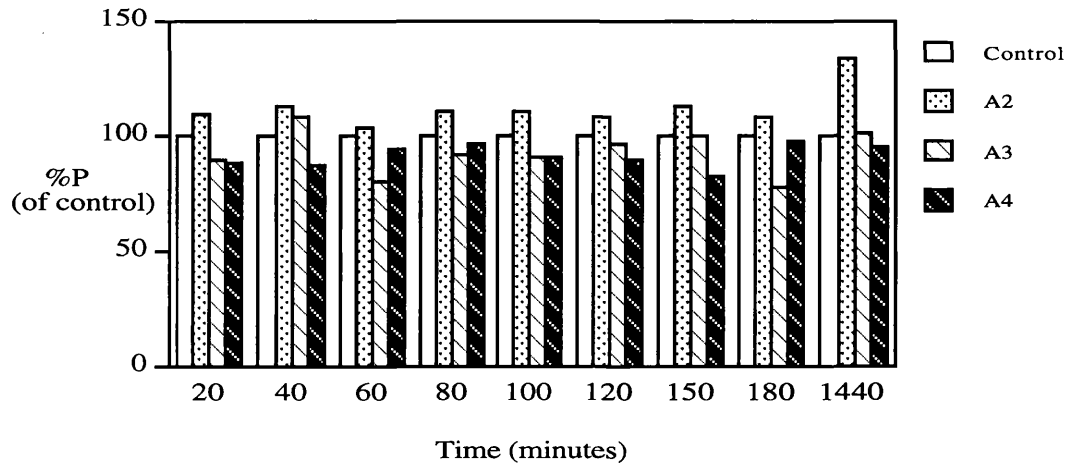


Figure 3.10.1. The effect of CRF (100 nmol/l) on P production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

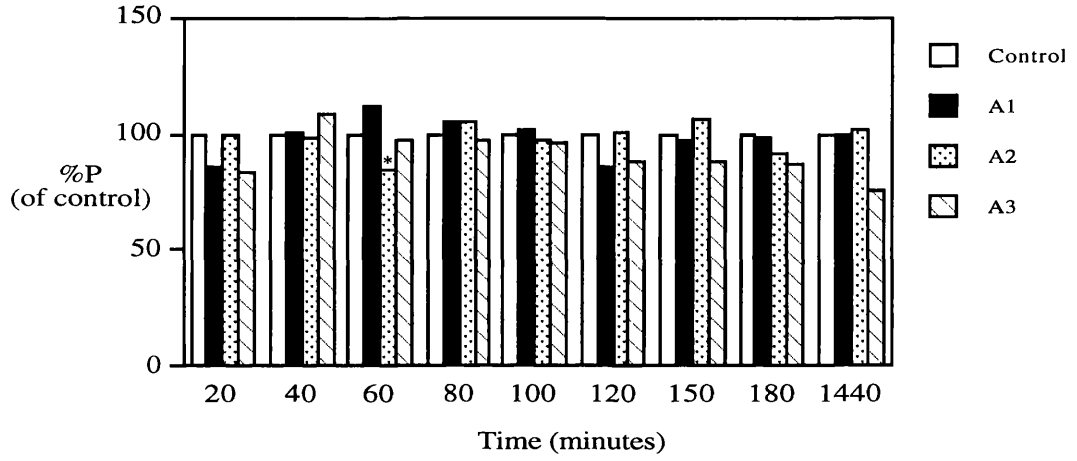


Figure 3.10.2. The effect of ACTH (10 nmol/l) on P production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

Time (minutes)		Placenta A2 Control	Placenta A2 CRF	Placenta A3 Control	Placenta A3 CRF	Placenta A4 Control	Placenta A4 CRF
20	mean ± SE	58.58 ± 2.13	63.87 ± 8.81	23.76 ± 1.07	21.21 ± 1.65	32.74 ± 5.34	28.73 ± 3.59
	median	57.33	58.58	24.76	19.72	29.32	27.76
40	mean ± SE	69.42 ± 4.58	77.65 ± 9.60	16.40 ± 1.44	17.73 ± 1.62	43.64 ± 5.06	37.73 ± 5.18
	median	69.48	68.21	17.21	16.62	45.41	39.70
60	mean ± SE	66.20 ± 7.09	68.59 ± 7.73	24.19 ± 2.23	19.37 ± 2.70	43.75 ± 5.25	41.01 ± 6.49
	median	66.27	70.88	24.26	15.79	42.48	37.06
80	mean ± SE	63.39 ± 4.71	69.86 ± 5.37	25.02 ± 1.05	22.94 ± 1.65	52.21 ± 4.00	50.16 ± 9.13
	median	62.71	67.48	24.73	22.43	49.42	44.84
100	mean ± SE	71.14 ± 5.76	78.57 ± 9.83	27.22 ± 1.05	24.65 ± 4.58	56.26 ± 4.55	50.72 ± 6.23
	median	74.51	73.62	25.27	22.81	56.92	50.21
120	mean ± SE	76.68 ± 5.63	82.38 ± 13.42	31.22 ± 2.73	29.87 ± 6.49	56.16 ± 6.14	49.91 ± 7.28
	median	77.24	77.78	31.79	25.17	57.18	46.69
150	mean ± SE	71.36 ± 4.90	79.81 ± 6.23	30.73 ± 1.88	30.63 ± 6.71	62.23 ± 7.05	51.00 ± 7.31
	median	69.90	80.55	29.91	24.63	58.19	50.02
180	mean ± SE	80.97 ± 3.69	87.62 ± 10.21	37.27 ± 4.12	29.11 ± 3.37	61.66 ± 6.81	59.81 ± 9.25
	median	80.42	82.08	34.88	27.16	57.27	51.02
1440	mean ± SE	81.78 ± 5.41	108.67 ± 34.41	33.22 ± 3.08	33.47 ± 3.12	62.11 ± 8.97	59.28 ± 8.40
	median	77.88	77.66	32.42	33.03	59.69	56.35

Table 3.10.1. Mean ± SE and median values for P levels (pmol/mg protein) produced by placental tissue explants incubated for 24 hours with/without CRF (100 nmol/l).

Time (minutes)		Placenta A1 Control	Placenta A1 ACTH	Placenta A2 Control	Placenta A2 ACTH	Placenta A3 Control	Placenta A3 ACTH
20	mean ± SE	27.43 ± 4.38	23.71 ± 3.86	58.58 ± 2.13	58.24 ± 3.97	23.76 ± 1.07	19.94 ± 1.23
	median	25.73	22.93	57.33	60.04	24.76	19.56
40	mean ± SE	32.41 ± 4.37	32.48 ± 4.34	69.42 ± 4.58	68.52 ± 5.60	16.40 ± 1.44	17.83 ± 1.23
	median	27.56	28.23	69.48	68.31	17.21	18.10
60	mean ± SE	28.80 ± 3.35	32.33 ± 5.97	66.20 ± 7.09	56.55 ± 3.19	24.19 ± 2.24	23.36 ± 1.32
	median	25.95	58.79	66.27	54.44	24.26	23.09
80	mean ± SE	36.38 ± 6.78	38.33 ± 8.07	63.39 ± 4.71	66.63 ± 2.60	25.02 ± 1.05	24.13 ± 1.98
	median	33.67	30.84	62.71	65.64	24.73	23.99
100	mean ± SE	32.70 ± 5.82	33.22 ± 4.96	71.14 ± 5.76	69.56 ± 4.48	27.22 ± 2.37	26.13 ± 1.16
	median	27.39	34.32	74.51	67.13	25.27	26.21
120	mean ± SE	45.38 ± 9.03	39.01 ± 3.57	76.68 ± 5.63	77.27 ± 2.43	31.22 ± 2.73	27.39 ± 0.51
	median	37.48	38.43	77.24	80.01	31.79	27.29
150	mean ± SE	39.57 ± 5.06	38.70 ± 4.44	71.36 ± 4.90	75.63 ± 3.42	30.73 ± 1.88	27.32 ± 1.86
	median	36.94	39.96	69.89	76.26	29.91	28.17
180	mean ± SE	47.31 ± 6.80	40.28 ± 4.81	80.96 ± 3.69	74.09 ± 3.87	37.27 ± 4.12	32.36 ± 2.50
	median	42.11	46.27	80.42	74.86	34.88	32.40
1440	mean ± SE	52.57 ± 4.68	52.19 ± 4.35	81.78 ± 5.41	83.91 ± 3.29	33.22 ± 3.08	25.29 ± 2.00
	median	49.68	56.76	77.88	85.32	32.42	26.96

Table 3.10.2. Mean ± SE and median values for P levels (pmol/mg protein) produced by tissue explants incubated for 24 hours with/without ACTH (10 nmol/l).

3.11. The effects of CRF and ACTH on P production by isolated cytotrophoblast cells

There was no significant change in P levels produced by cytotrophoblast cells when incubated with CRF compared to those levels produced by controls in seven of the placentae studied. However a significant decrease in progesterone synthesis was observed in one placenta (B4 $p < 0.01$) as can be seen in figure 3.11.1.

The addition of ACTH did not appear to alter the production of P in cell preparations from five of the placentae studied. Two placentae showed a significant increase in P synthesis (B2 $p< 0.01$ and B3 $p < 0.02$) but one placenta showed a significant decrease in synthesis (B4 < 0.01) (figure 3.11.2). The increase in synthesis occurred in cells incubated at a concentration of pregnenolone of 1 µmol/l whilst those cells which produced less P than controls were incubated at a concentration of 10 µmol/l.

The mean ± SE and median levels for those placentae incubated with/without CRF or ACTH are given in tables 3.11.1. One placenta, B8 was incubated with pregnenolone at concentrations of 10 µmol/l and 20 µmol/l.

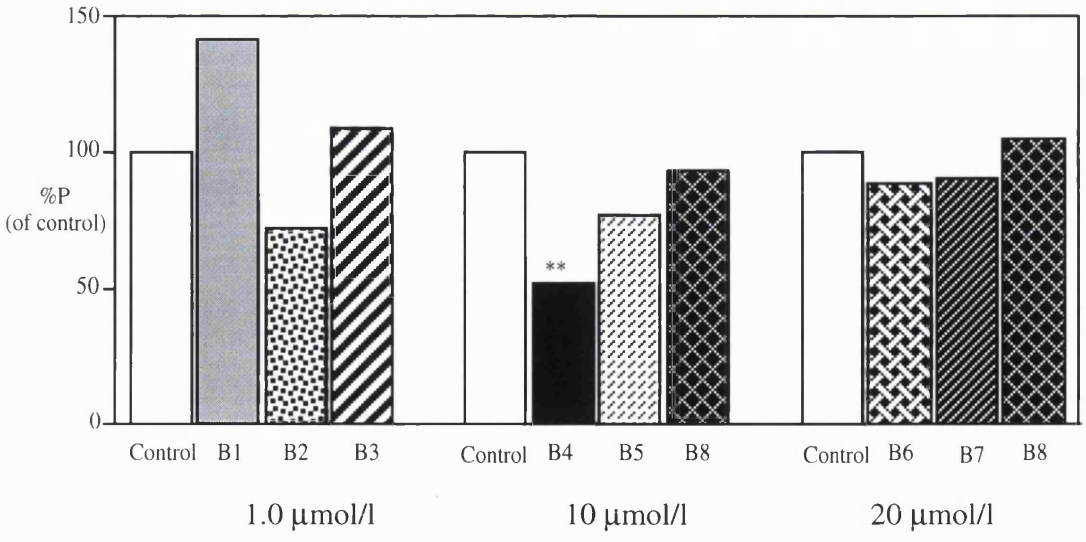


Figure 3.11.1. The effect of CRF (100 nmol/l) on P production by isolated cytotrophoblast cells incubated with various concentrations of pregnenolone.

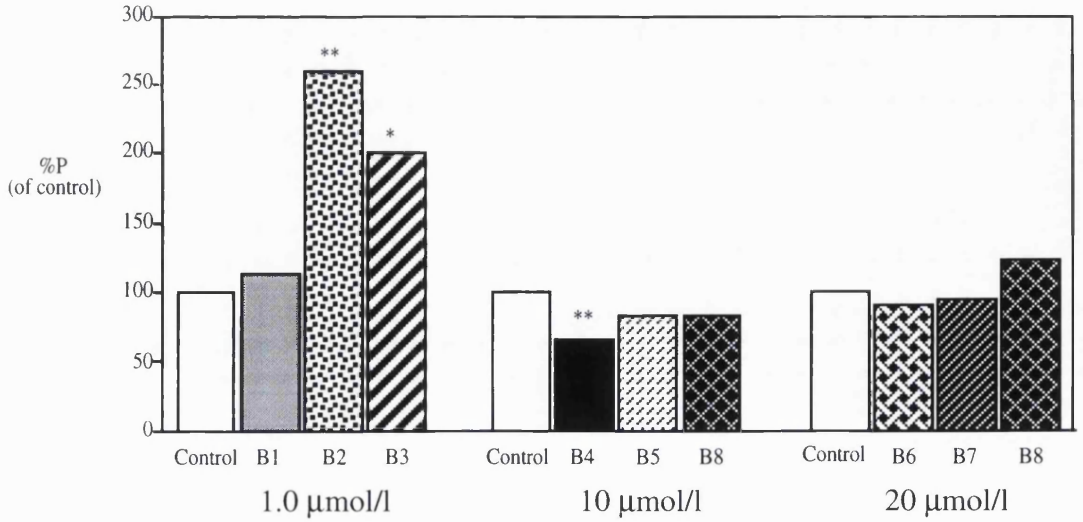


Figure 3.11.2. The effects of ACTH (10 nmol/l) on P levels production by isolated cytotrophoblast cells incubated with various concentrations of pregnenolone.

Placenta		Mean ± SE	Median
B1 Control	1 µmol /l	44.44 ± 2.95	45.16
CRF		59.66 ± 5.45	62.42
ACTH		50.90 ± 2.72	49.29
B2 Control	1 µmol /l	35.19 ± 4.08	29.34
CRF		25.43 ± 1.01	24.68
ACTH		91.24 ± 8.89	85.25
B3 Control	1 µmol /l	33.46 ± 4.66	34.24
CRF		36.57 ± 9.90	28.25
ACTH		67.10 ± 7.93	75.68
B4 Control	10 µmol /l	311.35 ± 6.85	311.30
CRF		160.43 ± 17.19	174.81
ACTH		206.88 ± 10.51	162.81
B5 Control	10 µmol /l	160.65 ± 19.84	156.11
CRF		122.98 ± 10.05	131.46
ACTH		134.27 ± 6.02	133.06
B6 Control	20 µmol /l	272.55 ± 24.33	266.30
CRF		239.97 ± 9.45	233.10
ACTH		247.29 ± 21.03	233.93
B7 Control	20 µmol /l	932.56 ± 62.20	905.79
CRF		851.66 ± 45.07	875.95
ACTH		884.49 ± 84.14	883.15
B8 Control	10 µmol /l	306.13 ± 23.22	314.90
CRF		285.45 ± 21.14	285.30
ACTH		253.57 ± 12.38	240.23
B8 Control	20 µmol /l	211.59 ± 26.29	206.29
CRF		223.36 ± 13.99	213.33
ACTH		261.93 ± 16.02	278.28

Table 3.11. Mean ± SE and median values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with pregnenolone alone (control) or pregnenolone with CRF (100 nmol/l)/ACTH (10 nmol/l).

3.12. The effects of CRF and ACTH on P levels produced by placental tissue explants compared to isolated cytotrophoblast cells from the same placentae

Similar experiments on placental tissue from 3 placentae (B1, B2 and B3 from which cells were also isolated, see section 3.11) indicated that there was no consistent effect of CRF or ACTH on minced preparations (incubated with 1.0 µmol/l pregnenolone) although one placenta (see figures 3.12.1, and 3.12.2) showed an increase in P production in the presence of CRF ($p < 0.01$) and ACTH ($p < 0.01$). The mean ± SE and median levels for those placentae incubated with/without CRF or ACTH are given in table 3.12.

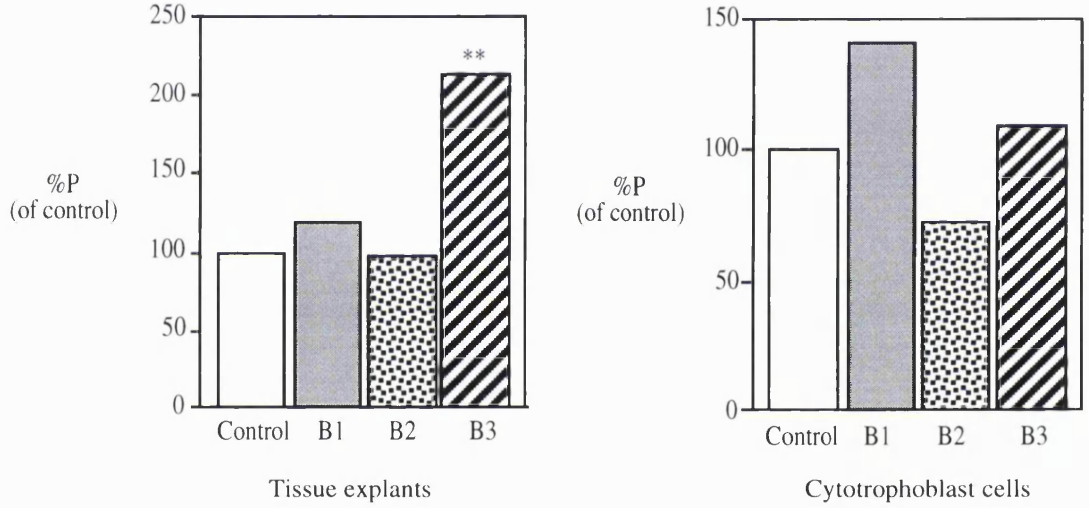


Figure 3.12.1. The effect of CRF (100 nmol/l) on P production by both tissue explants and cytotrophoblast cell preparations obtained from three placentae.

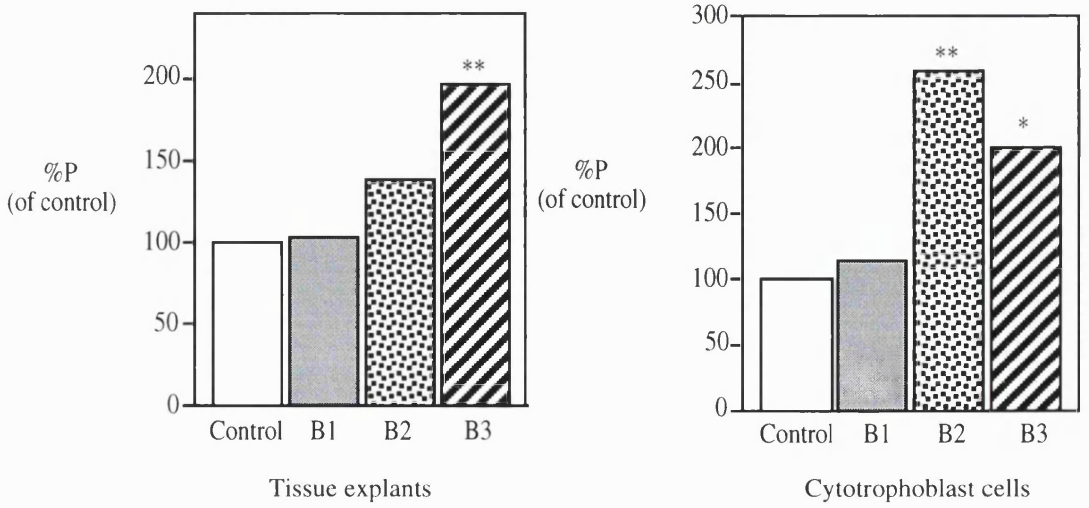


Figure 3.12.2. The effect of ACTH (10 nmol/l) on P production by both tissue explants and cytotrophoblast cell preparations obtained from three placentae.

Placenta	Mean ± SE	Median
B1 Control	54.25 ± 7.47	47.83
CRF	64.43 ± 5.94	59.31
ACTH	55.75 ± 9.19	49.93
B2 Control	133.38 ± 14.82	113.91
CRF	132.05 ± 18.73	121.13
ACTH	186.90 ± 24.61	185.49
B3 Control	91.92 ± 5.82	93.21
CRF	197.19 ± 58.70	120.74
ACTH	180.65 ± 1 6.22	162.75

Table 3.12. Mean ± SE and median values for P levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with pregnenolone alone (control) or pregnenolone with CRF (100 nmol/l) /ACTH (10 nmol/l) for 24 hours.

3.13. The effect of IGF-I on E2 production by placental tissue explants

On incubation with IGF-I (20 ng/ml) variable responses were observed in the tissue minces obtained from three individual placentae as can be seen from see figure 3.13.1. In one placenta there were no significant differences between the control explants incubated in DMEM alone and those incubated with IGF-I (20 ng/ml). However in placentae A3 and A4, levels were lower than the control throughout and there was a significant decrease in E2 levels produced by placenta A3 at five time points ($t = 20$ m $p < 0.01$; $t = 40$ m, $p \leq 0.02$; $t = 100$ m, $p < 0.01$; $t = 120$ m, $p < 0.01$; $t = 180$ m, $p < 0.05$), and at one time point for placenta A4 ($t = 20$ m, $p < 0.05$). When the concentration of IGF-I was increased to 400 ng/ml there were no significant differences from control values for two of the three placentae studied but there was a significant decrease in levels with placenta A4 (time = 20 m, $p < 0.02$).

The mean ± SE and median values for E2 levels produced by those placenta incubated with IGF-I (20 ng/ml) or IGF-I (400 ng/ml) and those of the controls for each placentae are given in tables 3.13.1 and 3.13.2 respectively .

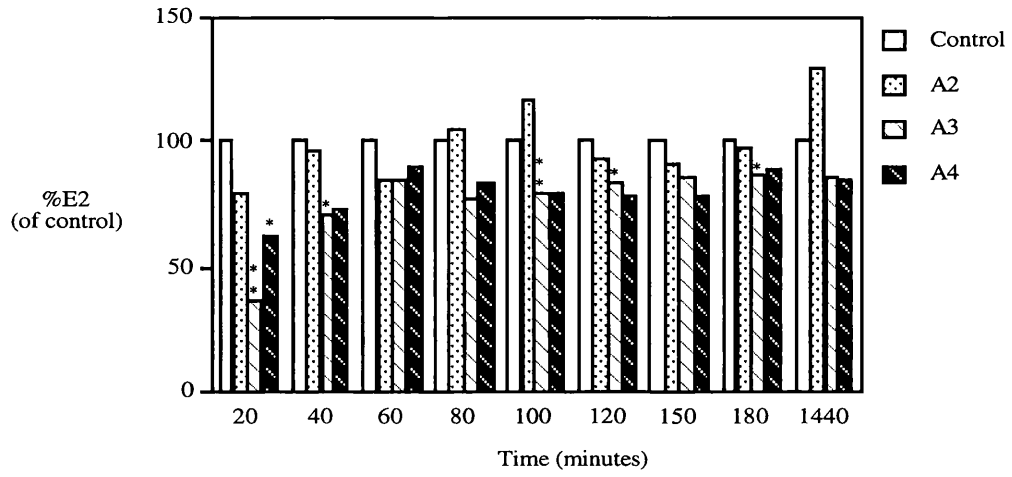


Figure 3.13.1. The effect of IGF-I at 20 ng/ml on E2 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

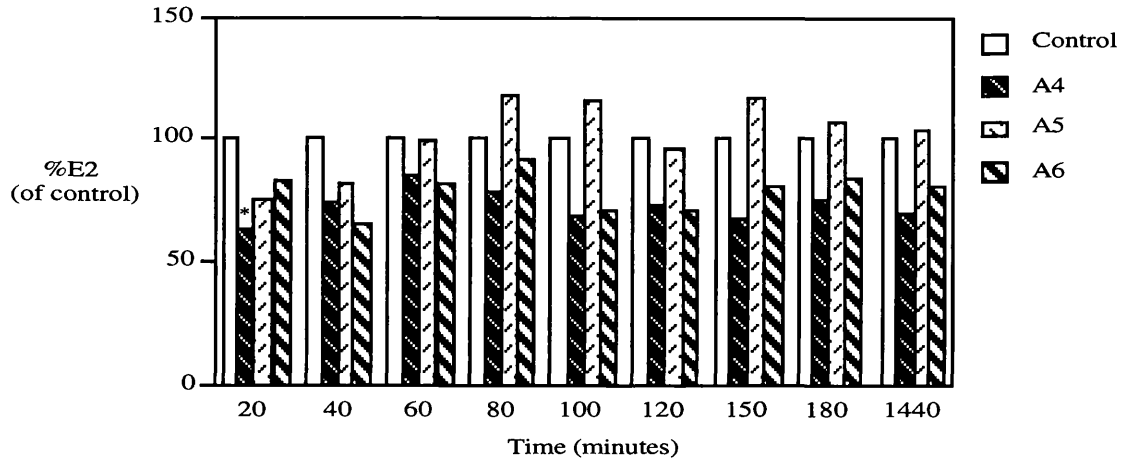


Figure 3.13.2. The effect of IGF-I at 400 ng/ml on E2 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

Time (minutes)		Placenta A2 Control	Placenta A2 IGF-I	Placenta A3 Control	Placenta A3 IGF-I	Placenta A4 Control	Placenta A4 IGF-I
20	mean ± SE	282.1 ± 40.6	224.8 ± 27.2	1144.8 ± 126.7	428.1 ± 82.7	920.8 ± 91.9	575.1 ± 47.9
	median	280.7	209.4	1140.3	344.6	909.1	570.8
40	mean ± SE	263.9 ± 40.1	236.4 ± 13.8	582.0 ± 47.8	411.8 ± 20.3	837.8 ± 146.3	610.8 ± 64.5
	median	239.7	223.8	571.7	436.63	681.0	602.4
60	mean ± SE	299.0 ± 19.0	253.7 ± 22.2	561.3 ± 126.7	476.8 ± 32.4	786.5 ± 137.9	716.0 ± 60.4
	median	292.8	257.3	561.2	478.6	705.4	671.4
80	mean ± SE	252.2 ± 15.4	263.9 ± 25.9	743.2 ± 131.8	575.3 ± 36.5	826.9 ± 84.9	693.8 ± 63.1
	median	244.5	255.9	652.7	576.2	757.7	688.9
100	mean ± SE	284.5 ± 17.6	332.9 ± 29.7	592.8 ± 34.2	470.8 ± 19.9	866.5 ± 142.7	690.0 ± 66.1
	median	278.4	327.3	574.7	594.3	776.1	648.5
120	mean ± SE	380.8 ± 46.9	353.9 ± 46.9	645.3 ± 23.2	537.5 ± 17.4	1155.9 ± 142.9	900.6 ± 68.9
	median	337.0	324.5	644.2	538.7	1161.3	815.7
150	mean ± SE	314.2 ± 16.6	286.2 ± 8.0	575.2 ± 17.8	494.3 ± 55.8	1139.0 ± 238.6	891.3 ± 67.2
	median	305.4	277.7	578.2	505.6	1001.8	839.2
180	mean ± SE	306.3 ± 16.7	297.8 ± 52.2	564.2 ± 33.7	489.1 ± 29.2	759.5 ± 92.8	676.6 ± 46.2
	median	300.5	279.7	534.6	467.1	708.5	629.4
1440	mean ± SE	323.6 ± 16.0	418.1 ± 52.9	820.1 ± 42.3	704.2 ± 50.7	950.5 ± 74.9	806.2 ± 80.4
	median	334.2	373.7	828.2	702.2	962.7	733.1

Table 3.13.1. Mean ± SE and median values for E2 levels (fmol/mg protein) produced by placental tissue explants incubated with/without IGF-I at 20 ng/ml for 24 hours.

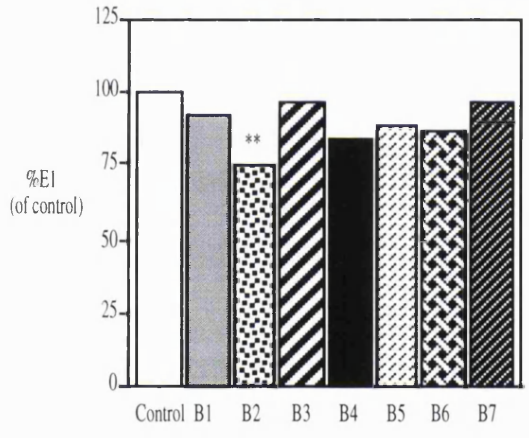
Time (minutes)		Placenta A4 Control	Placenta A4 IGF-I	Placenta A5 Control	Placenta A5 IGF-I	Placenta A6 Control	Placenta A6 IGF-I
20	mean ± SE	0.92 ± 0.09	0.59 ± 0.04	1.92 ± 0.22	1.45 ± 0.21	3.88 ± 0.93	3.23 ± 0.60
	median	0.91	0.56	1.91	1.34	3.85	3.30
40	mean ± SE	0.84 ± 0.15	0.62 ± 0.04	1.84 ± 0.35	1.52 ± 0.28	4.36 ± 0.68	2.86 ± 0.70
	median	0.68	0.62	1.81	1.51	4.96	2.76
60	mean ± SE	0.79 ± 0.14	0.67 ± 0.09	1.12 ± 0.18	1.10 ± 0.29	3.21 ± 0.54	2.65 ± 0.55
	median	0.71	0.61	0.95	0.95	2.94	2.54
80	mean ± SE	0.83 ± 0.09	0.65 ± 0.06	0.88 ± 0.11	1.06 ± 0.22	1.97 ± 0.24	1.81 ± 0.30
	median	0.76	0.67	0.77	0.87	1.80	1.66
100	mean ± SE	0.87 ± 0.14	0.60 ± 0.05	0.88 ± 0.17	1.03 ± 0.28	2.45 ± 0.47	1.74 ± 0.21
	median	0.78	0.59	0.75	0.74	1.86	1.58
120	mean ± SE	1.16 ± 0.14	0.85 ± 0.08	1.05 ± 0.19	1.01 ± 0.28	2.31 ± 0.35	1.65 ± 0.17
	median	1.16	0.08	0.96	0.77	1.88	1.64
150	mean ± SE	1.14 ± 0.24	0.77 ± 0.07	1.06 ± 0.15	1.25 ± 0.29	2.03 ± 0.27	1.64 ± 0.15
	median	1.00	0.77	0.92	1.06	1.73	1.57
180	mean ± SE	0.76 ± 0.09	0.57 ± 0.08	1.26 ± 0.24	1.34 ± 0.38	2.08 ± 0.33	1.76 ± 0.16
	median	0.71	0.51	1.08	1.12	1.74	1.84
1440	mean ± SE	0.95 ± 0.08	0.67 ± 0.11	1.32 ± 0.12	1.36 ± 0.22	1.95 ± 0.28	1.73 ± 0.12
	median	0.96	0.69	1.19	1.22	1.69	1.55

Table 3.13.2. Mean ± SE and median values for E2 levels (pmol/mg protein) produced by placental tissue explants incubated with/without and IGF-I at 400 ng/ml for 24 hours.

3.14. The effects of IGF-I and IGF-II on E1 and E2 production by isolated cytotrophoblast cells

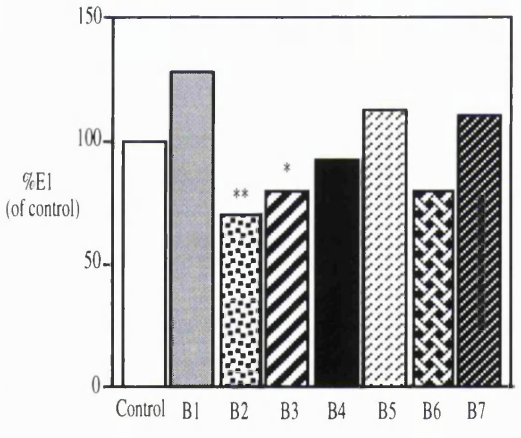
3.14.1. The effect of IGF-I on E1 production by isolated cytotrophoblast cells

There were no significant differences in E1 levels produced by cells incubated with IGF-I (20 ng/ml) as compared to those of the controls in six of the seven placentae studied but a significant decrease was observed in one placenta (B2, $p < 0.005$, figure 3.14.1a). At a higher concentration of IGF-I (400 ng/ml) there were no significant differences in E1 levels produced by five of the placentae compared to levels produced by controls, with the exception of cells prepared from placenta B2 and placenta B3 in which levels were significantly decreased ($p < 0.005$, < 0.02 respectively, figure 3.14.1b). The mean ± SE and median levels for E1, are shown in table 3.14a.



IGF-I 20 ng/ml

Figure 3.14.1.a .



IGF-I 400 ng/ml

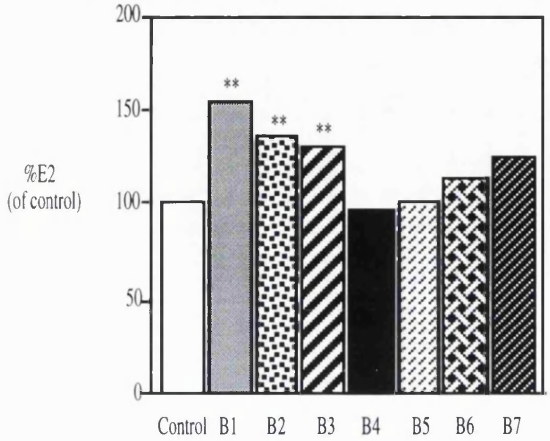
Figure 3.14.1b.

Figures 3.14.1a and 3.14.1b. The effect of IGF-I at 20 ng/ml and 400 ng/ml respectively on E1 production by isolated cytotrophoblast cells obtained from seven placentae.

3.14.2. The effect of IGF-I on E2 production by isolated cytotrophoblast cells.

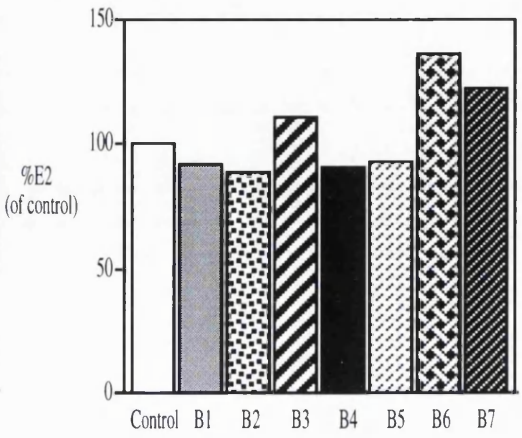
There were no significant differences in E2 levels in placental cell preparations from four individual placentae when incubated with IGF-I (20 ng/ml) in comparison to controls but E2 levels were significantly greater in 3 of the placentae studied (B1 $p < 0.005$, B2 $p < 0.005$, B3 $p < 0.005$, figure 3.14.2a). On incubating cell preparations with

IGF-I (400 ng/ml) there were no significant differences in E2 levels in comparison to those produced by controls in any of the seven placentae studied (figure 3.14.2b). The mean ± SE and median levels for E2 are given in table 3.14a.



IGF-I 20 ng/ml

Figure 3.14.2a



IGF-I 400 ng/ml

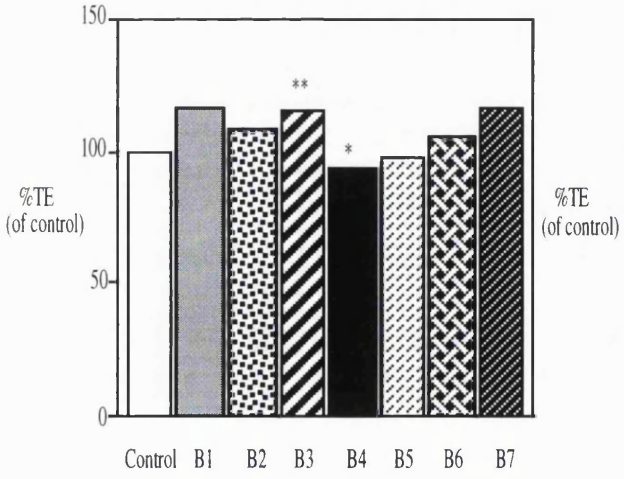
Figure 3.14.2b

Figures 3.14.2a and 3.14.2b. The effect of IGF-I at 20 ng/ml and 400 ng/ml respectively on E2 production by isolated cytotrophoblast cells obtained from seven placentae.

3.14.3. The effects of IGF-I on TE production by isolated cytotrophoblast cells

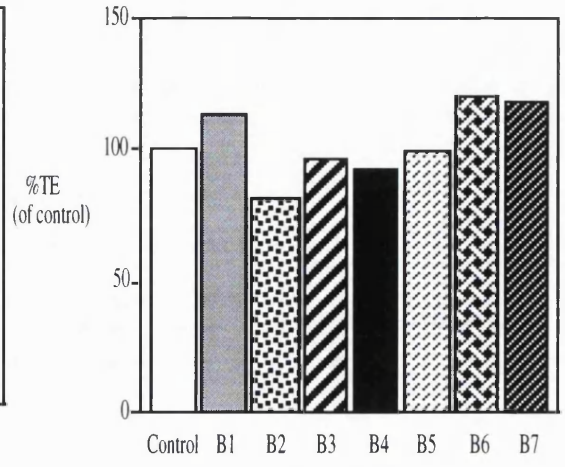
When the total E1 and E2 levels (TE) were calculated there were no significant differences in five of the placentae studied between isolated cell preparations incubated with IGF-I at 20 ng/ml compared to those levels produced by controls. However one of the placentae had significantly increased TE levels whilst another had significantly decreased TE levels when compared to those produced by the controls (B3, $p < 0.002$, B4, $p < 0.05$, respectively, figure 3.14.3a). Addition of IGF-I at 400 ng/ml had no significant effect on TE production when compared to the controls in any of the seven placentae studied (figure 3.14.3b.).

The mean ± SE and median levels for TE are given in tables 13.4b.



IGF-I 20 ng/ml

Figure 3.14.3a.



IGF-I 400 ng/ml

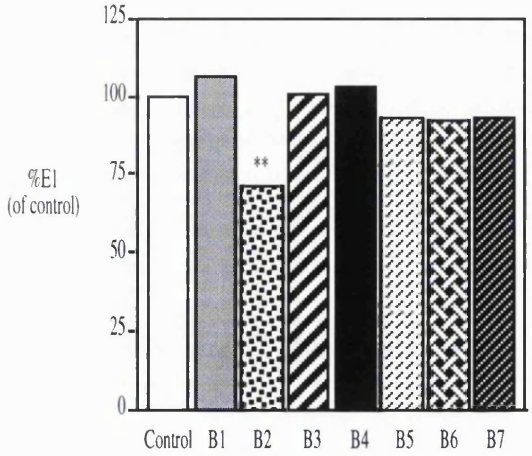
Figure 3.14.3b.

Figures 3.14.3a and 3.14.3b. The effects of IGF-I at 20 ng/ml and 400 ng/ml respectively on TE production by isolated cytotrophoblast cells obtained from seven placentae.

3.14.4. The effects of IGF-II on E1 production by isolated cytotrophoblast cells

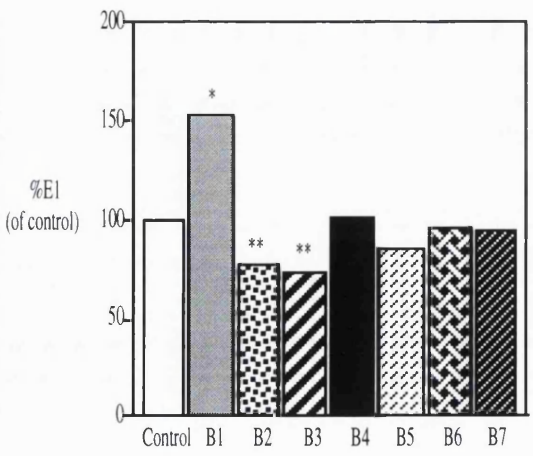
Levels of E1 produced from cell preparations incubated with A and IGF-II at 20 ng/ml (figure 3.14.4a) were similar to control values in six placentae. However levels produced by placenta B2 were significantly lower than the controls ($p < 0.01$). On incubation with IGF-II at 400 ng/ml E1 levels were not significantly different from those of the controls in four of the placentae studied (figure 3.14.4b). More variable responses were observed in three placentae with B1 being significantly higher than the respective control levels (B1, $p < 0.05$), whilst in placenta B2 and B3 the levels of E1 were markedly reduced in comparison to controls (B2 $p < 0.005$, B3 $p= 0.005$).

The mean ± SE and median values for E1 levels produced by the placental cell preparations incubated with IGF-II are given in table 3.14a.



IGF-II 20 ng/ml

Figure 3.14.4a.



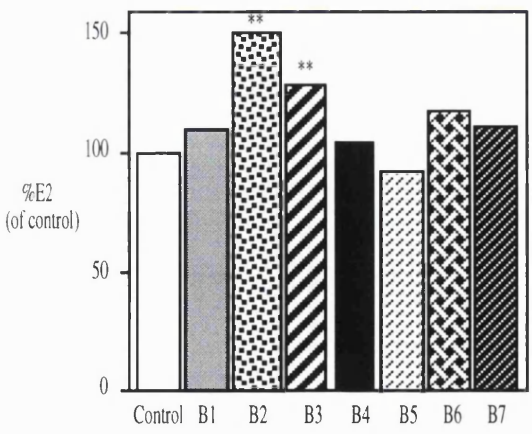
IGF-II 400 ng/ml

Figure 3.14.4b.

Figures 3.14.4a and 3.14.4b. The effects of IGF-II at 20 ng/ml and 400 ng/ml respectively on E1 production by isolated cytotrophoblast cells obtained from seven placentae.

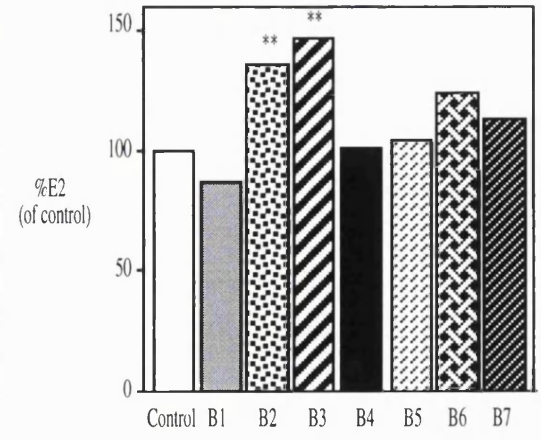
3.14.5. The effect of IGF-II on E2 production by isolated cytotrophoblast cells.

In five of the placentae studied E2 levels in cell preparations incubated with either IGF-II at 20 ng/ml or at 400 ng/ml were not significantly different from controls but they were markedly raised in placentae B2 ($p < 0.005$) and B3 ($p < 0.005$) at both IGF-II concentrations as can be seen in figures 3.14.5a and 3.14.5b. The mean ± SE and median values for these levels are given in table 3.14a.



IGF-II 20 ng/ml

Figure 3.14.5a.



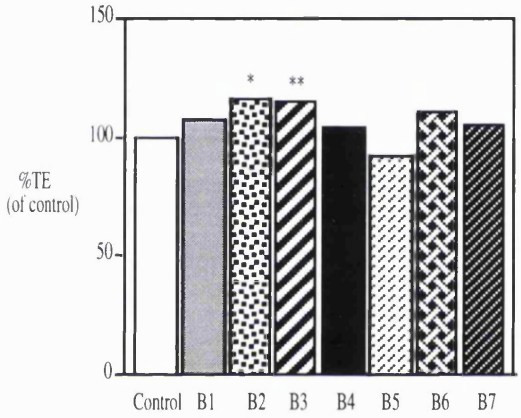
IGF-II 400 ng/ml

Figure 3.14.5b.

Figures 3.14.5a and 3.14.5b. The effect of IGF-II at 20 ng/ml and 400 ng/ml respectively on E2 production by isolated cytotrophoblast cells obtained from seven placentae.

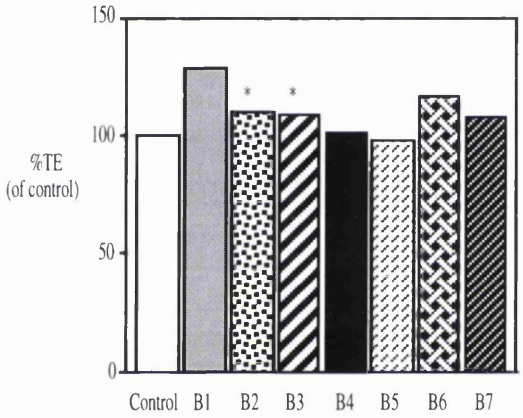
3.14.6. The effect of IGF-II on TE production by isolated cytotrophoblast cells

No significant differences were observed in TE levels produced by cell preparations incubated with IGF-II (20 ng/ml) with the exception of placentae B2 and B3 in which levels were significantly raised ($p < 0.05$ and $p < 0.005$ respectively) as compared to those of the controls (figure 3.14.6a). On incubation with IGF-II at 400 ng/ml TE levels were not significantly different from controls in experiments in five placenta but were again significantly raised in two (B2 $p < 0.05$, B3 $p < 0.05$) and are shown in figure 3.14.6b. The mean ± SE and median values are given in table 3.14b.



IGF-II 20 ng/ml

Figure 3.14.6a



IGF-II 400 ng/ml

Figure 3.14.6b.

Figures 3.14.6a and 3.14.6b. The effect of IGF-II at 20 ng/ml and 400 ng/ml respectively on TE production by isolated cytotrophoblast cells obtained from seven placentae.

Placenta		E1 Mean ± SE	E1 Median	E2 Mean ± SE	E2 Median
B1	Control	20.30 ± 2.04	19.73	11.80 ± 0.73	11.62
	IGF-I (20 ng/ml)	18.73 ± 0.93	17.94	18.19 ± 0.49	18.46
	IGF-I (400 ng/ml)	25.87 ± 0.86	26.42	10.82 ± 0.35	11.10
	IGF-II (20 ng/ml)	21.66 ± 0.75	22.55	12.87 ± 0.42	12.60
	IGF-II (400 ng/ml)	31.05 ± 3.43	31.17	10.24 ± 0.36	10.42
B2	Control	2.30 ± 0.07	2.30	3.03 ± 0.11	3.10
	IGF-I (20 ng/ml)	1.71 ± 0.16	1.73	4.10 ± 0.17	4.15
	IGF-I (400 ng/ml)	1.56 ± 0.13	1.61	2.69 ± 0.22	2.61
	IGF-II (20 ng/ml)	1.62 ± 0.13	1.61	4.53 ± 0.48	4.64
	IGF-II (400 ng/ml)	1.73 ± 0.09	1.76	4.09 ± 0.11	4.10
B3	Control	3.80 ± 0.08	3.78	3.81 ± 0.10	3.86
	IGF-I (20 ng/ml)	3.65 ± 0.10	3.73	4.97 ± 0.12	4.89
	IGF-I (400 ng/ml)	3.00 ± 0.16	2.82	4.26 ± .0.19	4.29
	IGF-II (20 ng/ml)	3.82 ± 0.14	3.77	4.85 ± 0.19	4.97
	IGF-II (400 ng/ml)	2.76 ± 0.07	2.75	5.53 ± 0.17	5.67
B4	Control	2.08 ± 0.06	2.05	5.73 ± 0.11	5.67
	IGF-I (20 ng/ml)	1.74 ± 0.17	1.87	5.50 ± 0.15	5.63
	IGF-I (400 ng/ml)	1.92 ± 0.17	1.75	5.21 ± 0.32	5.04
	IGF-II (20 ng/ml)	2.15 ± 0.17	2.18	5.94 ± 0.18	5.95
	IGF-II (400 ng/ml)	2.11 ± 0.15	1.90	5.77 ± 0.27	5.81
B5	Control	2.64 ± 0.17	2.81	5.25 ± 0.17	5.34
	IGF-I (20 ng/ml)	2.34 ± 0.17	2.33	5.29 ± 0.39	4.90
	IGF-I (400 ng/ml)	2.94 ± 0.11	2.90	4.86 ± 0.21	4.72
	IGF-II (20 ng/ml)	2.44 ± 0.11	2.44	4.81 ± 0.37	5.17
	IGF-II (400 ng/ml)	2.31 ± 0.28	2.36	5.52 ± 0.19	5.38
B6	Control	3.58 ± 0.20	3.75	9.23 ± 1.05	9.53
	IGF-I (20 ng/ml)	3.09 ± 0.16	2.76	10.41 ± 0.45	10.51
	IGF-I (400 ng/ml)	2.85 ± 0.12	3.75	12.65 ± 0.13	12.00
	IGF-II (20 ng/ml)	3.28 ± 0.20	3.12	10.77 ± 0.47	10.65
	IGF-II (400 ng/ml)	3.47 ± 0.27	3.49	11.40 ± 0.94	11.23
B7	Control	2.75 ± 0.13	2.82	6.68 ± 0.18	7.83
	IGF-I (20 ng/ml)	2.63 ± 0.12	2.64	8.28 ± 0.13	8.28
	IGF-I (400 ng/ml)	3.00 ± 2.28	3.04	8.23 ± 0.42	8.09
	IGF-II (20 ng/ml)	2.55 ± 1.09	2.52	7.33 ± 0.22	7.55
	IGF-II (400 ng/ml)	2.61 ± 0.09	2.53	7.56 ± 0.27	7.38

Table 3.14a. Mean ± SE and median E1 and E2 levels (pmol/100,000 viable cells/24 hours) produced by cytotrophoblast cells incubated with IGF-I or IGF-II and A or with A only (control).

Placenta		Mean ± SE	Median
B1	Control	32.10 ± 2.31	31.98
	IGF-I (20 ng/ml)	36.91 ± 1.15	36.90
	IGF-I (400 ng/ml)	36.68 ± 1.02	37.07
	IGF-II (20 ng/ml)	34.53 ± 1.76	35.38
	IGF-II (400 ng/ml)	41.30 ± 1.02	41.54
B2	Control	5.32 ± 0.17	54.60
	IGF-I (20 ng/ml)	5.80 ± 0.09	31.46
	IGF-I (400 ng/ml)	4.25 ± 1.55	21.61
	IGF-II (20 ng/ml)	6.15 ± 2.30	32.83
	IGF-II (400 ng/ml)	5.81 ± 0.10	32.65
B3	Control	7.61 ± 0.13	7.70
	IGF-I (20 ng/ml)	8.62 ± 0.35	8.64
	IGF-I (400 ng/ml)	7.26 ± 0.08	7.12
	IGF-II (20 ng/ml)	8.67 ± 0.10	8.86
	IGF-II (400 ng/ml)	8.29 ± 0.21	8.42
B4	Control	7.81 ± 0.07	7.92
	IGF-I (20 ng/ml)	7.24 ± 0.18	7.07
	IGF-I (400 ng/ml)	7.12 ± 0.48	6.79
	IGF-II (20 ng/ml)	8.08 ± 0.31	8.06
	IGF-II (400 ng/ml)	7.88 ± 0.20	7.91
B5	Control	7.89 ± 0.30	8.07
	IGF-I (20 ng/ml)	7.63 ± 0.50	7.24
	IGF-I (400 ng/ml)	7.80 ± 0.31	7.56
	IGF-II (20 ng/ml)	7.25 ± 0.45	7.52
	IGF-II (400 ng/ml)	7.83± 0.27	8.06
B6	Control	12.81 ± 1.07	13.04
	IGF-I (20 ng/ml)	13.50 ± 0.40	13.51
	IGF-I (400 ng/ml)	15.50 ± 1.42	14.69
	IGF-II (20 ng/ml)	14.05 ± 0.46	14.08
	IGF-II (400 ng/ml)	14.87 ± 1 .10	14.84
B7	Control	9.43 ± 0.11	10.68
	IGF-I (20 ng/ml)	10.91 ± 0.14	10.93
	IGF-I (400 ng/ml)	11.23 ± 0.39	11.32
	IGF-II (20 ng/ml)	9.88 ± 0.24	9.89
	IGF-II (400 ng/ml)	10.16 ± 0.31	9.98

Table 3.14b. Mean ± SE and median values for TE levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with A (control) or A and IGF-I or IGF-II.

3.15.1. The effects of IGF-I on E1 and E2 levels produced by tissue explants compared to cell preparations obtained from the same placentae

Figures 3.15.1a-3.15.1f show the comparison of the effects of IGF-I on E1, E2 and TE levels produced by cells and tissue obtained from three placentae. Levels of E1, E2 and TE produced by tissue minces from these three placentae were not significantly different compared to controls when incubated with IGF-I at 20 ng/ml or IGF-I at 400 ng/ml unlike those of the cell preparations obtained from these same placentae B1, B2 and B3 discussed in section 3.14.1.

The mean ± SE and median values for those levels produced by the tissue minces are shown in table 3.15a and 3.15b .

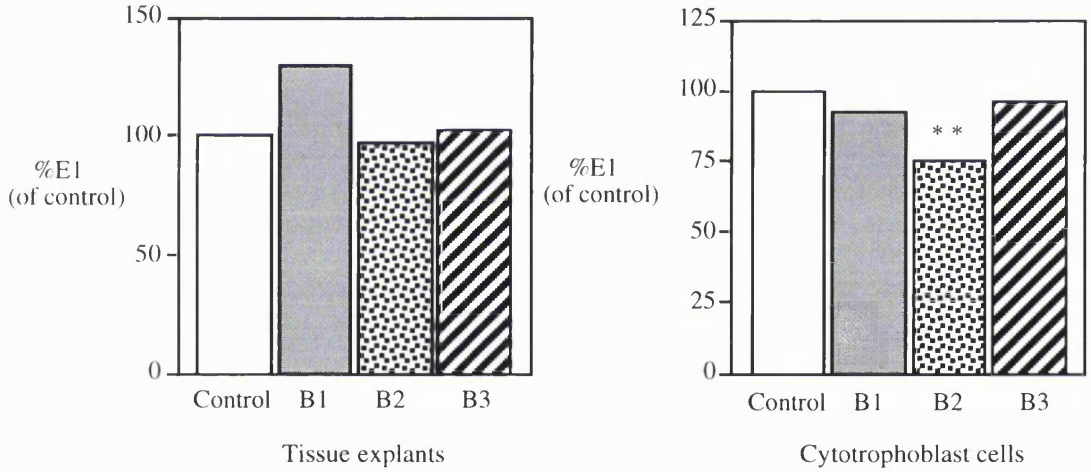


Figure 3.15.1a. The effect of IGF-I at 20 ng/ml on E1 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

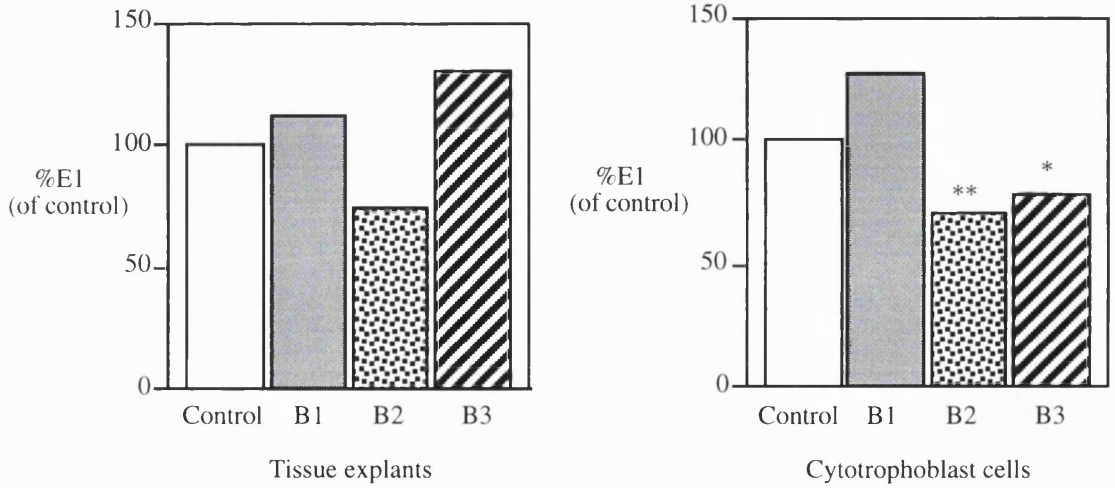


Figure 3.15.1b. The effect of IGF-I at 400 ng/ml on E1 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

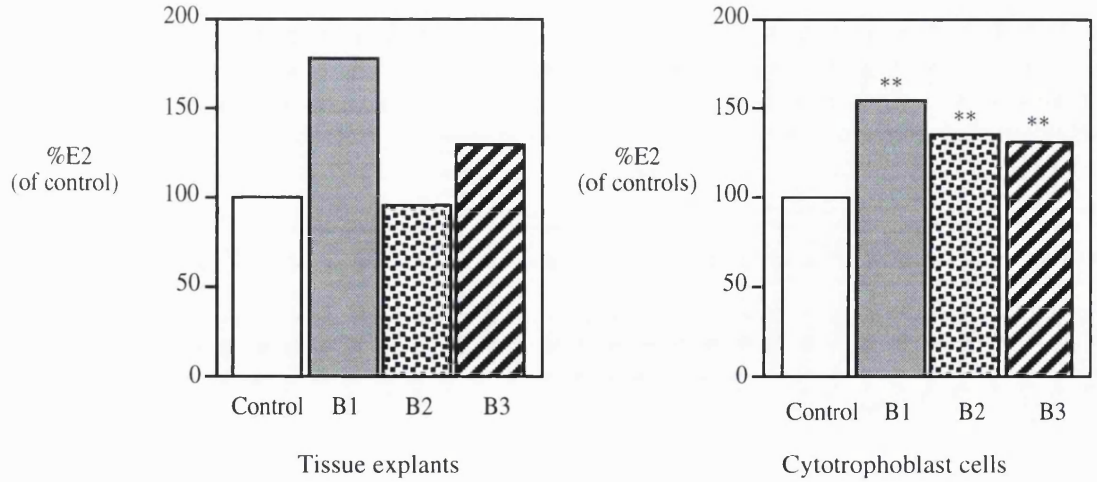


Figure 3.15.1c. The effect of IGF-I at 20 ng/ml on E2 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

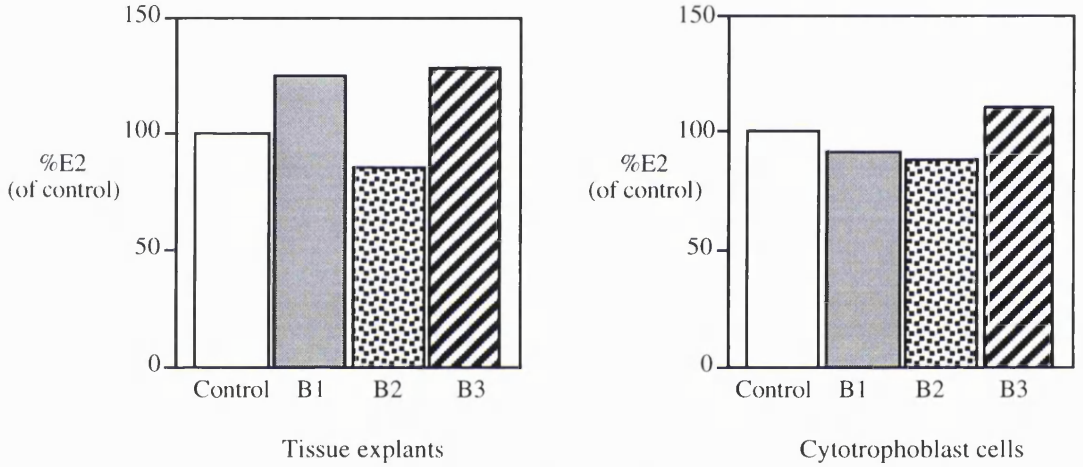


Figure 3.15.1d. The effect of IGF-I at 400 ng/ml on E2 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

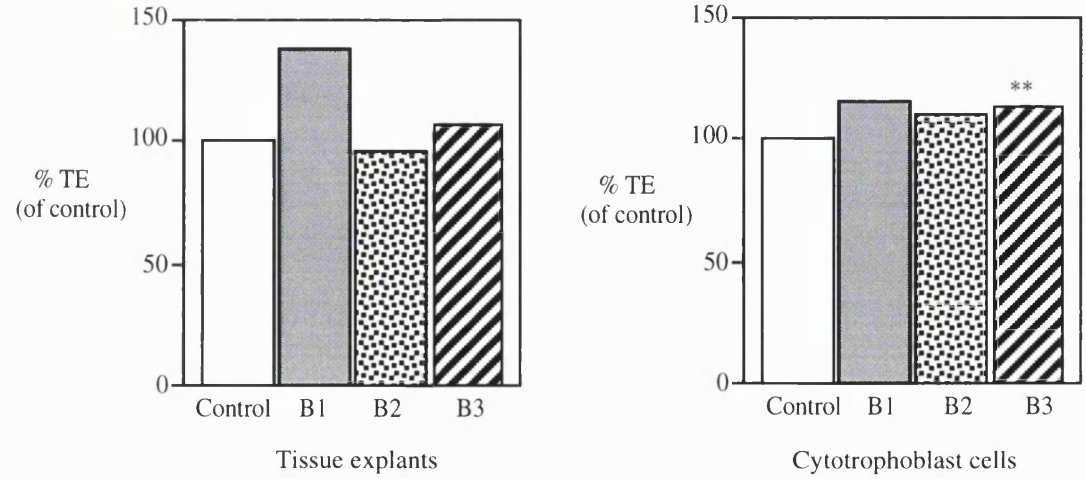


Figure 3.15.1e. The effect of IGF-I at 20 ng/ml on TE production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

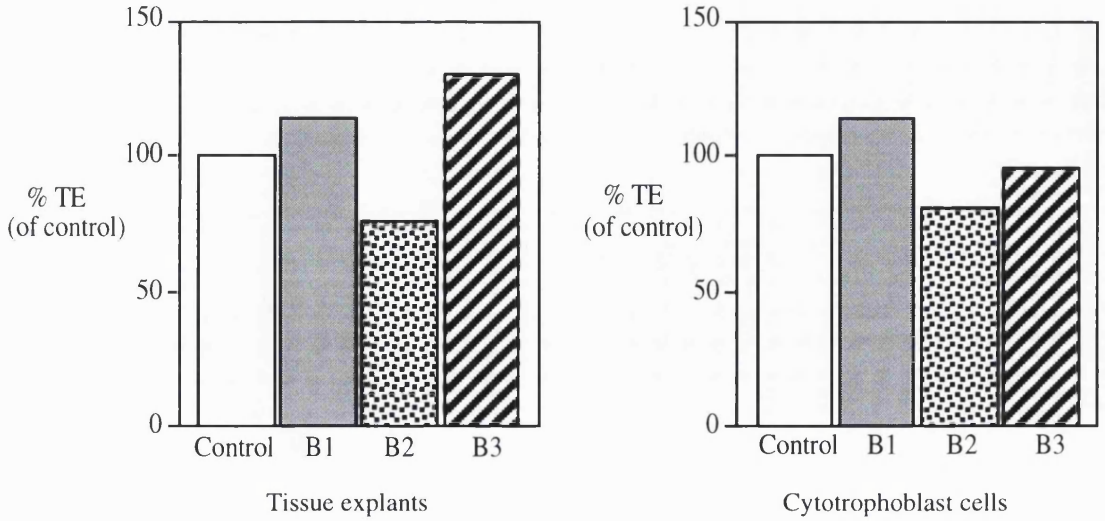


Figure 3.15.1f. The effect of IGF-I at 400 ng/ml on TE production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

3.15.2. The effects of IGF-II on E1 and E2 production by tissue explants compared to that of isolated cytotrophoblast cells obtained from the same placentae

The levels of E1, E2 and TE produced by tissue explants were not significantly different from those of controls with the exception of placenta B2 ($p < 0.05$) in which the TE was significantly lower on incubation with IGF-II at 400 ng/ml. In the cell preparations from this particular placenta the TE levels were significantly higher than the controls on incubation with IGF-II at 400 ng/ml. Figures 3.15.2a-3.15.2f show the comparison of tissue and cell preparations. The mean ± SE and median values for levels of E1, E2 and TE produced by tissue explants incubated with/without IGF-II are shown in tables 3.15a and 3.15b.

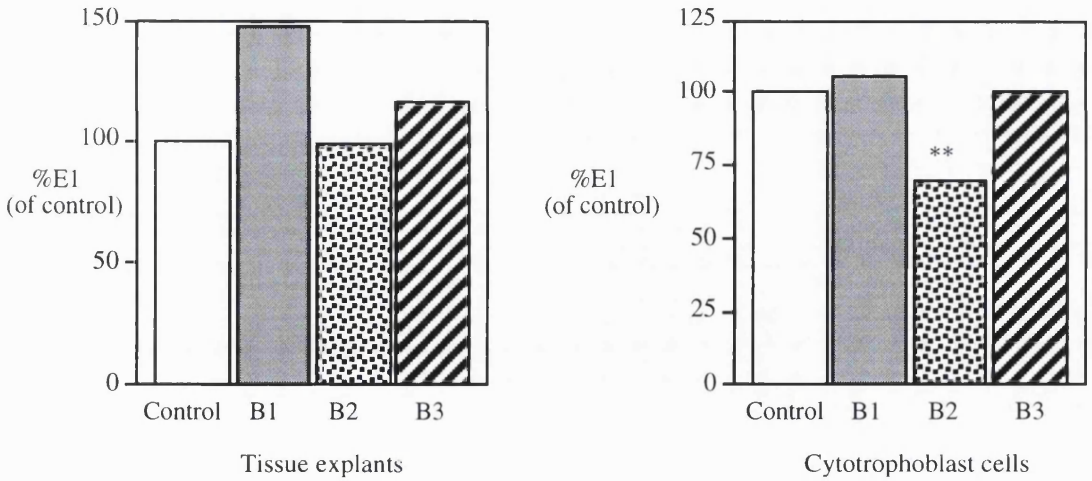


Figure 3.15.2a. The effect of IGF-II at 20 ng/ml on E1 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

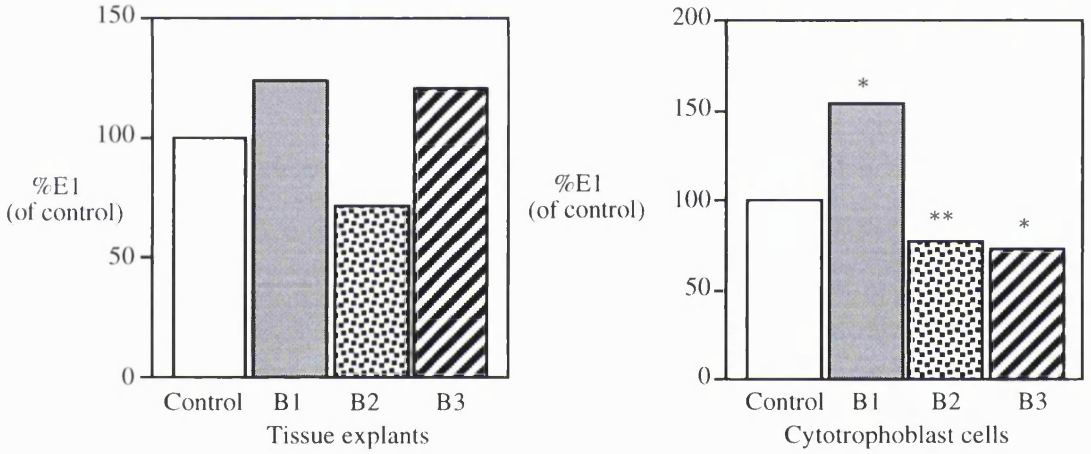


Figure 3.15.2b. The effect of IGF-II at 400 ng/ml on E1 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

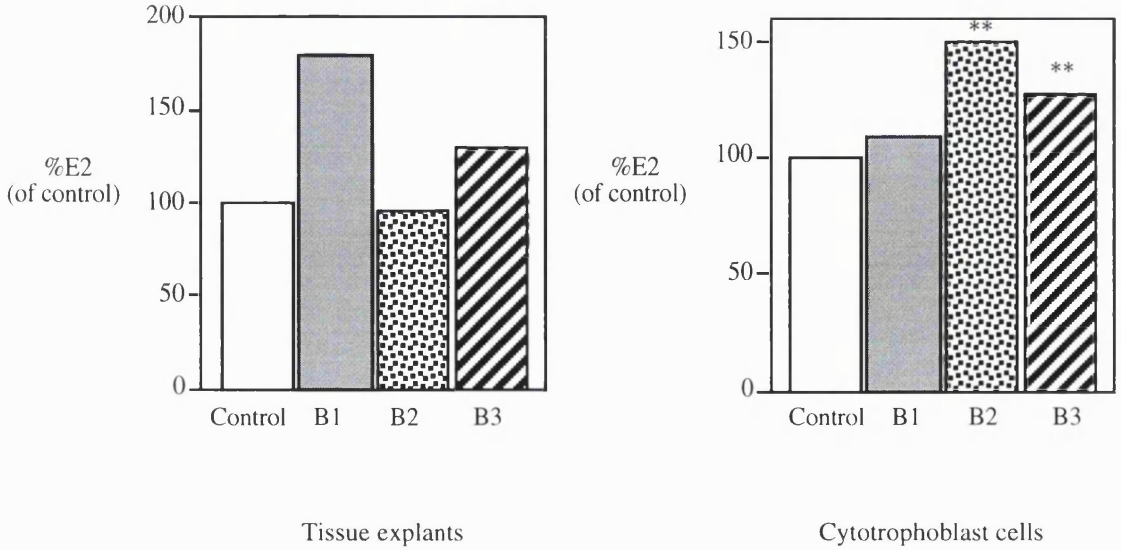


Figure 3.15.2c. The effect of IGF-II at 20 ng/ml on E2 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

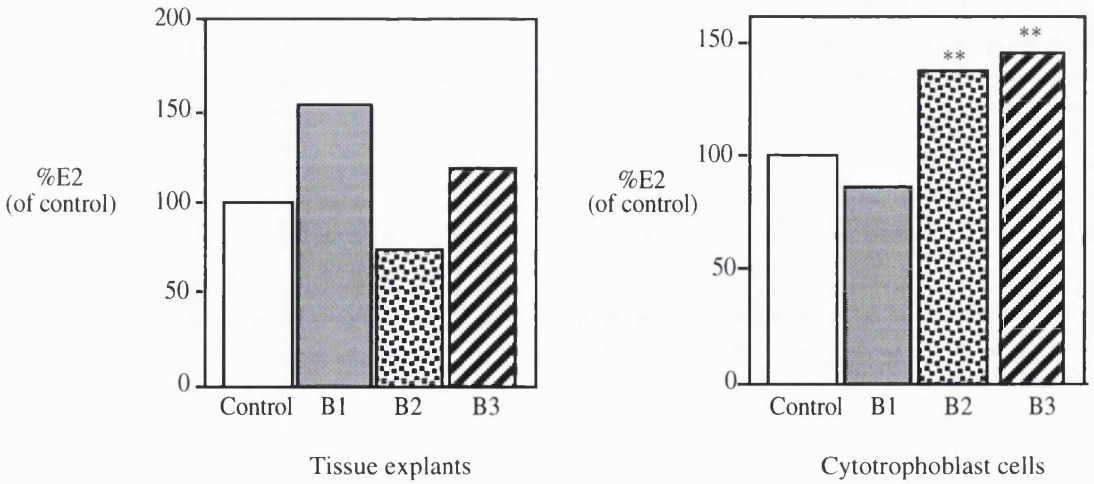


Figure 3.15.2d. The effect of IGF-II at 400 ng/ml on E2 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

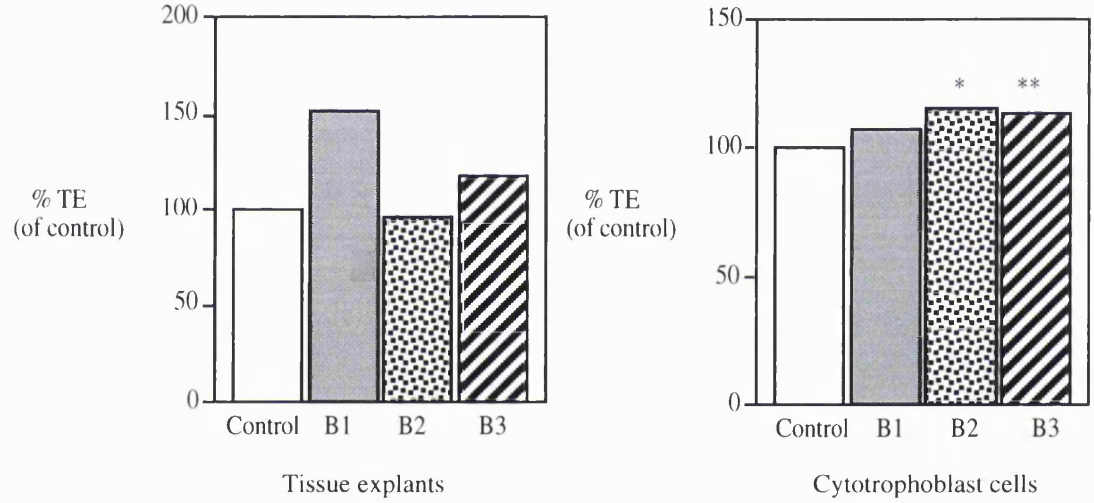


Figure 3.15.2e. The effect of IGF-II at 20 ng/ml on TE production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

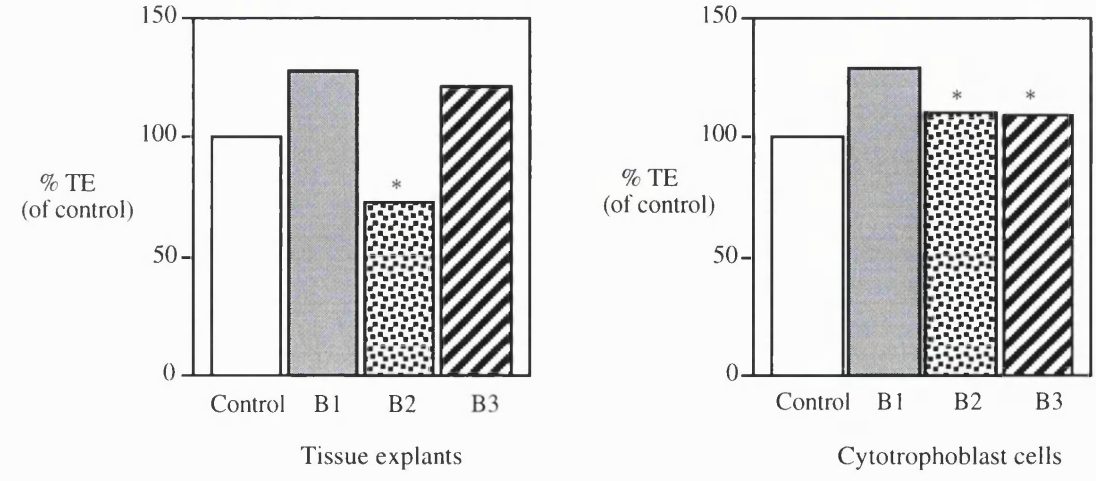


Figure 3.15.2f. The effects of IGF-II at 400 ng/ml on TE production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

	E1		E2	
Placenta	**Mean ± SE**	**Median**	**Mean ± SE**	**Median**
B1 Control	3.13 ± 0.34	2.73	0.53 ± 0.05	0.54
IGF-I (20ng/ml)	4.09 ± 0.66	3.85	0.94 ± 0.24	0.67
IGF-I (400ng/ml)	3.52 ± 0.50	3.38	0.65 ± 0.14	0.60
IGF-II (20ng/ml)	4.62 ± 1.34	3.45	0.95 ± 0.36	0.57
IGF-II (400ng/ml)	3.86 ± 0.36	4.16	0.81 ± 0.12	0.73
B2 Control	25.53 ± 0.28	23.91	3.47 ± 0.36	3.43
IGF-I (20ng/ml)	24.66 ± 0.36	27.80	3.29 ± 0.57	3.05
IGF-I (400ng/ml)	19.07 ± 0.31	18.89	2.96 ± 0.29	2.87
IGF-II (20ng/ml)	25.10 ± 0.70	18.13	2.92 ± 0.87	1.82
IGF-II (400ng/ml)	18.39 ± 1.65	18.45	2.58 ± 0.23	2.54
B3 Control	4.45 ± 0.67	4.23	0.78 ± 0.07	0.72
IGF-I (20ng/ml)	4.58 ± 0.56	4.30	1.01 ± 0.19	0.86
IGF-I (400ng/ml)	5.82 ± 1.03	5.26	1.00 ± 0.10	1.01
IGF-II (20ng/ml)	5.20 ± 0.65	5.11	0.97 ± 0.15	1.03
IGF-II (400ng/ml)	5.40 ± 0.72	5.14	0.94 ± 0.10	0.97

Table 3.15a. Mean ± SE and median values for E1 and E2 levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with A alone (control) or A and IGF-I or IGF-II.

	TE	
Placenta	**Mean ± SE**	**Median**
B1 Control	3.66 ± 0.37	3.27
IGF-I (20ng/ml)	5.03 ± 0.88	4.52
IGF-I (400ng/ml)	4.17 ± 0.64	3.98
IGF-II (20ng/ml)	5.56 ± 1.70	4.02
IGF-II (400ng/ml)	4.68 ± 0.45	4.89
B2 Control	29.00 ± 3.01	27.22
IGF-I (20ng/ml)	27.95 ± 4.03	31.68
IGF-I (400ng/ml)	22.03 ± 3.23	21.97
IGF-II (20ng/ml)	28.02 ± 7.82	19.76
IGF-II (400ng/ml)	20.97 ± 1.74	20.77
B3 Control	5.24 ± 0.73	4.91
IGF-I (20ng/ml)	5.59 ± 0.74	5.16
IGF-I (400ng/ml)	6.82 ± 1.11	6.27
IGF-II (20ng/ml)	6.17 ± 0.79	6.14
IGF-II (400ng/ml)	6.34 ± 0.77	6.11

Table 3.15b. Mean ± SE and median values for TE levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with A alone (control) or A and IGF-I or IGF-II.

3.16. The effect of IGF-I on E3 production by placental tissue explants

There were no significant effects of IGF-I on E3 production in comparison to those of the controls in tissue incubates from all three placentae incubated for 24 hours except for a significant decrease in E3 levels produced by placenta A4 at one time point (t = 100 m, $p < 0.02$).

The mean ± SE and median values for E3 levels produced by tissue incubated with/without IGF-I at 20 ng/ml and IGF-I at 400 ng/ml are given in tables 3.16.1 and 3.16.2 respectively.

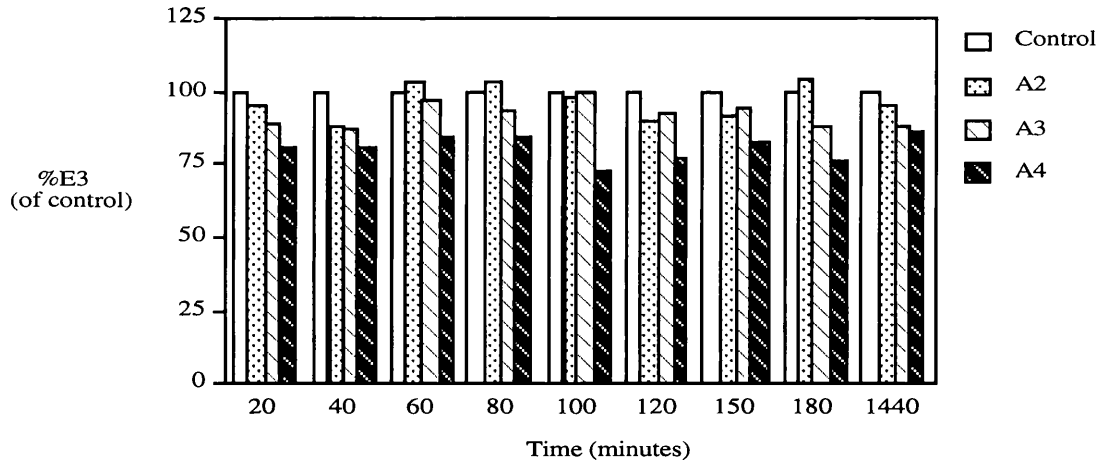


Figure 3.16.1. The effect of IGF-I at 20 ng/ml on E3 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

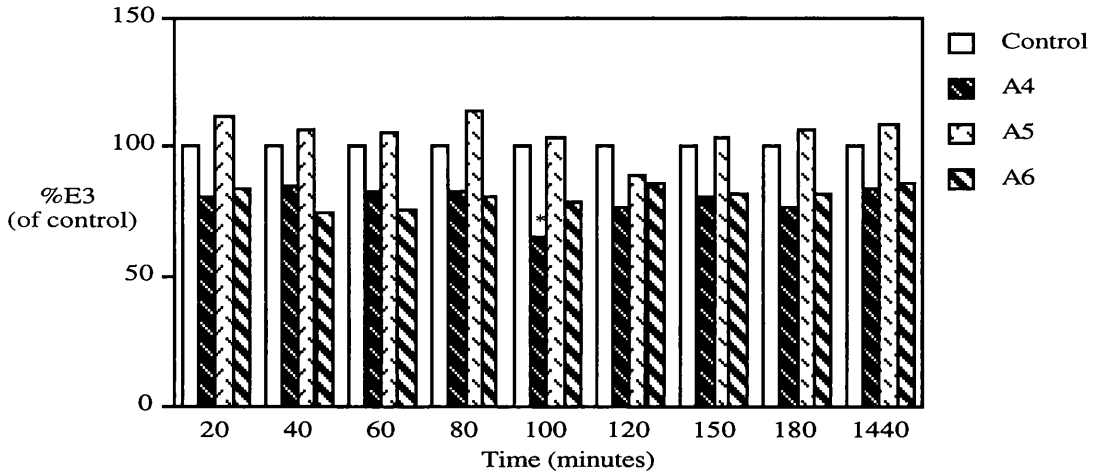


Figure 3.16.2. The effect of IGF-I at 400 ng/ml on E3 production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

Time (minutes)		Placenta A2 Control	Placenta A2 IGF-I	Placenta A3 Control	Placenta A3 IGF-I	Placenta A4 Control	Placenta A4 IGF-I
20	mean ± SE	2.28 ± 0.07	2.17 ± 0.06	0.72 ± 0.12	0.64 ± 0.04	1.86 ± 0.38	1.51 ± 0.25
	median	2.33	2.20	0.68	0.66	1.84	1.57
40	mean ± SE	2.06 ± 0.20	1.82 ± 0.07	0.89 ± 0.09	0.77 ± 0.07	2.16 ± 0.26	1.74 ± 0.15
	median	2.23	1.78	0.83	8.22	2.04	1.69
60	mean ± SE	2.26 ± 0.15	2.33 ± 0.15	0.92 ± 0.03	0.89 ± 0.06	2.06± 0.19	1.74 ± 0.16
	median	2.27	2.14	0.89	0.91	1.99	1.54
80	mean ± SE	2.51 ± 0.17	2.61 ± 0.19	0.94 ± 0.05	0.88 ± 0.05	2.12 ± 0.24	1.79 ± 0.20
	median	2.55	2.67	0.94	0.90	1.99	1.59
100	mean ± SE	2.55 ± 0.20	2.51 ± 0.12	0.92 ± 0.07	0.92 ± 0.04	2.54 ± 0.25	1.84 ± 0.18
	median	2.41	2.50	0.91	0.94	2.35	1.82
120	mean ± SE	2.58 ± 0.20	2.31 ± 0.28	0.98 ± 0.05	0.91 ± 0.04	2.49 ± 0.27	1.92 ± 0.14
	median	2.58	2.45	1.00	0.88	2.35	2.01
150	mean ± SE	2.99 ± 0.38	2.75 ± 0.15	0.90 ± 0.04	0.85 ± 0.02	2.28 ± 0.18	1.88 ± 0.15
	median	2.69	2.73	0.90	0.85	2.20	1.88
180	mean ± SE	2.54 ± 0.19	2.65± 0.15	1.29 ± 0.5	1.14 ± 0.06	2.66 ± 0.27	2 .02 ± 0.20
	median	2.42	2.73	1.25	1.12	2.55	2.20
1440	mean ± SE	3.14 ± 0.34	3.00 ± 0.42	1.45 ± 0.11	1.28 ± 0.05	2.50 ± 0.16	2.23 ± 0.18
	median	3.02	3.12	1.40	1.26	2.57	2.37

Table 3.16.1. Mean ± SE and median values for E3 levels (pmol/mg protein) produced by placental tissue explants incubated with/without IGF-I at 20 ng/ml for 24 hours.

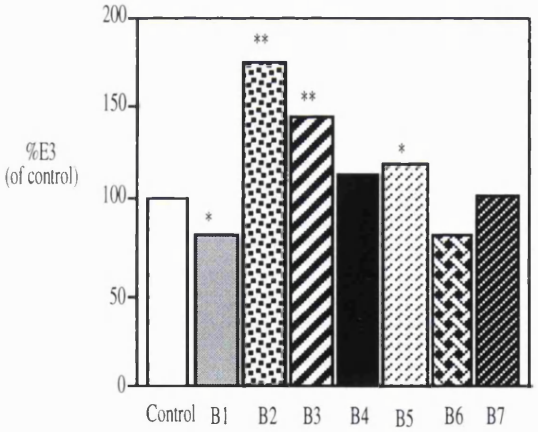
Time (minutes)		Placenta A4 Control	Placenta A4 IGF-I	Placenta A5 Control	Placenta A5 IGF-I	Placenta A6 Control	Placenta A6 IGF-I
20	mean ± SE	1.86 ± 0.38	1.48± 0.34	5.02 ± 0.79	5.57 ± 0.81	9.96 ± 0.98	8.23 ± 0.45
	median	1.84	1.49	4.51	4.98	9.62	7.96
40	mean ± SE	2.16 ± 0.26	1.81 ± 0.25	5.36 ± 0.81	5.68 ± 0.98	13.46± 1.52	9.99 ± 0.51
	median	2.04	1.61	4.59	4.94	12.89	10.07
60	mean ± SE	2.06 ± 0.19	1.69± 0.19	5.03 ± 0.41	5.25 ± 0.71	13.21 ± 1.81	9.92 ± 0.51
	median	1.99	1.52	4.58	4.81	12.07	9.88
80	mean ± SE	2.12 ± 0.24	1.74 ± 0.22	4.49 ± 0.76	5.08 ± 0.71	13.96 ± 1.54	11.18 ± 0.49
	median	1.99	1.69	4.69	4.81	12.56	11.36
100	mean ± SE	2.54 ± 0.25	1.66 ± 0.16	5.87 ± 0.74	6.06 ± 0.91	14.68 ± 1.21	11.33 ± 0.50
	median	2.35	1.68	5.39	5.40	13.79	11.30
120	mean ± SE	2.49 ± 0.27	1.89 ± 0.23	5.80 ± 0.90	5.08 ± 0.91	11.64 ± 1.02	9.85 ± 0.77
	median	2.35	1.77	5.31	5.10	11.46	9.55
150	mean ± SE	2.28 ± 0.18	1.83 ± 0.14	5.23 ± 0.51	5.43 ± 0.71	12.75 ± 1.39	10.40 ± 0.56
	median	2.20	1.74	5.12	4.99	12.25	10.31
180	mean ± SE	2.66 ± 0.27	2.02 ± 0.22	5.63 ± 0.56	5.95 ± 0.86	15.45 ± 1.82	12.57 ± 0.77
	median	2.55	1.82	5.29	5.62	14.36	12.40
1440	mean ± SE	2.57 ± 0.16	2.14 ± 0.21	6.60 ± 0.62	7.11 ± 1.07	14.65 ± 2.21	12.45 ± 1.31
	median	2.50	1.95	6.11	6.25	13.43	11.73

Table 3.16.2. The mean ± SE and median E3 levels (pmol/mg protein) produced by placental explants incubated with/without IGF-I at 400 ng/ml for 24 hours.

3.17.1. The effects of IGF-I on E3 production by isolated cytotrophoblast cells

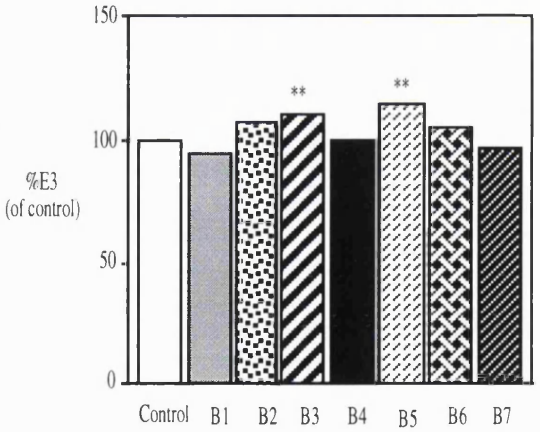
There was no significant difference in levels of E3 produced by isolated cell preparations incubated with IGF-I (20 ng/ml) in comparison to those of the controls in three of the seven placentae studied. In the other four placentae variable responses were observed. Cytotrophoblast cells isolated from placenta B1 produced significantly less E3 ($p < 0.05$), whilst placentae B2, B3, B5 had markedly increased E3 levels ($p < 0.01$, $p < 0.01$, $p < 0.02$ respectively) compared to those of the controls as can be seen in figure 3.17.1a. On incubation with IGF-I (400 ng/ml) there was no change in E3 levels measured in five placentae compared to controls whereas cells isolated from placentae B3 and B5 showed significantly raised levels ($p = 0.003$ for both placentae, see figure 3.17.1b).

The mean ± SE and median values for E3 levels incubated with IGF-I are given in table 3.17.



IGF-I 20 ng/ml

Figure 3.17.1a.



IGF-I 400 ng/ml

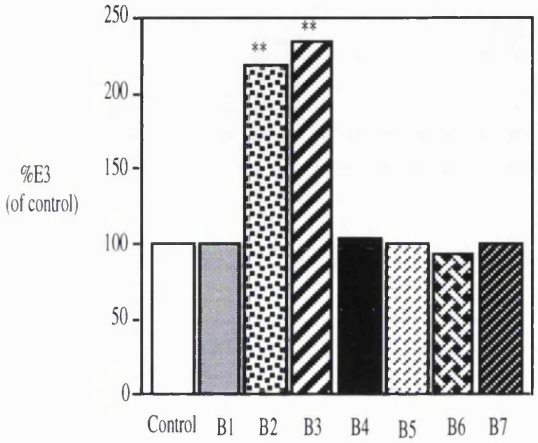
Figure 3.17.1b.

Figure 3.17.1a and 3.17.1b. The effects of IGF-I at 20 ng/ml and 400 ng/ml respectively on E3 levels produced by isolated cytotrophoblast cells obtained from seven placentae.

3.17.2. The effect of IGF-II on E3 production by isolated cytotrophoblast cells

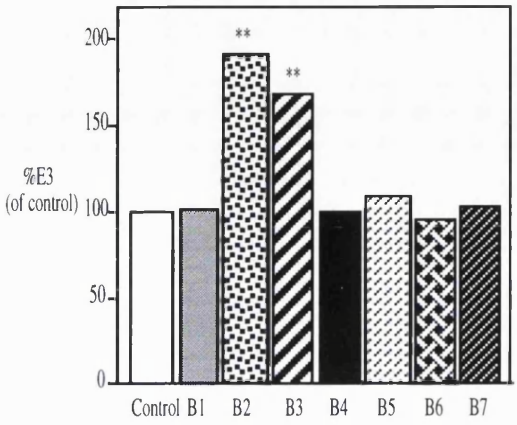
It can be seen from figure 3.17.2a that the levels of E3 produced by placental cells incubated with IGF-II at 20 ng/ml and 400 ng/ml were not significantly different from

those of the control levels in five placentae whereas placentae B2 and B3 had markedly raised E3 levels ($p < 0.004$ in all cases). The mean ± SE and median values for E3 levels produced by cells incubated with IGF-II are given in table 3.17.



IGF-II 20 ng/ml

Figure 3.17.2a



IGF-II 400 ng/ml

Figure 3.176.2b.

Figures 3.17.2a and 3.17.2b. The effect of IGF-II at 20 ng/ml and 400 ng/ml respectively on E3 production by isolated cytotrophoblast cells obtained from seven placentae.

Placenta		Mean ± SE	Median
B1	Control	1.53 ± 0.06	1.51
	IGF-I (20 ng/ml)	1.30± 0.06	1.26
	IGF-I (400 ng/ml)	1.46 ± 0.03	1.43
	IGF-II (20 ng/ml)	1.54 ± 0.10	1.49
	IGF-II (400 ng/ml)	1.55 ± 0.09	1.62
B2	Control	0.18 ± 0.01	0.18
	IGF-I (20 ng/ml)	0.31 ± 0.05	0.03
	IGF-I (400 ng/ml)	0.19 ± 0.01	0.20
	IGF-II (20 ng/ml)	0.39 ± 0.02	0.38
	IGF-II (400 ng/ml)	0.34 ± 0.02	0.35
B3	Control	0.69 ± 0.01	0.69
	IGF-I (20 ng/ml)	1.00 ± 0.06	1.50
	IGF-I (400 ng/ml)	0.76 ± 0.03	0.74
	IGF-II (20 ng/ml)	1.62 ± 0.05	1.60
	IGF-II (400 ng/ml)	1.16 ± 0.11	1.21
B4	Control	0.91 ± 0.07	0.89
	IGF-I (20 ng/ml)	1.02 ± 0.03	1.02
	IGF-I (400 ng/ml)	0.90 ± 0.04	0.91
	IGF-II (20 ng/ml)	0.95 ± 0.03	0.94
	IGF-II (400 ng/ml)	0.90 ± 0.05	0.91
B5	Control	0.70 ± 0.01	0.70
	IGF-I (20 ng/ml)	0.82 ± 0.03	0.82
	IGF-I (400 ng/ml)	0.79 ± 0.02	0.79
	IGF-II (20 ng/ml)	0.70 ± 0.03	0.71
	IGF-II (400 ng/ml)	0.76 ± 0.05	0.76
B6	Control	3.07 ± 0.11	3.17
	IGF-I (20 ng/ml)	2.62 ± 0.13	2.69
	IGF-I (400 ng/ml)	3.21 ± 0.21	3.03
	IGF-II (20 ng/ml)	2.89 ± 0.16	2.94
	IGF-II (400 ng/ml)	2.91 ± 0.17	2.78
B7	Control	0.99 ± 0.08	1.07
	IGF-I (20 ng/ml)	0.99 ± 0.04	1.01
	IGF-I (400 ng/ml)	0.95 ± 0.10	1.03
	IGF-II (20 ng/ml)	0.97 ± 0.05	0.96
	IGF-II (400 ng/ml)	1.02 ± 0.06	1.08

Table 3.17. Mean ± SE and median values for E3 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with 16α-OHA or 16α-OHA and IGF-I or IGF-II.

3.18. The effects of IGF-I and IGF-II on E3 production by tissue explants compared to those produced by cytotrophoblast cells isolated from the same three placentae

Whereas cell preparations from placentae B1, B2 and B3 showed variable responses to both IGF-I and IGF-II there appeared to be no significant increase or decrease in E3 production from tissue explants from these placentae incubated with either IGF-I or IGF-II (Figures 3.18a-3.18d). The mean ± SE and median values for E3 levels produced by tissue explants incubated with IGF-II are shown in table 3.18.

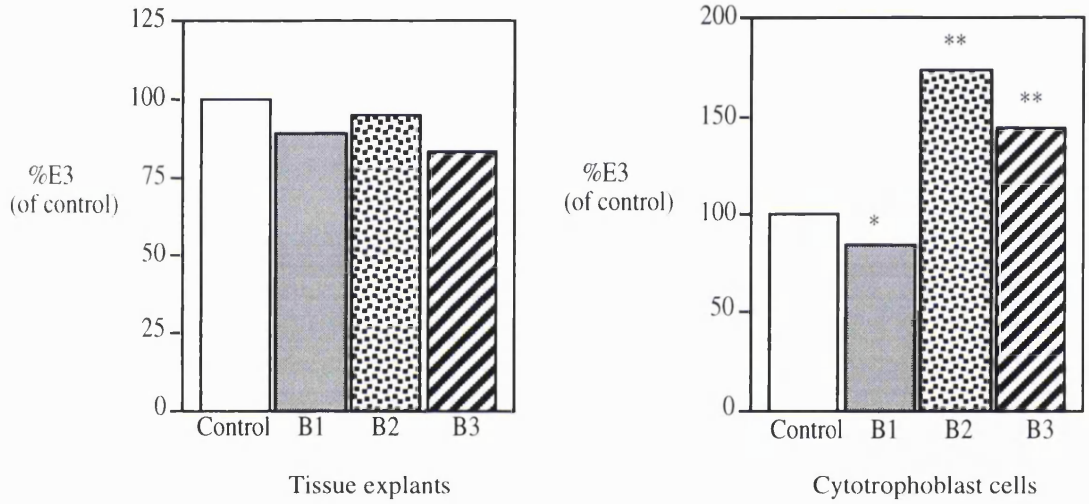


Figure 3.18a. The effects of IGF-I at 20 ng/ml on E3 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

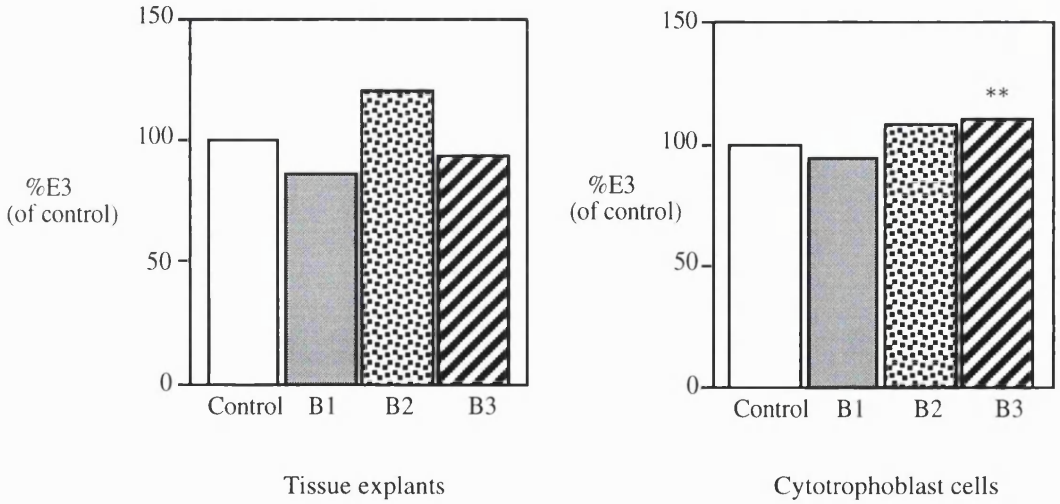


Figure 3.18b. The effects of IGF-I at 400 ng/ml on E3 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

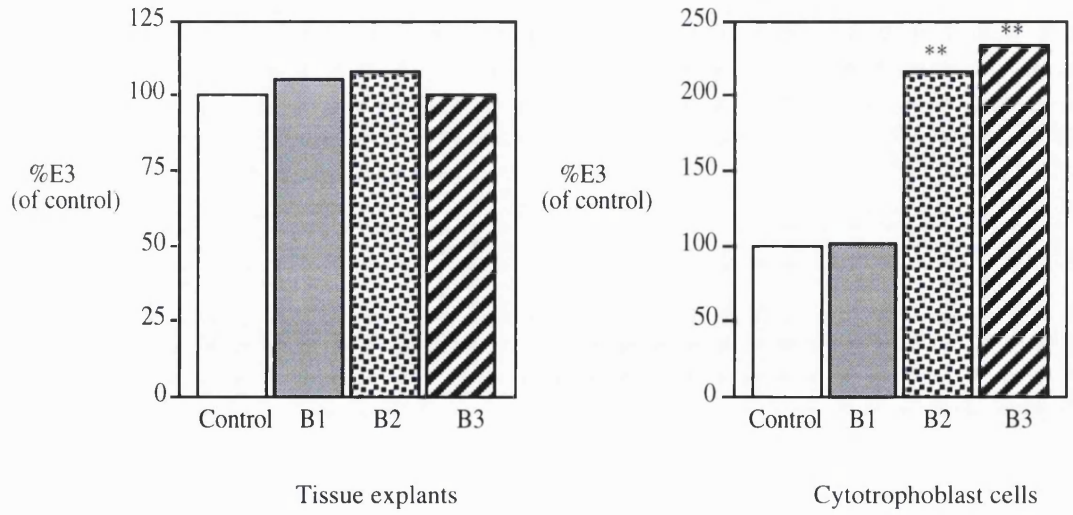


Figure 3.18c. The effects of IGF-II at 20 ng/ml on E3 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

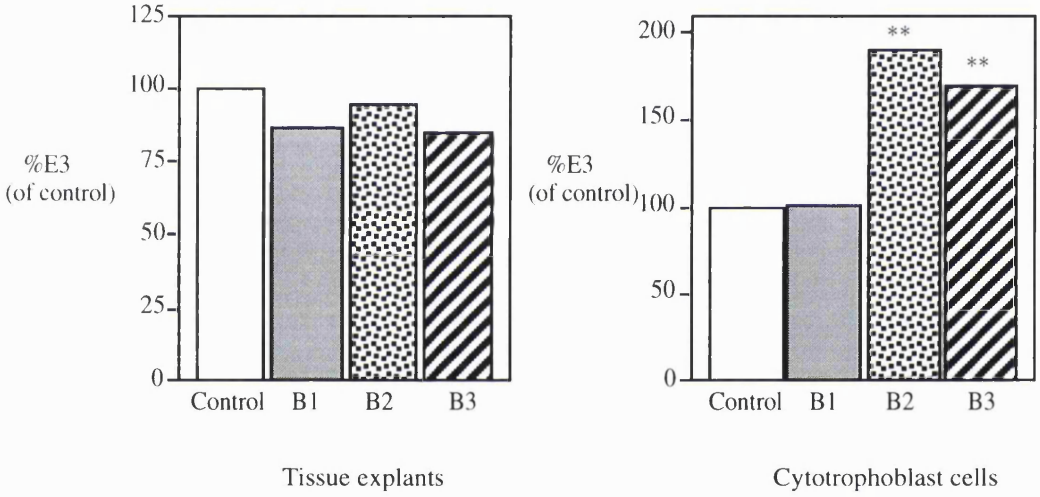


Figure 3.18d. The effects of IGF-II at 400 ng/ml on E3 production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

Placenta	Mean ± SE	Median
B1 Control	4.66 ± 0.08	4.02
IGF-I (20 ng/ml)	4.15 ± 0.56	3.40
IGF-I (400 ng/ml)	4.05 ± 0.43	4.08
IGF-II (20 ng/ml)	4.93 ± 0.29	5.03
IGF-II (400 ng/ml)	4.03 ± 037	3.95
B2 Control	8.89 ± 0.67	8.86
IGF-I (20 ng/ml)	8.48 ± 0.82	8.35
IGF-I (400 ng/ml)	10.73 ± 1.41	10.27
IGF-II (20 ng/ml)	9.61 ± 1.11	9.75
IGF-II (400 ng/ml)	8.42 ± 1.03	7.76
B3 Control	6.06 ± 0.46	5.92
IGF-I (20 ng/ml)	5.06 ± 0.44	4.69
IGF-I (400 ng/ml)	5.66 ± 1.04	4.90
IGF-II (20 ng/ml)	6.10 ± 0.82	6.15
IGF-II (400 ng/ml)	5.17 ± 0.55	4.99

Table 3.18. Mean ± SE and median values for E3 levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with 16α-OHA or 16α-OHA and IGF-I or IGF-II.

3.19. The effects of IGF-I on P production by placental tissue explants

There were no significant differences in P levels produced by tissue incubated with IGF-I (20 ng/ml) compared to those of the controls (incubated in DMEM alone) in the three placentae studied with the exception of placenta A3 which had significantly lower levels at just one time point ($p < 0.03$) which can be seen in Figure 3.19.1.

When minces were incubated with IGF-I at 400 ng/ml there was no significant differences from control values in two of the placentae studied, whereas in the third placenta (A4) there was a significant decrease in P produced when IGF-I was present at various time points, as can be seen in figure 3.19.2 ($t = 40, 60, 100, 120, 150$ m, $p < 0.05$; $t = 80$ m, $p < 0.02$). This significant decrease was not present at 24 hours.

The mean ± SE and median values for P levels produced by tissue explants incubated with/without IGF-I at 20 ng/ml and 400 ng/ml are shown in tables 3.19.1 and 3.19.2 respectively.

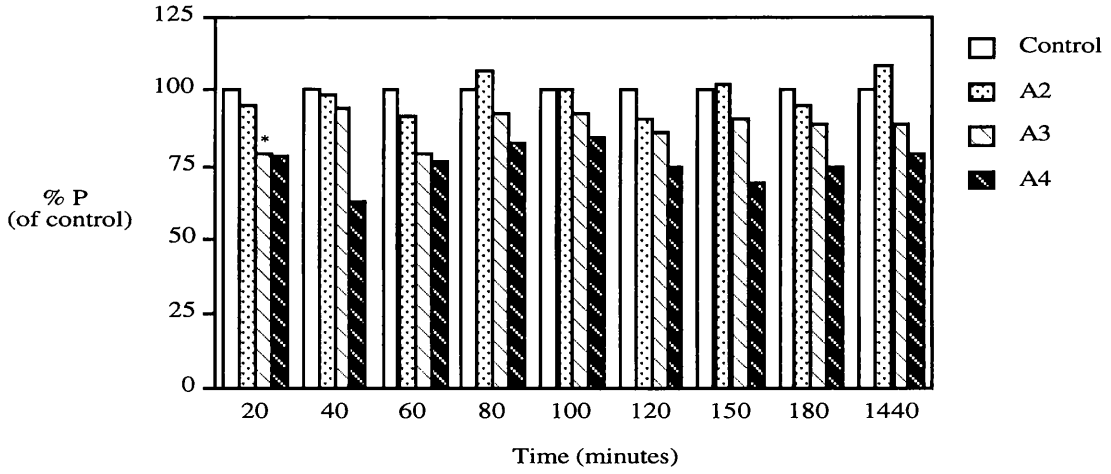


Figure 3.19.1. The effects of IGF-I at 20 ng/ml on P production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

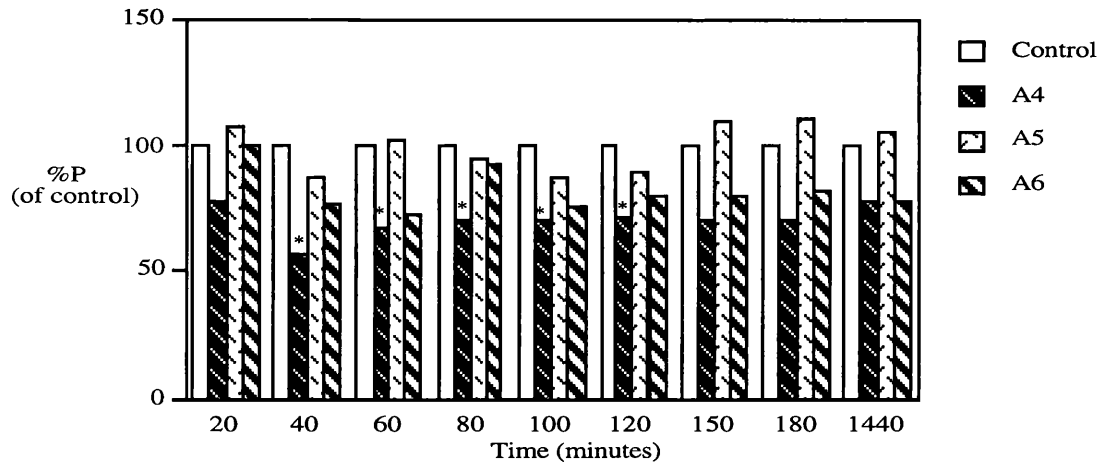


Figure 3.19.2. The effect of IGF-I at 400 ng/ml on P production by tissue explants obtained from three placentae compared to controls incubated with DMEM alone.

Time (minutes)		Placenta A2 Control	Placenta A2 IGF-I	Placenta A3 Control	Placenta A3 IGF-I	Placenta A4 Control	Placenta A4 IGF-I
20	mean ± SE	58.58 ± 2.13	55.82 ± 2.48	23.76 ± 1.07	18.78 ± 1.34	32.74 ± 5.34	27.40 ± 1.88
	median	57.33	62.36	24.78	19.43	29.32	25.76
40	mean ± SE	69.42 ± 4.58	68.42 ± 4.10	16.40 ± 1.43	15.46 ± 0.15	43.64 ± 5.06	27.31 ± 2.58
	median	69.48	71.26	17.21	15.17	45.41	30.72
60	mean ± SE	66.20 ± 7.09	60.53 ± 5.76	24.19 ± 2.24	19.20 ± 3.40	43.75 ± 5.25	33.67 ± 3.02
	median	66.27	58.35	24.26	21.31	42.48	37.46
80	mean ± SE	63.39 ± 4.71	67.70 ± 5.82	25.02 ± 1.05	23.30 ± 1.97	52.21 ± 4.00	43.19 ± 4.07
	median	62.71	63.09	24.74	23.95	49.42	42.17
100	mean ± SE	71.14 ± 5.76	71.44 ± 6.07	27.22 ± 2.35	25.33 ± 2.39	56.26 ± 4.54	47.48 ± 6.20
	median	74.51	67.35	25.27	25.22	56.92	44.17
120	mean ± SE	76.68 ± 5.63	69.72 ± 5.22	31.22 ± 2.73	27.15 ± 2.16	56.16 ± 6.14	41.94 ± 4.01
	median	77.24	64.68	31.79	27.63	57.18	39.97
150	mean ± SE	71.36 ± 4.93	72.94 ± 6.39	30.73 ± 1.88	27.70 ± 2.13	52.23 ± 7.06	42.54 ± 5.18
	median	69.89	67.67	29.91	26.37	58.19	41.50
180	mean ± SE	80.96 ± 3.69	77.38 ± 5.76	37.27 ± 4.10	33.15 ± 3.56	61.66 ± 6.81	45.87 ± 4.84
	median	80.42	71.64	34.88	29.89	57.27	42.80
1440	mean ± SE	81.78 ± 5.41	88.63 ± 5.76	33.22 ± 3.08	29.67 ± 1.84	62.11 ± 8.97	48.79 ± 4.77
	median	77.88	86.81	32.42	30.59	59.69	50.31

Table 3.19.1. Mean ± SE and median values for P levels (pmol/mg protein) produced by placental tissue explants incubated with/without IGF-I at 20 ng/ml for 24 hours.

Time (minutes)		Placenta A4 Control	Placenta A4 IGF-I	Placenta A5 Control	Placenta A5 IGF-I	Placenta A6 Control	Placenta A6 IGF-I
20	mean ± SE	32.75 ± 5.34	25.51 ± 3.18	34.31 ± 6.36	36.76 ± 4.32	73.71 ± 6.36	73.30 ± 5.28
	median	29.32	23.56	30.50	35.84	72.41	72.09
40	mean ± SE	43.63 ± 5.06	24.80 ± 3.40	86.36 ± 17.78	75.62 ± 20.26	110.22 ± 5.77	84.62 ± 9.31
	median	45.41	23.56	89.93	35.84	106.37	72.09
60	mean ± SE	43.76 ± 5.25	29.61 ± 2.70	91.23 ± 25.03	93.78 ± 23.85	141.67 ± 18.83	102.14 ± 7.22
	median	42.48	28.46	75.05	80.23	127.11	102.30
80	mean ± SE	52.22 ± 4.00	36.54 ± 3.24	110.60 ± 36.54	104.46 ± 26.14	132.86 ± 21.27	123.38 ± 9.02
	median	49.42	36.25	77.78	88.02	119.98	126.56
100	mean ± SE	56.25 ± 4.54	39.88 ± 6.26	89.58 ± 23.63	78.36 ± 11.07	137.44 ± 17.84	104.53 ± 9.00
	median	56.92	35.74	76.00	71.26	125.71	101.70
120	mean ± SE	56.16 ± 6.14	40.23 ± 4.23	84.11 ± 15.30	75.43 ± 12.66	167.52 ± 24.14	134.90 ± 12.24
	median	57.18	36.38	68.18	65.70	151.62	134.86
150	mean ± SE	62.23 ± 7.06	43.66 ± 6.68	85.38 ± 15.53	94.16 ± 18.89	149.94 ± 17.39	119.89 ± 7.79
	median	58.19	39.05	69.96	77.27	150.41	118.07
180	mean ± SE	61.66 ± 6.81	43.70 ± 6.11	90.09 ± 19.02	100.11 ± 18.44	159.76 ± 20.92	131.43 ± 11.86
	median	57.27	39.97	73.33	97.12	155.03	141.03
1440	mean ± SE	62.11 ± 8.97	48.59 ± 5.15	95.21 ± 15.90	100.58 ± 19.37	172.07 ± 17.30	135.01 ± 10.46
	median	59.69	44.58	79.02	86.43	175.25	132.19

Table 3.19.2. Mean ± SE and median values for P levels (pmol/mg protein) produced by placental tissue explants incubated with/without IGF-I at 400 ng/ml for 24 hours.

3.20.1. The effect of IGF-I on P production by isolated cytotrophoblast cells

There were no significant differences in P levels produced by cytotrophoblast cells incubated with IGF-I at both 20 ng/ml and 400 ng/ml in six of the seven placentae studied. However levels produced by placenta B4 were significantly lower when cells were incubated with IGF-I at 20 ng/ml ($p < 0.005$) and IGF-I at 400 ng/ml ($p < 0.05$) as illustrated in figures 3.20.1a and 3.20.1b. The mean ± SE and median values for P levels produced by cells incubated with pregnenolone alone or with the addition of IGF-I are shown in table 3.20a-c .

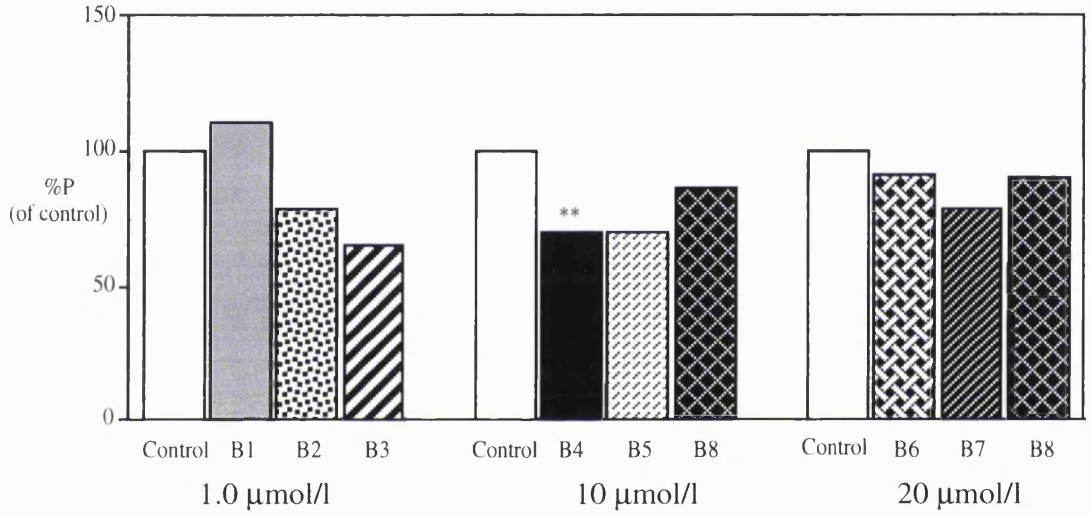


Figure 3.20.1a. The effect of IGF-I at 20 ng/ml on P production by cytotrophoblast cells isolated from seven placentae and incubated with various concentrations of pregnenolone.

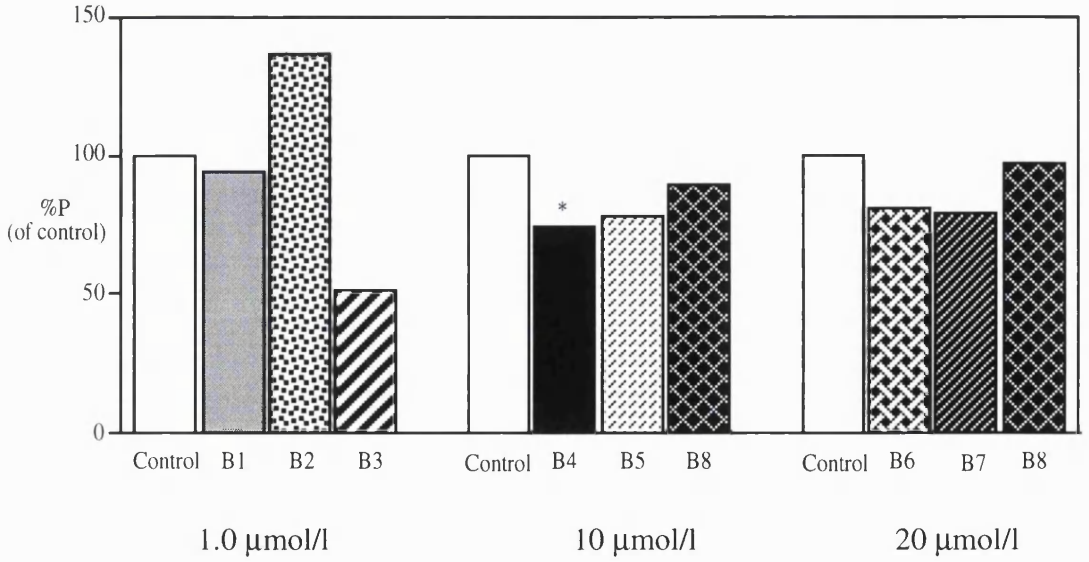


Figure 3.20.1b. The effect of IGF-I at 400 ng/ml on P production by cytotrophoblast cells isolated from seven placentae and incubated with various concentrations of pregnenolone.

3.20.2 The effects of IGF-II on P production by isolated cytotrophoblasts

On incubation with IGF-II (20 ng/ml) there was no significant difference in levels of P as compared to those of the controls in seven of the eight placentae studied whereas the levels produced by placenta B4 were significantly lower than those of the controls ($p < 0.005$, see figure 3.20.2a). When cells were incubated with IGF-II at a concentration of 400 ng/ml P levels were not significantly different to controls in four placentae. However P levels produced by cell preparations obtained from B4 and B8 incubated with IGF-II at 400 ng/ml and 10 μmol/l pregnenolone, were significantly lower than controls ($p < 0.005$, $p < 0.04$ respectively). When the cells were incubated with IGF-II at 400 ng/ml and 20 μmol/l pregnenolone, levels obtained from placenta B6 were significantly higher ($p < 0.005$, see figure 3.20.2b) but those levels produced by placenta B7 were significantly lower ($p \geq 0.01$) than those of controls.

The mean ± SE and median values for P levels produced by cells incubated with IGF-II are in table 3.20.2a-c.

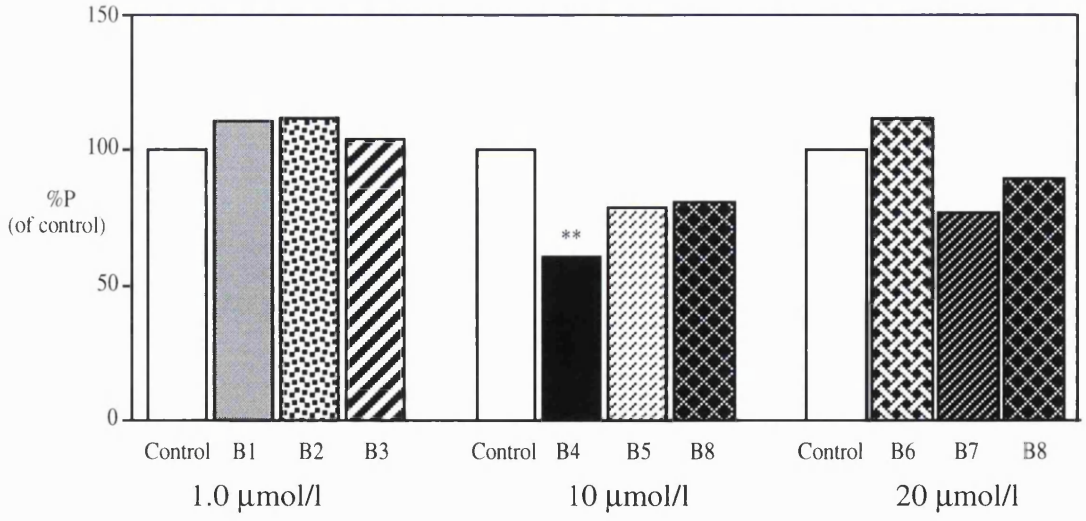


Figure 3.20.2a. The effects of IGF-II at 20 ng/ml on P production by cytotrophoblast cells isolated from seven placentae and incubated with various concentrations of pregnenolone.

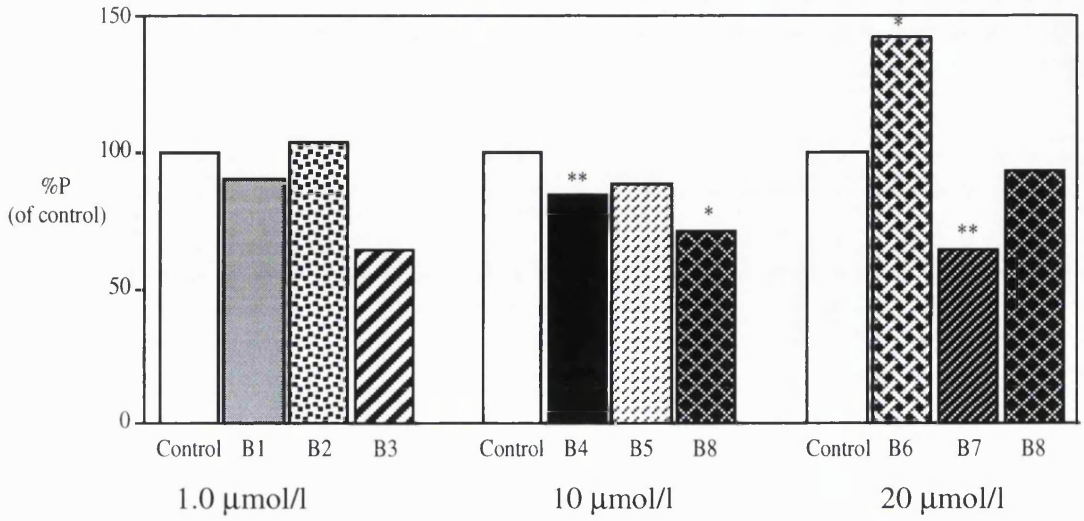


Figure 3.20.2b. The effects of IGF-II at 400 ng/ml on P production by cytotrophoblast cells isolated from seven placentae and incubated with various concentrations of pregnenolone.

Placenta		Mean ± SE	Median
B1	Control	44.44 ± 2.95	45.16
	IGF-I (20 ng/ml)	48.84 ± 4.70	62.42
	IGF-I (400 ng/ml)	41.61 ± 2.01	41.66
	IGF-II (20 ng/ml)	49.02 ± 5.56	51.59
	IGF-II (400 ng/ml)	39.92 ± 3.57	35.67
B2	Control	35.19 ± 4.08	29.34
	IGF-I (20 ng/ml)	27.47 ± 2.56	24.68
	IGF-I (400 ng/ml)	47.96 ± 13.84	27.18
	IGF-II (20 ng/ml)	39.53 ± 6.95	38.83
	IGF-II (400 ng/ml)	36.56 ± 6.83	31.71
B3	Control	33.46 ± 4.66	34.24
	IGF-I (20 ng/ml)	21.81 ± 4.19	18.18
	IGF-I (400 ng/ml)	17.34 ± 3.57	16.64
	IGF-II (20 ng/ml)	34.84 ± 5.71	29.99
	IGF-II (400 ng/ml)	21.09 ± 4.30	16.64

Table 3.20a. Mean ± SE and median values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with 1.0 µmol/l pregnenolone alone (control) or pregnenolone and IGF-I/IGF-II.

Placenta		Mean ± SE	Median
B4	Control	311.35 ± 6.85	311.30
	IGF-I (20 ng/ml)	217.50 ± 13.67	228.17
	IGF-I (400 ng/ml)	230.75 ± 22.22	230.75
	IGF-II (20 ng/ml)	189.01 ± 20.28	201.48
	IGF-II (400 ng/ml)	264.43 ± 9.94	260.77
B5	Control	160.65 ± 19.84	156.11
	IGF-I (20 ng/ml)	111.92 ± 3.29	113.07
	IGF-I (400 ng/ml)	125.66 ± 9.35	131.80
	IGF-II (20 ng/ml)	126.33 ± 23.85	139.47
	IGF-II (400 ng/ml)	142.84 ± 14.00	141.16
B8	Control	306.13 ± 23.22	314.90
	IGF-I (20 ng/ml)	264.98 ± 18.73	274.53
	IGF-I (400 ng/ml)	273.03 ± 36.77	276.12
	IGF-II (20 ng/ml)	247.58 ± 27.08	232.00
	IGF-II (400 ng/ml)	217.48 ± 16.04	226.43

Table 3.20b. Mean ±SE and median values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with 10 μmol /l pregnenolone alone (control) or pregnenolone and IGF-I/IGF-II.

Placenta		Mean ± SE	Median
B6	Control	272.55 ± 24.33	266.30
	IGF-I (20 ng/ml)	250.43 ± 10.70	259.52
	IGF-I (400 ng/ml)	219.95 ± 18.57	229.07
	IGF-II (20 ng/ml)	304.63 ± 20.89	308.21
	IGF-II (400 ng/ml)	388.75 ± 19.61	400.67
B7	Control	932.56 ± 62.20	905.79
	IGF-I (20 ng/ml)	731.06 ± 119.60	721.97
	IGF-I (400 ng/ml)	734.89 ± 120.86	718.47
	IGF-II (20 ng/ml)	712.29 ± 120.30	706.82
	IGF-II (400 ng/ml)	598.40 ± 68.67	581.99
B8	Control	211.59 ± 26.29	206.29
	IGF-I (20 ng/ml)	190.06 ± 14.33	186.21
	IGF-I (400 ng/ml)	204.36 ± 22.65	190.15
	IGF-II (20 ng/ml)	189.34 ± 17.76	190.75
	IGF-II (400 ng/ml)	197.02 ± 14.62	195.00

Table 3.20c. Mean ± SE and median values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with 20 μmol/l pregnenolone alone (control) or pregnenolone and IGF-I/IGF-II.

3.21 The effects of IGF-I and IGF-II on P levels produced by tissue explants compared to cells isolated from the same three placentae

The addition of IGF-I at 20 ng/ml or 400 ng/ml had no significant effect on P levels produced by either isolated cell preparations or tissue minces from three placentae B1, B2, B3, which were incubated with 1 µmol/l pregnenolone. Figures 3.21.1 and 3.21.2 show the effects of IGF-I on both tissue minces and cytotrophoblast cells obtained from these placentae.

Similarly in the isolated cell preparations the addition of IGF-II at 20 ng/ml had no effect on P levels produced by tissue minces from two placentae. However, one placenta, B1, showed significantly raised P levels ($p < 0.05$) although no significant effect was observed from cells isolated from this same placentae. There were no significant differences in P levels produced by minces in any of the three placentae incubated with IGF-II at a concentration of 400 ng/ml compared with controls. Figures 3.21.3 and 3.21.4 show the effects IGF-II on P produced by both tissue minces and cytotrophoblast cells obtained from three placentae.

The means ± SE and median values for these P levels and those of the controls are given in table 3.21.

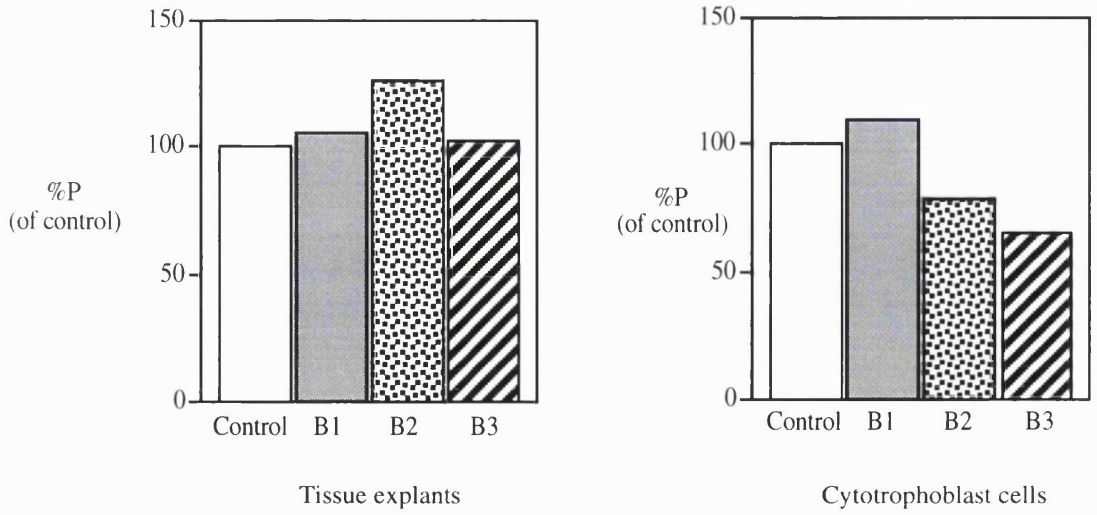


Figure 3.21.1. The effect of IGF-I at 20 ng/ml on P production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

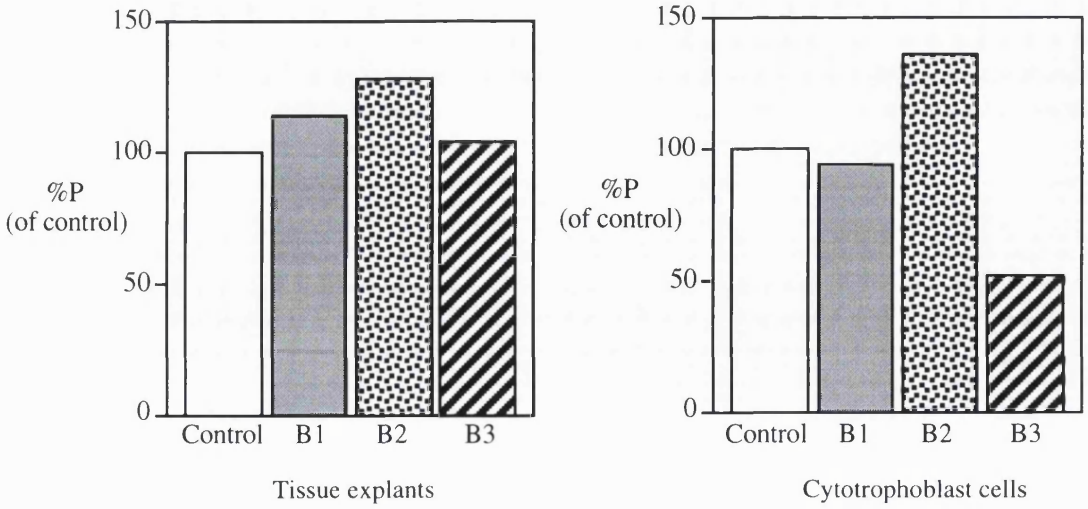


Figure 3.21.2. The effect of IGF-I at 400 ng/ml on P production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

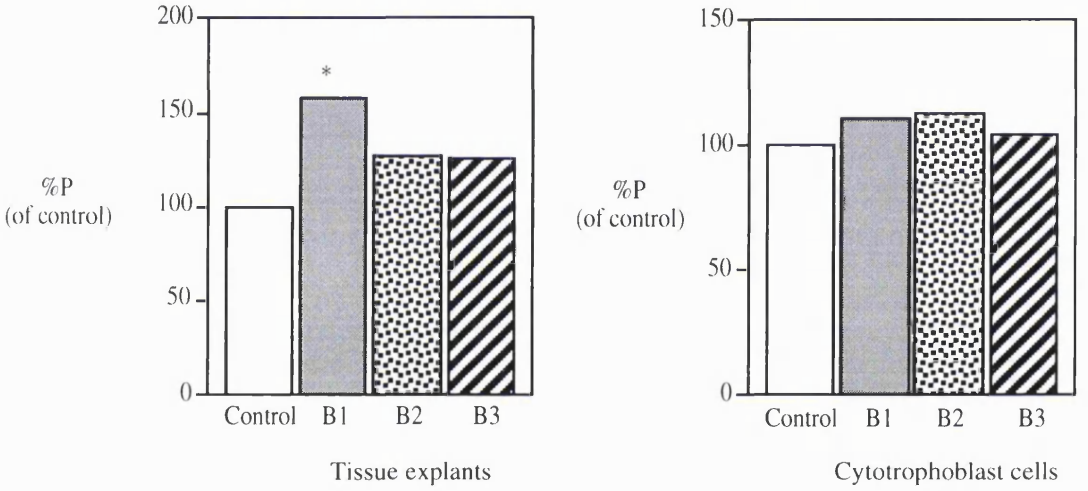


Figure 3.21.3. The effect of IGF-II at 20 ng/ml on P production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

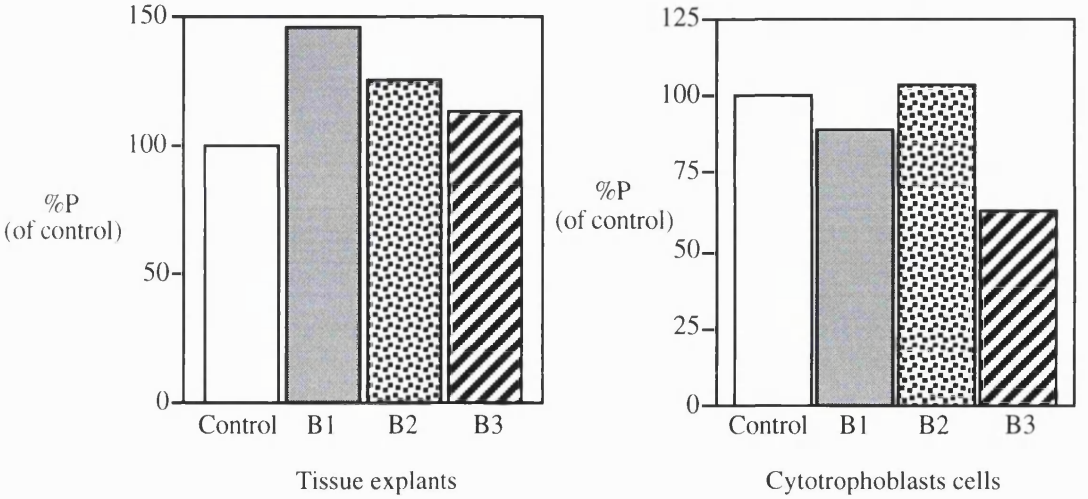


Figure 3.21.4. The effect of IGF-II at 400 ng/ml on P production by tissue explants and isolated cytotrophoblast cells obtained from three placentae.

Placenta	Mean ± SE	Median
B1 Control	54.25 ± 7.47	47.83
IGF-I (20 ng/ml)	57.30 ± 8.68	51.99
IGF-I (400 ng/ml)	62.08 ± 7.63	63.85
IGF-II (20 ng/ml)	85.97 ± 11.23	83.38
IGF-II (400 ng/ml)	79.54 ± 9.22	84.59
B2 Control	133.38 ± 14.82	113.91
IGF-I (20 ng/ml)	169.28 ± 25.34	150.64
IGF-I (400 ng/ml)	170.75 ± 30.31	172.67
IGF-II (20 ng/ml)	169.90 ± 30.59	138.11
IGF-II (400 ng/ml)	167.82 ± 27.22	146.06
B3 Control	91.92 ± 5.83	93.21
IGF-I (20 ng/ml)	93.59 ± 21.88	71.42
IGF-I (400 ng/ml)	94.90 ± 7.35	98.99
IGF-II (20 ng/ml)	115.70 ± 14.69	114.10
IGF-II (400 ng/ml)	104.38 ± 9.29	96.93

Table 3.21. Mean ± SE and median values for P levels (pmol/mg protein/24 hours) produced by placental tissue explants incubated with 1 μmol/l pregnenolone alone (control) or pregnenolone and IGF-I/IGF-II.

3.22. The effects of the addition of NADPH on steroid production by placental tissue explants

The production of E1 was measured in experiments on minces from three placentae. Oestrone levels were not affected by the addition of NADPH in two placentae but were significantly raised in placenta A3 at two time points as compared to those incubated with DMEM alone (t = 150 minutes, $p < 0.05$; t 180 minutes, $p \leq 0.01$, see figure 3.22.1).

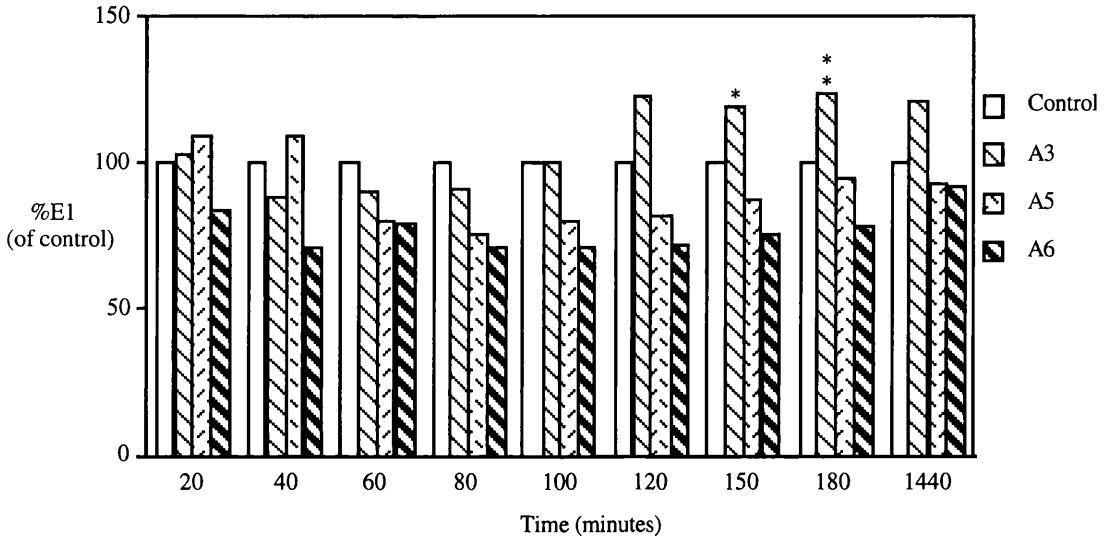


Figure 3.22.1. Production of E1 by tissue explants (obtained from three individual placentae) incubated with NADPH compared to controls incubated with DMEM alone.

The addition of NADPH to placental explants stimulated E2 production significantly in four of the five placentae studied at various time points throughout the incubatory period. However E2 levels were not significantly different (although the levels were raised at most time points) to control levels for minced preparations obtained from placentae A5 (see figure 3.22.2).

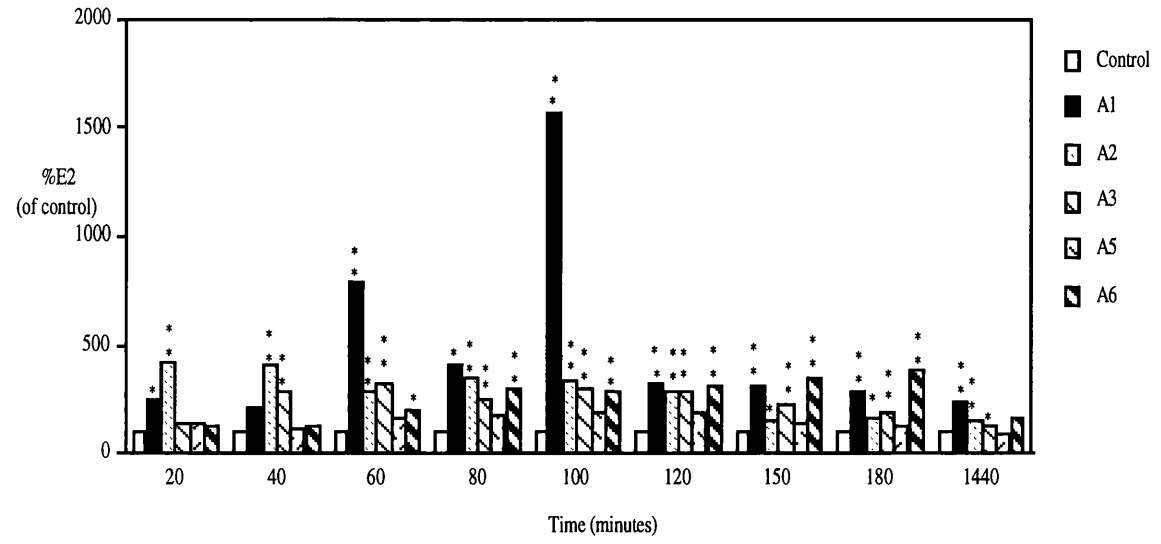


Figure 3.22.2. Production of E2 by tissue explants (obtained from five individual placentae) incubated with NADPH compared to controls incubated with DMEM alone.

Levels of E3 in all five placentae were not affected by the addition of NADPH with the exception of one time point in placenta A3 (t = 80 m, $p < 0.05$) as shown in figure 3.22.3.

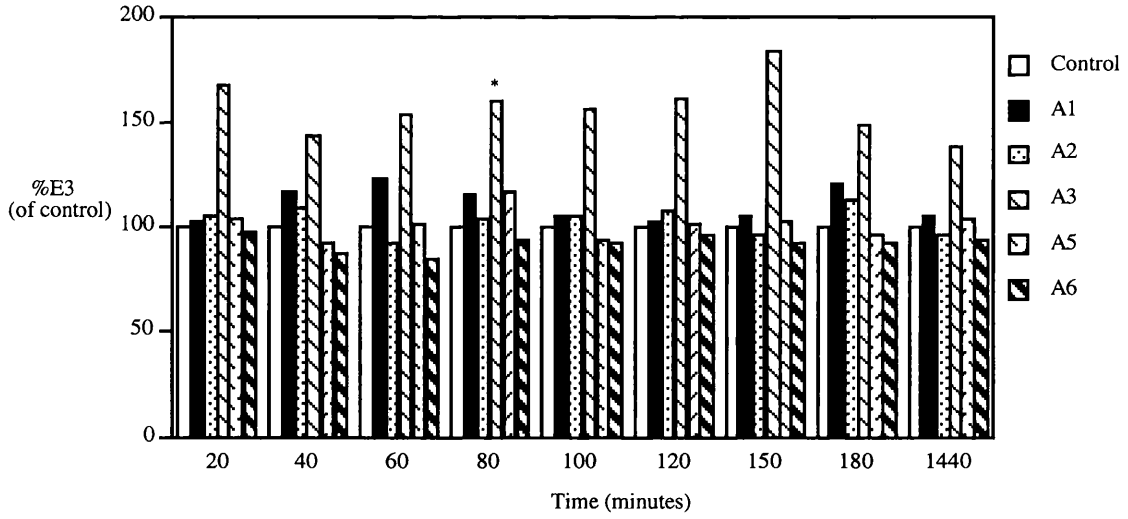


Figure 3.22.3. Production of E3 by tissue explants (obtained from five individual placentae) incubated with NADPH compared to controls incubated with DMEM alone.

Progesterone production was significantly increased in four of the placentae studied compared to the controls following the addition of NADPH. However the addition of NADPH to the fifth placenta A5 had no significant effect on P production although the levels were raised at most time points compared with controls (see figure 3.22.4).

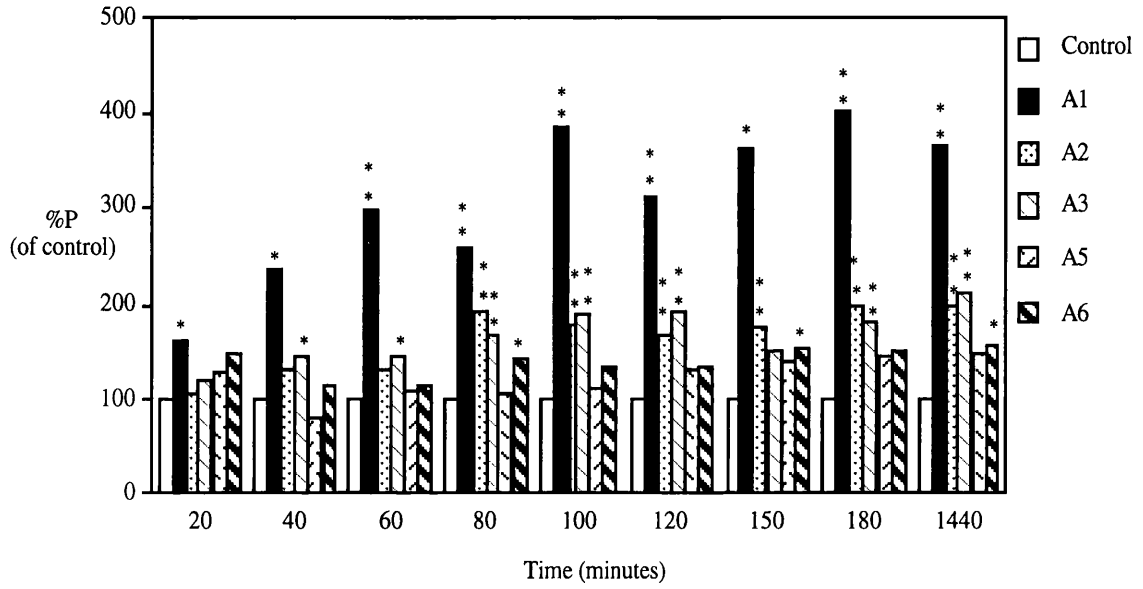


Figure 3.22.4. Production of P by tissue explants (obtained from five individual placentae) incubated with NADPH compared to controls incubated with DMEM alone.

The addition of CRF, ACTH, and IGF-I in the presence of NADPH had no significant effect on the production of E1, E2, E3 or P. The mean ± SE and median values for E1, E2, E3 and P incubated with/without NADPH and NADPH with CRF/ACTH/IGF-I are given in tables 3.22.1-3.22.4 . The p values for those levels of E2 and P which were significantly different are tabulated in table 3.22.5 and 3.22.6 .

Time (minutes)		Placenta A3 Control	Placenta A3 NADPH	Placenta A5 Control	Placenta A5 NADPH	Placenta A6 Control	Placenta A6 NADPH
20	mean ± SE	2.04 ± 0.27	2.11 ± 0.29	2.79 ± 0.37	3.05 ± 0.43	2.86 ± 0.51	2.42 ± 0.34
	median	1.87	2.20	2.35	2.67	2.56	2.42
40	mean ± SE	1.61 ± 0.17	1.42 ± 0.12	2.80 ± 0.58	3.08 ± 0.67	4.13 ± 0.74	2.95 ± 0.73
	median	1.62	1.45	2.42	2.53	3.50	3.12
60	mean ± SE	2.21 ± 0.16	2.00 ± 0.13	3.66 ± 0.50	2.93 ± 0.70	4.04 ± 0.51	3.21 ± 0.38
	median	2.03	2.01	3.33	2.50	3.79	2.92
80	mean ± SE	2.43 ± 0.42	2.21 ± 0.49	3.38 ± 0.35	2.56 ± 0.48	4.84 ± 0.82	3.45 ± 0.50
	median	2.33	1.85	3.12	2.25	4.50	3.41
100	mean ± SE	2.83 ± 0.22	2.85 ± 0.23	3.79 ± 0.52	3.07 ± 0.68	4.55 ± 0.63	3.25 ± 0.34
	median	2.76	2.60	3.51	2.51	4.23	3.02
120	mean ± SE	2.00 ± 0.19	2.47 ± 0.24	3.79 ± 0.04	3.12 ± 5.56	4.84 ± 0.49	3.52 ± 0.36
	median	1.98	2.66	3.53	2.71	4.71	3.29
150	mean ± SE	2.22 ± 0.12	2.66 ± 0.13	4.16 ± 0.59	3.65 ± 0.43	4.95 ± 0.18	3.76 ± 0.33
	median	2.33	2.74	3.67	3.42	4.54	3.40
180	mean ± SE	2.29 ± 0.08	2.83 ± 0.14	4.10 ± 0.46	3.89 ± 0.51	5.09 ± 0.58	4.07 ± 0.29
	median	2.27	2.71	3.69	3.76	4.94	4.03
1440	mean ± SE	2.66 ± 0.11	3.22 ± 0.37	4.76 ± 0.47	4.45 ± 0.45	5.95 ± 0.91	5.50 ± 0.64
	median	2.62	2.96	4.25	4.20	5.74	5.36

Table 3.22.1. Mean ± SE and median values for E1 levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH for 24 hours..

	20 minutes	40 minutes	60minutes	80 minutes	100 minutes	120 minutes	150 minutes	180 minutes	1440 minutes
A1 Control	1.43 ± 0.44	0.73 ± 0.14	0.60 ± 0.07	1.07 ± 0.42	0.65 ± 0.09	0.62 ± 0.05	0.73 ± 0.07	0.73 ± 0.07	0.73 ± 0.10
A1 NADPH	3.42 ± 0.59	1.51 ± 0.55	4.69 ± 0.69	4.29 ± 0.87	1.01 ±2.05	2.03 ± 0.52	2.20 ± 0.14	2.02 ± 1.70	1.70 ± 0.16
A1 NADPH+ACTH	3.64 ± 0.76	0.93 ± 0.22	5.76 ± 0.58	3.73 ± 0.61	4.18 ± 1.27	1.52 ± 0.29	2.08 ± 0.30	2.11 ± 0.45	1.74 ± 0.34
A2 Control	0.28 ± 0.05	0.26 ± 0.04	0.30 ± 0.02	0.25 ± 0.02	0.29 ± 0.02	0.38 ± 0.05	0.31 ± 0.02	0.30 ± 0.02	0.32 ± 0.02
A2 NADPH	1.17 ± 0.17	1.08 ± 0.10	0.82 ± 0.06	0.87 ± 0.09	0.95 ± 0.10	1.11 ± 0.16	0.48 ± 0.05	0.48 ± 0.09	0.50 ± 0.03
A2 CRF+NADPH	0.99 ± 0.15	1.02 ± 0.11	0.73 ± 0.07	0.73 ± 0.08	1.05 ± 0.08	1.30 ± 0.06	0.48 ± 0.20	0.51 ± 0.06	0.48 ± 0.03
A2ACTH+NADPH	0.79 ± 0.10	1.01 ± 0.15	0.75 ± 0.13	0.80 ± 0.11	0.86 ± 0.12	1.21 ± 0.10	0.43 ± 0.31	0.53 ± 0.12	0.47 ± 0.04
A2 IGF+NADPH	1.26 ± 0.85	1.02 ± 0.89	0.82 ± 0.08	0.87 ± 0.09	1.12 ± 0.03	1.12 ± 0.18	0.51 ± 0.05	0.65 ± 0.11	0.51 ± 0.03
A3 Control	1.12 ± 0.13	0.58 ± 0.05	0.56 ± 0.02	0.74 ± 0.13	0.59 ± 0.03	0.65 ± 0.02	0.58 ± 0.02	0.56 ± 0.04	0.82 ± 0.04
A3 NADPH	1.51 ± 0.10	1.61 ± 0.16	1.80 ± 0.15	1.78 ± 0.13	1.75 ± 0.17	1.86 ± 0.11	1.29 ± 0.17	1.08 ± 0.13	0.99 ± 0.05
A3 NADPH+CRF	1.63 ± 0.34	1.76 ± 0.26	1.48 ± 0.14	1.84 ± 0.27	1.58 ± 0.20	1.55 ± 0.18	1.11 ± 0.12	0.81 ± 0.10	0.86 ± 0.13
A3 NADPH+ACTH	1.13 ± 0.20	1.52 ± 0.18	1.56 ± 0.15	1.36 ± 0.19	1.41 ± 0.11	1.63 ± 0.19	0.91 ± 0.15	0.83 ± 0.07	0.86 ± 0.09
A3 NADPH+IGF-I	1.21 ± 0.07	1.24 ± 0.12	1.43 ± 0.05	1.51 ± 0.08	1.14 ± 0.09	1.57 ± 0.15	0.94 ± 0.12	0.74 ± 0.03	0.84 ± 0.02
A4 Control	0.92 ± 0.92	0.84 ± 0.15	0.79 ± 0.14	0.83 ± 0.14	0.87 ± 0.09	1.16 ± 0.14	1.14 ± 0.24	0.76 ± 0.09	0.95 ± 0.08
A4 NADPH+CRF	2.42 ± 0.48	2.32 ± 5.97	1.68 ± 0.33	1.57 ± 0.33	1.17 ± 0.22	1.29 ± 0.26	1.28 ± 0.25	1.01 ± 0.20	1.19 ± 0.21
A4 NADPH+IGF-I	1.64 ± 0.38	2.19 ± 0.60	1.26 ± 0.32	1.02 ± 0.16	0.99 ± 0.23	1.33 ± 0.40	1.46 ± 0.36	1.07 ± 0.24	1.08 ± 0.30
A5 Control	1.92 ± 0.22	1.84 ± 0.35	1.11 ± 0.18	0.88 ± 0.11	0.88 ± 0.17	1.05 ± 0.19	1.06 ± 0.15	1.26 ± 0.24	1.32 ± 0.12
A5 NADPH	2.49 ± 0.529	2.16 ± 0.42	1.74 ± 0.40	1.50 ± 0.33	1.69 ± 0.47	1.87 ± 0.48	1.46 ± 0.33	1.53 ± 0.39	1.22 ± 0.17
A6 Control	3.88 ± 0.09	4.36 ± 0.68	3.21 ± 0.55	1.97 ± 0.24	2.45 ± 0.47	2.32 ± 0.35	2.03 ± 0.27	2.08 ± 0.33	1.95 ± 0.28
A6 NADPH	4.97 ± 0.73	5.60 ± 0.48	6.28 ± 6.66	5.76 ± 0.55	6.83 ± 0.78	7.03 ± 0.66	6.92 ± 1.00	7.84 ± 0.84	3.08 ± 0.33

Table 3.22.2a. Mean ± SE values for E2 levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH or NADPH in combination with CRF (100nmol/l)/ACTH (10nmol/l)/IGF-I (20 ng/ml) for 24 hours.

Placenta	20 minutes	40 minutes	60 minutes	80 minutes	100 minutes	120 minutes	150 minutes	180 minutes	1440 minutes
A1 Control	1.22	0.59	0.59	0.59	0.55	0.61	0.67	0.73	0.68
A1 NADPH	3.52	1.01	4.72	4.24	8.64	1.65	2.25	1.62	1.64
A1 NADPH+ACTH	3.21	0.88	5.21	4.20	3.09	1.38	1.96	1.92	1.51
A2 Control	0.27	0.24	0.29	0.25	0.28	0.34	0.31	0.30	0.33
A2 NADPH	1.32	1.12	0.80	0.88	1.03	1.12	0.43	0.43	0.51
A2 NADPH+CRF	0.97	1.01	0.67	0.71	0.95	1.36	0.43	0.47	0.44
A2 NADPH+ACTH	0.79	0.89	0.64	0.83	0.93	1.30	0.41	0.44	0.45
A2 NADPH+IGF-I	1.34	1.02	0.77	0.90	1.11	1.21	0.52	0.63	0.50
A3 Control	1.14	0.57	0.56	0.65	0.57	0.64	0.58	0.53	0.83
A3 NADPH	1.44	1.62	1.61	1.73	1.74	1.78	1.23	0.98	0.98
A3 NADPH+CRF	1.30	1.62	1.54	1.76	1.61	1.47	1.05	0.82	0.83
A3 NADPH+ACTH	1.19	0.00	1.75	1.32	1.41	1.48	0.84	0.82	0.83
A3 NADPH+IGF-I	1.20	1.27	1.40	1.52	1.11	1.47	0.89	0.75	0.84
A4 Control	0.91	0.68	0.71	0.76	0.78	1.16	1.00	0.71	0.96
A4 NADPH+CRF	2.24	2.03	1.58	1.51	1.02	1.19	1.15	0.88	1.19
A4 NADPH+IGF-I	1.30	1.64	1.00	1.03	0.79	0.99	1.12	8.62	0.82
A5 Control	1.91	1.81	0.95	0.77	0.75	0.96	0.92	1.08	1.19
A5 NADPH	2.22	1.67	1.34	1.46	1.51	1.66	1.15	1.06	1.14
A6 Control	3.85	4.96	2.94	1.80	1.86	1.88	1.73	1.74	1.69
A6 NADPH	5.02	5.43	5.62	5.04	5.91	6.36	5.64	7.55	2.70

Table 3.22.2b. Median values for E2 levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH or NADPH in combination with CRF (100nmol/l)/ACTH (10nmol/l)/IGF-I (20 ng/ml) for 24 hours.

Placenta	20minutes	40 minutes	60 minutes	80 minutes	100 minutes	120 minutes	150 minutes	180 minutes	1 440minutes
A1 Control	0.68±0.07	0.61±0.06	0.68±0.07	0.68±0.05	0.82±0.05	0.73 ± 0.07	0.80±0.05	0.85±0.09	1.02±0.09
A1 NADPH	0.70±0.11	0.70±0.89	0.83±0.08	0.78±0.04	0.86±0.05	0.75 ± 0.06	0.83±0.06	1.01±0.16	1.07±0.13
A1 NADPH+ACTH	0.72±0.12	0.65±0.10	0.73±0.81	0.73±0.67	0.82±0.07	0.87 ± 0.14	0.90±0.12	0.98±0.17	1.08±0.15
A2 Control	2.28±0.07	2.06±0.20	2.26±0.23	2.51±0.17	2.54±0.20	2.59 ± 0.14	3.00±0.38	2.54± 0.19	3.14±0.34
A2 NADPH	2.40±0.18	2.24±0.13	2.08±0.10	2.59±0.16	2.65±0.14	2.78 ± 0.18	2.87±0.15	2.84±0.17	3.03±0.35.
A2 NADPH+CRF	2.28±0.16	2.09±0.19	2.50±0.29	2.78±0.08	2.65±0.37	2.83 ± 0.34	3.00±0.16	2.79±0.19	3.35±0.38
A2 NADPH+ACTH	2.18±0.12	2.05± 0.12	2.40±0.21	2.69±0.17	2.71 ±0.19	2.81 ± 0.22	3.05±0.16	2.87±0.23	3.17±0.43.
A2 NADPH+IGF-I	2.51±0.19	2.21±0.14	2.41±0.05	2.90±0.16	2.85±0.17	2.98 ± 0.18	2.98±0.15	3.00±0.18	3.84±0.59.
A3 Control	0.72±0.12	0.89±0.09	0.92±0.03	0.94±0.05	0.92±0.07	0.98 ± 0.05	0.90±0.04	1.29±0.05	1.45±0.11.
A3 NADPH	1.20±0.21	1.26±0.25	1.41±0.25	1.50±0.28	1.43±0.42	1.58 ± 0.26	1.66±0.33	1.91±0.32	1.99±0.31.
A3 NADPH+CRF	0.70±0.19	1.00±0.24	1.16±0.16	1.10±0.14	0.99±0.15	1.09 ± 0.14	1.00±0.16	1.40±0.23	1.50±0.20
A3 NADPH+ACTH	0.81±0.13	0.90±0.08	1.13±0.13	0.98±0.11	0.87±0.13	1.01 ± 0.14	0.95±0.85	1.29±0.14	1.45±0.14
A3 NADPH+IGF-I	0.65± 0.11	0.77±0.06	0.93±0.04	0.88±0.05	0.77±0.05	0.88 ± 0.07	0.81±0.67	1.35±0.24	1.25±0.55
A4 Control	1.86±0.38	2.16±0.26	2.06±0.19	2.12±0.24	2.54±0.25	2.49 ± 0.27	2.28±0.18	2.66±0.27	2.50±0.16
A4 CRF+NADPH	2.08±0.43	2.27±0.39	2.08±0.33	2.19±0.31	2.66 ±0.38	2.48 ± 0.37	2.34±0.38	2.54±0.34	2.65±0.42.
A4 CRF+IGF-I	1.91±0.65	2.30± 0.59	1.91±0.38	2.22±0.37	2.54 ±0.46	2.42 ± 0.49	2.27±0.42	2.64±0.50	2.52±0.37
A5 Control	5.02±0.79	5.36±0.81	5.03±0.41	4.49±0.76	5.87±0.74	5.80 ± 0.90	5.23±0.51	5.63±0.56	6.60±0.62
A5 NADPH	5.19± 1.10	4.93±0.79	5.07±0.79	5.23±0.67	5.51 ±0.76	5.83 ± 0.86	5.31±0.67	5.43±0.63	6.83±0.86
A6 Control	9.96±0.98	13.46±1.52	13.21±1.81	13.96±1.54	14.67±1.21	11.64 ± 1.02	12.75±1.39	15.45±1.82	14.65±2.21
A6 NADPH	9.65±1.05	11.72±1.06	11.05±1.10	12.91±0.70	13.52± 1.07	11.11 ± 0.83	11.77±0.93	14.17±1.33	13.79±0.13

Table 3.22.3a. Mean ± SE values for E3 levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH or NADPH in combination with CRF (100nmol/l)/ACTH (10nmol/l)/IGF-I (20 ng/ml) for 24 hours.

Placenta	20 minutes	40 minutes	60 minutes	80 minutes	100 minutes	120 minutes	150 minutes	180 minutes	1440 minutes
A1 Control	0.63	0.58	0.65	0.70	0.83	0.77	0.82	0.76	1.00
A1 NADPH	0.63	0.65	0.82	0.75	0.86	0.73	0.80	0.89	1.00
A1 NADPH+ACTH	0.68	0.66	0.75	0.78	0.83	0.85	0.93	0.96	1.00
A2 Control	2.33	2.23	2.27	2.55	2.41	2.58	2.69	2.42	3.02
A2 NADPH	2.50	2.39	2.06	2.64	2.61	2.67	2.91	2.82	3.10
A2 NADPH+CRF	2.28	2.01	2.13	2.79	2.82	2.82	2.93	2.76	3.40
A2 NADPH+ACTH	2.12	2.04	2.37	2.69	2.58	2.67	3.05	2.72	3.30
A2 NADPH+IGF-I	2.43	2.14	2.40	2.84	2.77	3.01	2.99	2.79	3.40
A3 Control	0.68	0.83	0.89	0.94	0.91	1.00	0.90	1.25	1.40
A3 NADPH	1.05	1.14	1.12	1.24	1.05	1.39	1.42	1.64	0.19
A3 NADPH+CRF	0.59	0.78	1.10	1.01	0.85	0.99	0.89	1.20	0.13
A3 NADPH+ACTH	0.79	0.99	1.21	0.98	0.85	0.94	1.00	1.38	0.15
A3 NADPH+IGF-I	0.66	0.73	0.91	0.92	0.74	0.91	0.75	1.10	0.12
A4 Control	1.84	2.04	1.99	1.99	2.35	2.35	2.20	2.55	2.57
A4 CRF+NADPH	1.67	1.96	1.94	2.01	2.58	2.28	2.15	2.53	2.30
A4 NADPH+IGF-I	1.55	1.75	1.50	1.83	2.11	2.06	1.99	2.32	2.90
A5 CONTROL	4.51	4.59	4.58	4.69	5.39	5.31	5.12	5.29	6.10
A5 NADPH	4.48	4.20	4.44	4.80	5.00	5.30	4.72	4.62	5.80
A6 Control	9.62	12.89	12.07	12.56	13.79	11.46	12.25	14.36	13.43
A6 NADPH	9.74	11.09	10.45	12.61	12.71	11.66	11.54	13.75	13.00

Table 3.22.3b. Median values for E3 levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH or NADPH in combination with CRF (100nmol/l)/ACTH (10nmol/l)/IGF-I (20 ng/ml) for 24 hours.

	20 minutes	40 minutes	60 minutes	80 minutes	100 minutes	120 minutes	150 minutes	180 minutes	1440 minutes
A1 Control	27.43 ± 4.38	32.41 ± 4.37	28.29 ± 3.35	36.38 ± 6.78	32.70± 5.82	45.38 ± 9.03	39.57 ± 5.06	47.31± 6.80	52.57 ± 4.68
A1 NADPH	43.95 ± 8.52	74.99 ± 14.64	85.05 ± 13.78	93.47 ± 21.22	125.43 ± 31.96	140.37 ± 22.51	143.81 ± 26.00	164.31 ± 20.62	191.24 ± 22.02
A1 NADPH+ACTH	25.18 ± 5.34	48.81 ± 9.38	56.72 ± 10.62	66.77 ± 13.90	82.71 ± 15.96	82.47 ± 15.96	86.59 ± 1 8.73	94.19 ± 18.06	112.68 ± 20.45
A2 Control	58.58 ± 2.13	69.42 ± 4.58	66.20 ± 7.09	63.39 ± 4.71	71.14 ± 5.76	76.68 ± 5.63	71.36 ± 4.90	80.96 ± 3.69	81.78 ± 5.41
A2 NADPH	61.85 ± 5.72	89.63 ± 7.66	88.00 ± 6.14	123.08 ± 12.08	126.16 ± 11.51	127.17 ± 11.42	124.38 ± 8.01	160.66 ± 15.84	164.08 ± 13.42
A2 NADPH+CRF	52.80 ± 4.13	82.15 ± 3.66	93.28 ± 11.64	109.03 ± 8.11	126.73 ± 16.03	113.62 ± 20.00	126.16 ± 16.57	129.70 ± 19.33	131.47 ± 15.33
A2 NADPH+ACTH	51.36 ± 6.58	96.06 ± 9.86	81.99 ± 5.60	95.40 ± 13.74	123.30 ± 11.64	123.34 ± 14.12	132.14 ± 13.17	144.04 ± 15.39	147.58 ± 15.58
A2 NADPH+IGF1	61.63 ± 4.26	88.74 ± 6.20	91.55 ± 9.32	119.02 ± 8.17	135.02 ± 10.88	134.03 ± 9.22	141.09 ± 9.29	146.13 ± 13.32	169.42 ± 16.60
A3 Control	23.76 ± 1.07	16.40 ± 1.43	24.19 ± 2.24	25.02 ± 1.05	27.22 ± 2.35	31.22 ± 2.73	30.73 ± 1.88	37.27 ± 4.10	33.22 ± 3.08
A3 NADPH	28.02 ± 1.59	23.90 ± 1.97	35.03 ± 3.12	41.56 ± 2.19	52.12 ± 3.75	60.04 ± 6.23	53.58 ± 8.74	66.58 ± 6.23	69.79 ± 8.59
A3 NADPH+CRF	27.17 ± 3.66	21.58 ± 2.23	33.73 ± 7.44	38.91 ± 3.53	46.61 ± 6.96	50.20 ± 6.46	55.00 ± 8.74	62.23 ± 9.00	67.96 ± 10.11
A3 NADPH+ACTH	20.73 ± 4.42	21.10 ± 3.08	30.62 ± 2.89	37.88 ± 4.04	39.52 ± 5.66	48.75 ± 6.46	47.48 ± 5.50	58.04 ± 5.02	62.71 ± 6.23
A3 NADPH+IGF-I	21.35 ± 2.61	19.61 ± 8.81	31.20 ± 2.73	35.46 ± 3.28	37.14 ± 4.29	40.76 ± 4.99	45.37 ± 3.37	50.55 ± 3.59	56.79 ± 5.69
A4 Control	32.74 ± 5.34	43.64 ± 5.06	43.75 ± 5.25	52.21 ± 4.00	56.26 ± 4.54	56.16 ± 6.14	62.23 ± 7.06	61.66 ± 6.81	62.11 ± 8.97
A4 NADPH+CRF	39.13 ± 6.74	47.40± 10.56	51.13 ± 11.96	59.15 ± 10.72	80.14 ± 12.91	73.13 ± 15.90	75.89 ± 12.78	82.86 ± 14.85	103.62 ± 19.43
A4 NADPH+IGF-I	33.86 ± 6.20	53.14 ± 25.15	51.76 ± 11.29	66.65 ± 14.60	80.25 ± 21.02	67.36 ± 14.06	70.82 ± 18.03	86.62 ± 23.09	82.03 ± 14.56
A5 Control	34.33 ± 6.36	86.38 ± 17.78	91.26 ± 25.03	110.60 ± 36.54	89.57 ± 23.63	84.10 ± 15.30	85.39 ± 15.30	90.07 ± 19.02	95.20 ± 15.90
A5 NADPH	43.33 ± 8.81	70.02 ± 20.26	98.48 ± 32.40	117.14 ± 41.75	98.86 ± 13.55	110.88 ± 20.45	120.20 ± 23.12	130.38 ± 26.90	139.42 ± 28.05
A6 Control	73.70 ± 6.36	110.21 ± 5.77	141.65 ± 18.83	132.87 ± 21.27	137.43 ± 17.43	167.52 ± 24.14	149.94 ± 17.39	159.76 ± 20.92	172.06 ± 17.30
A6 NADPH	108.93 ± 25.28	124.92 ± 14.98	159.39 ± 16.85	190.58 ± 13.01	186.77 ± 16.66	228.95 ± 22.48	232.41 ± 26.01	237.36 ± 30.27	271.06 ± 31.64

Table 3.22.4a. Mean ± SE values for P levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH or NADPH in combination with CRF (100nmol/l)/ACTH (10nmol/l)/IGF-I (20 ng/ml) for 24 hours.

	20 minutes	40 minutes	60 minutes	80 minutes	100 minutes	120 minutes	150 minutes	180 minutes	1440 minutes
A1 Control	25.73	27.56	25.95	33.67	27.39	37.48	36.94	42.11	49.68
A1 NADPH	35.14	63.60	68.72	75.62	86.72	125.74	115.88	153.75	163.93
A1 NADPH+ACTH	28.30	40.74	50.37	55.84	65.13	78.01	76.73	87.20	94.22
A2 Control	57.33	69.48	66.27	62.71	74.51	77.24	69.89	80.42	77.88
A2 NADPH	61.31	94.41	81.22	127.30	124.12	134.51	128.03	167.75	167.55
A2 NADPH+CRF	50.59	83.25	89.58	105.89	113.56	96.29	116.42	128.63	127.01
A2 NADPH+ACTH	54.60	94.51	81.15	94.32	123.03	106.85	123.07	136.23	136.90
A2 NADPH+IGF-I	58.26	86.53	92.89	121.57	148.92	131.78	140.62	142.81	176.52
A3 Control	24.76	17.21	24.26	24.73	25.27	31.79	29.91	34.88	32.42
A3 NADPH	28.62	23.25	31.51	41.44	48.56	54.22	51.67	66.02	62.65
A3 NADPH+CRF	24.65	20.54	27.25	34.98	39.75	45.79	53.01	53.90	58.73
A3 NADPH+ACTH	21.53	20.77	30.15	36.32	37.27	54.89	44.46	57.49	62.46
A3 NADPH+IGF-I	20.03	19.68	30.15	32.21	33.58	36.70	44.42	50.34	53.77
A4 Control	29.32	45.41	42.48	49.42	56.92	57.18	58.19	57.27	59.69
A4 NADPH+CRF	35.71	40.00	45.12	56.35	77.15	65.41	74.28	75.72	104.15
A4 NADPH+IGF-I	31.29	28.56	41.91	55.01	65.86	56.83	63.00	72.47	66.75
A5 Control	30.50	89.93	75.05	77.78	76.00	68.18	69.96	73.33	79.02
A5 NADPH	37.62	49.19	69.67	73.30	97.15	87.23	101.95	101.22	114.35
A6 Control	72.41	106.37	127.11	119.98	125.71	151.62	150.41	155.03	175.25
A6 NADPH	92.92	115.28	121.64	143.32	132.07	159.00	138.04	144.72	167.33

Table 3.22.4b. Median values for P levels (pmol/mg protein) produced by placental tissue explants incubated with DMEM alone (control) or with DMEM containing NADPH or NADPH in combination with CRF (100nmol/l)/ACTH (10nmol/l)/IGF-I (20 ng/ml) for 24 hours.

Time (minutes)	Placenta A1	Placenta A2	Placenta A3	Placenta A6
20	0.025	0.004	ns	ns
40	ns	0.004	0.004	ns
60	0.004	0.004	0.004	0.016
80	0.010	0.004	0.007	0.004
100	0.004	0.004	0.004	0.004
120	0.004	0.006	0.004	0.004
150	0.004	0.006	0.004	0.004
180	0.004	0.010	0.004	0.004
1440	0.004	0.004	0.040	ns

Table 3.22.5. Probability levels for those placentae which had significantly greater E2 levels than those of the controls on addition of NADPH (ns = not significant).

Time (minutes)	Placenta A1	Placenta A2	Placenta A3	Placenta A6
20	ns	ns	0.037	ns
40	0.016	ns	0.016	ns
60	0.004	ns	0.016	ns
80	0.010	0.004	0.004	0.040
100	0.004	0.004	0.004	ns
120	0.006	0.004	0.004	ns
150	0.040	0.004	ns	0.016
180	0.004	0.004	0.010	ns
1440	0.004	0.004	0.004	0.016

Table 3.22.6. Probability levels for those placentae which had significantly greater P levels than those of the controls on addition of NADPH (ns = not significant).

Discussion

4.1. Tissue versus cytotrophoblast studies as models for studying placental steroid synthesis

4.1.1. Tissue explant studies as models for studying placental function

An apparent advantage of using tissue explants rather than purified cell preparations is that the responses observed by minced tissue may well be more physiological than those of isolated cytotrophoblast cells. Tissue comprises both a mixture of cell types and the extracelluar matrix through which cells can communicate so that the response of cells within tissue as a whole may well offer a greater indication of what actually occurs in vivo.

There are however disadvantages in using tissue explants. It is difficult, for instance, to determine whether steroids which are produced by the placental explants are those that are being newly sythesized during incubation or are being released from pre-formed stores already present within the cells. It is not possible to determine the proportion of cells within the tissue that are actually steroid producing cells and it is also difficult to establish their viability. The fragments used in this study were fairly consistent in size (approximately 2-4mm^3) but it was noticeable that mincing caused the tissue fragments to stick together, probably due to a release of DNA from damaged cells. In static cultures such as those used in this study such clumping might reduce the overall surface area exposed to the culture medium. This might not only affect the viability of the cells at the core of the tissue (which might not receive nutrients or other factors required to sustain them) but could also reduce the chances of any substrate such as steroid precursors, or peptides such as CRF, ACTH, IGF-I or IGF-II added to the medium reaching a particular cell type on which they might have a regulatory effect. Furthermore uptake of oxygen and release of carbon dioxide by the tissue is likely to be compromised.

4.1.2. Purified and isolated cells as models for studying placental function

A system consisting of purified cytotrophoblast cells allows a relatively homogeneous population of cells to be isolated so that a fairly consistent and uniform number of the same cell type can be plated and studied. It is also far easier to determine the viablilty of isolated cells than that of tissue fragments. Percoll gradient centrifugation has been shown by Nestler (1987) to give greater than 95% purity of cytotrophoblast cells. The viability of cells isolated in this study was consistently greater than 90%. The major disadvantage of using purified cell lines is that they may not behave in the same manner as they would in vivo once they have been removed from their tissue matrix and their surrounding normal physiological environment.

4.1.3. Morphology and viablity of cells in this study

The cells isolated in this study when plated were small, round and mononuclear as seen under phase contrast microscopy and after staining with haematoxylin and eosin. At 24 hours a small number of cells appeared to have formed aggregates but it was not clear if these had fused to form syncytiotrophoblasts or whether the cells were still separate. The viability was still greater than 90% after 24 hours. The general morphology and staining for granulocytes suggest that the cells in this study were approximately 90% pure. In these studies the cells produced very little oestrogens or P unless a suitable steroid precursor was present, which strongly indicates that the levels of steroids measured is that which has been newly synthesized produced by the cells rather than released from intracellular stores. This further suggests that any effects that CRF, ACTH, IGF-I or IGF-II have on steroid production in the isolated cells in the present study are due to their action on enzymatic pathways involved in steroid synthesis. These hormones may modulate the enzymes synthesizing the steroids measured by either regulating the production of new enzymes or by enhancing or inhibiting enzymes already *in situ* .

Cells cultured in 10% fetal calf serum or 20% calf serum for a number of days are thought to form a syncytium and have been shown to produce HCG and HPL (Kliman, et al 1986; Richards, Hartmas and Handwerger 1994; Henson, Shi, Greene and Reggio 1996). Purified cells isolated in this study were also cultured in 20% calf serum and incubated up to 96 hours at 37°C in 5% carbon dioxide. As viewed under phase contrast microscopy these cells had begun to aggregate by 24 hours and by 96 hours most of the cells had formed a syncytium, although the degree of syncytium formation varied from placenta to placenta. Those placentae which had a greater degree of syncytium formation tended to produce higher levels of all steroids. Both HPL and HCG were measurable in aliquots of culture medium taken from these cell cultures. Mean levels of HCG in this study were found to rise with increasing time in culture. The levels produced by one placenta at 24 hours were 7.35 mIU/100,000 viable cells rising to 271.7 mIU/100,000 at 96 hours. In the second placenta mean levels were higher and were 20.44 mIU/100,000 viable cells at 24 hours and 6227.8 mIU/100,000 viable cells at 96 hours (number of wells assayed at each time point for each of the two placenta studied = 4). These results are in accordance with others who also found a gradual rise in HCG in placental cells cultured over a number of days (Kliman et al 1986; Henson et al 1996). The mean levels of HPL also rose over a four day culture. The mean levels were undetectable in one placenta at 24 hours but increased to 79.5 ng/100,000 viable cells at 96 hours. In the second placenta the levels of HCG produced by the cells were higher rising from 381.0 ng/100,000 viable cells at 24 hours to 1053.6 ng/100,000 viable cells at 96 hours. Interestingly it would appear that both the pattern and levels of these hormones produced by placental cell cultures are dependant upon whether the serum that the cells are cultured in is of bovine or human origin. Cells incubated in serum obtained from pregnant women produced more HCG and HPL than cells incubated with serum from non pregnant humans or 10% fetal bovine serum. The pattern of HCG and HPL release by cells cultured with maternal serum and 10% fetal bovine serum also differed with levels of both these hormones rising at days 3-4 and remaining elevated up to day 12 of culture in cells incubated with maternal serum,

however this pattern was absent in those cells cultured with fetal bovine serum. The actual batch of protease used to prepare the cell cultures also affected the amount of HPL and particularly HCG released by these cells in culture (Richards et al 1994).

4.2. Choice of precursors for cell studies

4.2.1. Activity of the enzyme systems P-450cscc, 3β-HSD, aromatase and 17β-HSD in placental cytotrophoblast cells

Cytotrophoblast cells incubated with DMEM alone produced undetectable amounts of the oestrogens and very little progesterone (levels which in most cases were below the sensitivity of the assay). It was therefore necessary to add the appropriate steroid precursor in order to study the production of these steroids by the cells.

4.2.2. Choice of precursor for E1 and E2 production by cytotrophoblast cells

Isolated cytotrophoblast cells obtained from two placentae were incubated in DMEM containing 20% calf serum with either DHEA, DHEAS, A or testosterone as precursors for E1 and E2 production. There was no consistent difference in production of either E1 or E2 by the cells isolated from two placentae irrespective of the precursor used.

In order to measure the activity of the aromatase system in cytotrophoblast cells A was chosen as the preferred substrate for the synthesis of both E1 and E2. The concentration used in all experiments was 700 nmol/l as at this concentration the enzyme system appeared to be saturated and the percentage conversion from A to these oestrogens under the conditions chosen was 1-2% in the preliminary experiments.

4.2.3. Choice of precursor for E3 production by cytotrophoblast cells

Isolated cytotrophoblast cells obtained from two placentae were incubated in DMEM containing 20% calf serum with either 16α-OHDHEA, 16α-OHA, 16α-OHT or 16α-OHE1 as precursors for E3 production. There were no significant differences in E3 production by cells incubated with 1μmol/l of 16α-OHA or 16α-OHDHEA. When the isolated cells were incubated with 16α-OHE1 mean levels (9972.3 and 1288.4 pmol/100,000 viable cells/ 24 hours for each placenta) were higher than those levels produced by cells incubated with the other precursors. The mean levels produced by the cells incubated with the other precursors were highest when 16α-OHDHEA was used as a precursor and were 55.5 and 353.6 pmol/100000 viable cells/24 hours (placenta 2 and placenta 1 respectively, values for all precursors are given in table 3.1.2b, section 3.1.2). In both of the placentae studied the conversion of 16α-OHT (1 μmol/l) to E3 was significantly less than the conversion of 16α-OHA to E3 at 24 hours of incubation. Mean E3 levels in the two placentae when incubated with 16α-OHA and 16α-OHT respectively for experiment 1 were 262.8 and 117.0 pmol/100,000/24 hours viable cells and for the second experiment these levels were 53.3 and 32.7 pmol/100,000 viable cells/24 hours.

In order to measure the activity of the aromatase enzyme and the production of E3 in cytotrophoblast cells 16α-OHA was chosen as the preferred substrate. The concentration used in all experiments was 1000 nmo/l as at this concentration the enzyme system appeared to be saturated and the percentage conversion from 16α-OHA to E3 in the preliminary experiments was 0.5-1%.

4.2.4. Aromatase and 17β HSD acitivity

The production of E1, E2 and E3 by cytotrophoblast cells incubated with the appropriate precursors, which produced very low amounts of these steroids when

incubated without precursors, confirmed that these steroids are being actively synthesized by the cells. Studies indicating that aromatase is located only in the syncytiotrophoblast of the placentae (Fournet-Dulguerov et al 1987; Kitawaki et al 1992) are suprising in view of the present findings and those by Nestler (Nestler et al 1987) who also demonstrated aromatase activity to be present in isolated cytotrophoblast cells. The levels of TE measured in this study were within a similar range to that of Nestler and Williams (1987), with means of controls ranging from 53.3 pmol/million cells to 320 pmol/million cells in this study (see table 3.4b). One study has however shown that aromatase levels in the placenta increase progressively during gestation (Kitawaki et al 1992) and that the increasing levels of oestrogens observed are due to an increase in quantity of the aromatase enzyme being produced. In this study isolated cells were also cultured for 96 hours, by which time they appeared to have formed syncytia. Levels of all three oestrogens were generally higher when precursors were added at 72 hours and measured at 96 hours compared to when precursors were added immediately after plating and measured at 24 hours (although one placenta showed little differences in E1 or E2 levels at either time).

It is possible that the aromatase present within the cytotrophoblast cells isolated in this study is a discrete form of aromatase which experimental techniques to date have not detected or this enzyme may be inactive in term placentae and has in some way been activated as the cells are being isolated. The cells may begin to synthesize the enzyme during the isolation process or once they have been plated. This may be due to some external trigger such as a chemical, or a factor present in the bovine serum, used during the isolation procedure. It may be that in the intact placenta the cells of the syncytium are somehow regulating the expression of this enzyme in cytotrophoblast cells, perhaps by producing some inhibitory factor. Once the syncytiotrophoblast cells are removed by the isolation and purification procedure the aromatase enzyme may then be activated or synthesized in the isolated cytotrophoblast cells.

The interconversion of E1 and E2 is catalysed by 17β-HSD and as A has been shown to be an obligatory intermediate in the conversion of DHEA to E1 (Anderson and Lieberman 1980) then E2 must be formed through the pathways shown in figure 4.2.4a.

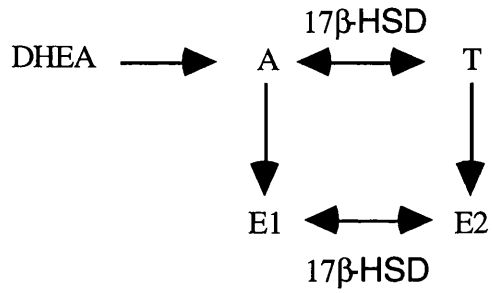


Figure 4.2.4a. The pathway of E2 formation from DHEA in human placental tissue.

Four forms of 17β-HSD have been isolated in the human placenta, types I, II, IV and V, with most research being performed on types I and II which appear to be the most abundant forms in this tissue (Blomquist et al 1985; Blomquist et al 1987; Adamski et al 1995; Labrie et al 1997). Earlier studies on the microsomal type II enzyme (preferentially oxidative form) have located it to the syncytiotrophoblast and not cytotrophoblast cells obtained from tissue taken during early or late gestation (Fournet-Dulguerov et al 1987, Dupont et al 1991). More recently mRNA has been isolated for the type I enzyme in cytotrophoblast cells (Beaudion 1995). Measurements of the tritiated inter-conversion of E1 and E2 by purified cytotrophoblast cells have shown that 17β–HSD is predominantly active in the reductive direction towards E2 with oxidative activity ranging from 0.2 to 0.6 nmol/50,000 viable cells/4 hours and reductive activity ranging from 0.7 to 1.8 nmol/50, 000 viable cells /4 hours (Shepherd, Beckett, Marchant, Serhal and McGarrigle 1995). The mean levels of E1 were lower than those of E2 produced by isolated cytotrophoblast cell preparations in six of the seven individual placentae in this study whilst the converse was true with levels measured from the tissue minces. This is in agreement with other studies in which the E2 formation by villus fragments is low (Tseng, Stolee and Gurpide 1972, Blomquist et al 1987). It has been found that the type 1 enzyme is equally reactive with both E1 and E2. Type II 17β–HSD has a relatively lower affinity for E1 (and A) so the reaction

tends to be in the oxidative direction favouring the formation of E1 (Blomquist et al 1985, Blomquist et al 1987, Wu et al 1993). This may indicate that type I activity is predominant in the cytotrophoblast cells in short term culture whilst in tissue minces, which contain syncytiotrophoblast as well as cytotrophoblast cells, the type II and enzyme may be the more active or more abundant so that the action is predominately in the oxidative direction. The oxidative pathway E2 to E1 may also be favoured by type IV 17β–HSD but the relative contribution of the type IV enzyme to E1 formation may be quite small as it is not found in the same abundance as type I and type II 17β–HSD (Adamski 1995). As the net E1 production in placental tissue in this study was greater than E2 production it might have been expected that the regulation of these enzymes is concomitant with the formation of the syncytium and that with time in culture the ratio E1:E2 might increase. However, this was not the case (see table 3.1.6 for E1 and E2 levels produced by cells incubated for 24 hours and 96 hours). The activity of the type II enzyme has been reported to be low in the syncytiotrophoblast (Beaudoin et al 1995). In agreement with these findings the measurement of the tritiated inter-conversion of E1 and E2 at 96 hours by syncytiotrophoblast cells cultured from two placentae was greater than the oxidative activity (reductive activity 5.04-7.14 nmol/50,000 viable cells/4hrs, oxidative activity 0.15-0.18 nmol/50,000 viable cells/4hrs (unpublished data)).

The findings in this study with isolated cells, however, may not be reflecting what is actually occurring *in vivo*. It is possible that the isolation procedure or cell culture conditions have in some way inhibited the expression of type II activity or have stimulated type I activity (or even type IV). It cannot be ruled out that culture conditions or the isolation procedures have in some way affected the expression of this enzyme in the isolated placental cells. It is not possible to determine if the cultured cells are behaving in an identical way to cells *in vivo* or whether the expression and functioning of the type II enzyme may require interactions with other cell types.

There is a scarcity of information regarding the form of 17β-HSD which may be involved in the conversion of 16α–hydroxylated DHEA to E3 as shown in figure 4.2.2b.

It is possible that a 17β–HSD exists which is distincte from types already characterized in the human placenta for the formation of E1 or E2 or that one or more of the types already characterized are responsible for E3 formation.

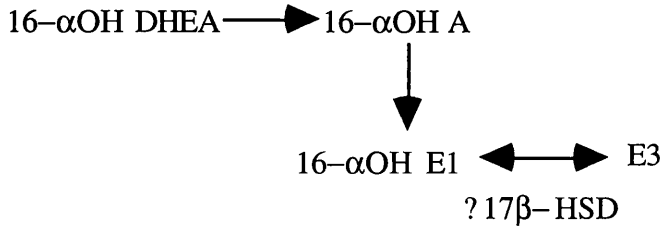


Figure 4.2.4b. The conversion of 16α–hydroxylated DHEA to E3.

4.2.5. Inhibition of E3 production by A

Some researchers have postulated that several species of aromatase exist (Canick and Ryan 1975; Canick and Ryan 1976; Cantineau et al 1982; Osawa et al 1982; Hagerman 1987; Harada 1988). However more recent research indicates that as the aromatase enzyme is encoded by a single copy gene, that only one enzyme is responsible for all the aromatase reactions (Vickery and Kellis 1987; Means et al 1989, Corbin et al 1989; Harada 1990). In the present study it was demonstrated that A (70 nmol/l) inhibited E3 production in cytotrophoblast cells (see section 3.8). This suggests that either A was binding to a common catalytic site on the same enzyme or binding to a separate site on the same enzyme and altering the conformation of the catalytic site for 16α–OHA so that this steroid was not able to bind. The results in this study do not however rule out the possibilty that two or more enzymes exist one of which binds both A and 16α–OHA and one which binds A, as first postulated by Canick and Ryan (1975, 1976). As the inhibitory effect was dose dependent it suggests that the enzyme has a greater affinity for A than it has for 16α-OHA. At higher concentrations of A more binding sites would be either occupied or their conformation changed (either way less 16α–OHA would bind and be converted to E3) than at lower concentrations of A. The converse was not the case as E2 production from A did not appear to be inhibited by 16α–OHA. These

findings are similar to those of Canick and Ryan who studied human placental microsomal aromatase and showed that the conversion of another 16α hydroxylated compound, 16α–OHT, to E3 was inhibited by A in a competitive manner, but that the conversion of A to E2 was not (Cannick and Ryan 1976). The results in this study are also in accord with the higher affinities shown by aromatase for A than 16α–OHA in studies on the purified microsomal enzyme system (Cantineau et al 1982; Purohit and Oakey 1989; Yoshida and Osawa 1991).

In contrast to the isolated cells this inhibition by A of E3 production was not apparent within the tissue explants studied. This may well have been due to the fact that the precursors which were added to the medium containing the explants were not reaching the appropriate cells within the tissue and that these cells were synthesizing E3 from readily obtainable precursors already present within the tissue. It may be the supply and uptake of precursors by the cells and the transport of these precursors within the cell to the enzyme systems which ultimately regulates the overall E1, E2 and E3 production.

These findings are interesting as they conflict with the well established observation that more E3 is produced in normal pregnancy than E2 or E1 (Brown 1956; Mujaji, Toumba and Oakey 1979; Fotsis 1987). In the experiments on tissue explants although E3 levels were sometimes higher than those of E1 and E2 this was not always the case and the isolated cells produced invariably less E3 than E1 and E2. Substrate availability present within the placental explants might explain the variablity in the tissue experiments from the ratios obtained in pregnancy when less E3 is produced than E1 or E2, but would not account for the lower ratio of E3 to E1 and E2 produced by isolated cells incubated with the appropriate precursors. It is possible that a distinct form of aromatase present elsewhere within the cell or a form of 17β-HSD that has a higher affinity for the 16α-hydroxylated precursors are somehow "switched off" in the isolated cells due to the methods involved in the isolation procedure, or the culture medium in which they are incubated.

4.2.6. Choice of precursor for P production by cytotrophblast cells.: P450cscc activity and 3β HSD activity

Cholesterol is converted to progesterone via the pathway shown in figure 4.2.6.

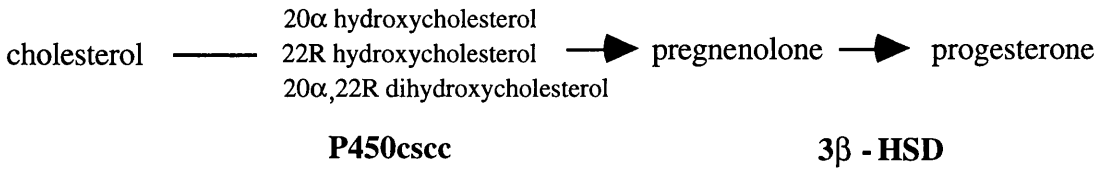


Figure 4.2.6. Pathway of P formation from cholesterol.

It was initially intended that both the conversion of cholesterol to pregnenolone and pregnenolone to P would be studied. This proved impossible because the amount of P formed from cholesterol, 25-OHcholesterol and LDL in theses studies was insufficient for accurate measurement of P450cscc. The reason for the low levels of P production on the addition of 25-OHcholesterol even when the number of cells was raised to 1,000,000 viable cells per well and the concentration of 25-OHcholesterol was as high as 80 μmol/l is uncertain. The levels of P produced by cells incubated in DMEM alone were not significantly different to those incubated with cholesterol or 25-OHcholesterol. These findings are in accord with another study which also found that cholesterol was a poor precursor for P production in human syncytiotrophoblast cell cultures (Paul, Das, Jailkhani and Talwar 1987). A possible explanation is that cholesterol or 25-OHcholesterol is not reaching the enzyme system either because its transfer across the cell membrane or its transport within the cell to the enzyme system within the mitochondria has been in some way affected. In the case of 25-OHcholesterol it is unlikely that it is the transfer across the cell membrane that is affected as this derivative of cholesterol has been used by other researchers because of its greater solubility than

cholesterol in aqueous medium and has been shown to be an effective steroidogenic substrate in dispersed rat luteal cells (Sinensky 1981; Toaff, Schleyer, and Strauss 1982). Indeed a study by Tuckey (1992) using isolated human placental mitchondria to measure P-450cscc activity has shown that 25-OHcholesterol is a poor substrate for pregnenolone synthesis in this cellular organelle. The other possibility is that the P-450cscc enzyme system is not functioning properly in isolated cells. However, it seems unlikely that the enzyme system was damaged by the isolation process as both the aromatase sytem and 3β-HSD sytem are functioning normally. The cytotrophoblast P-450cscc may not be "switched on'" or it may be present within the isolated cells but only at very low levels. Levels of P-450cscc have been reported to be lower in cultured cytotrophoblast cells than in placental homogenates suggesting that the synthesis of this enzyme could well be induced as syncytiotrophoblasts form (Tuckey, Kostadinovic and Cameron 1994). Kliman and colleagues (Kliman, Nestler, Sermasi, Sanyer and Strauss 1986) were able to show an increase in P levels produced by purified cytotrophoblast cells by 2-8 fold in four hours with the addition of 50 μmol/l 25-OHcholesterol. Nestler and Williams (1987) incubated cytotrophoblast cells with 50 μmol/l 25-OH cholesterol and also obtained measurable levels of P (approximately 52 pmoles/million cells/24 hours). In both cases much less than 1% of the substrate was converted to P in 24 hours. The values obtained by both sets of reseachers are very low considering the concentration of precursor used. The discrepancies between this study and those by Kliman et al (1986) and Nestler and Williams (1987) are difficult to explain. It is of course possible, though unlikely that the RIA assay employed in this study was not as sensitive as that used by Kliman et al (1986) and Nestler and Williams (1987) or it could be that there was an appreciable amount of cross-reactivity with other substances which resulted in the "measurable" levels obtained with the assays of other researchers.

The fact that LDL and 25-OHcholesterol proved to be poor precursors for P production in this cell system poses the possibility that the major precursor for P may well not be LDL but could be cholesterol sulphate, a substrate that may be readily available to placental cells *in vivo*. Indeed Tuckey et al (1994) found that cholesterol sulphate was a

better precursor for P production by cytotrophoblast cells and mitochondria than LDL or other intermediates in cholesterol side chain cleavage.

Because of the low level of conversion of cholesterol to pregnenolone it was not possible to measure P450cscc activity in these studies. The addition of pregnenolone did result in P production showing that the 3β-HSD enzyme was functioning within these cells. The studies by Mason et al (1993) indicated that the levels of mRNA for 3β-HSD in cytotrophoblasts were undectable after isolation of these cells but increased with time in culture. However their findings do not completely accord with preliminary experiments in this study in which substantial progesterone formation was detectable in the media from cells incubated for only 2 hours with pregnenolone (mean levels from two placentae incubated in triplicate wells were 560 fmoles/100,000 viable cells). However this does not exclude the possibilty that synthesis or activation of 3β-HSD is occurring with time in culture. In this study cytotrophoblast cells were isolated from three placenta and cultured for up 96 hours. Cytotrophoblast cells from each of the three placentae were incubated immediately after purification with pregnenolone for 24 hours and then frozen. Other cell preparations from the three placentae were incubated with DMEM alone for 72 hours before the addition of pregnenolone to the culture medium. The cells were then incubated with the precursors for a further 24 hours. The levels of P produced by those cells incubated in DMEM alone for 72 hours before the addition of pregnenolone were higher (means ranged from 2.71 to 10.47 nmol/100,000 viable cells/24 hour) than those produced by cells incubated with this precursor immediately after purification (means ranged from 1.12-8.65 nmol/100,000 viable cells/24 hour), suggesting that the activity or the amount of enzyme is increasing in time in culture. In the cell preparations obtained from one of the placentae studied the enzyme system was not saturated even at pregnenolone concentrations of 80 μmol/l. However even at concentrations as low as 5 nmol only around 10% of the precursor was being transformed to P with the percentage dropping to about 3% at concentrations of 10 nmol/l which is in agreement with the results of Nestler (Nestler and Williams 1987) and suggests that it is the entry of pregnenolone into the cell or its transport within the

cell that is the rate limiting factor in this cell system. On incubating cytotrophoblast cells at a concentration of 6 μmol/l pregnenolone Nestler obtained mean levels of 649 pmol/million cells. In the present study mean levels of P produced by isolated cytotrophoblast incubated with 1 μmol/l pregnenolone were 330-440 pmol/million viable cells/24hours and those incubated with 10 μmol/l pregnenolone were in the range 1600-3110 pmol /million viable cells/24 hours (results given in table 3.11).

4.3. A role for CRF and ACTH in steroid production in the human placenta?

Despite the fact that both CRF and ACTH are synthesized in placental tissue and their modulatory roles on steroid synthesis in the adrenal are well known, there is a scarcity of information regarding any modulatory role that they might have on steroidogenesis in the placenta. In view of the findings that P and E2 were enhanced by addition of ACTH to placental minecs (Barnea et al 1986) the present study attempted to clarify whether or not these hormones have any regulatory function on the production of E1, E2, E3 and P in human placental tissue explants and isolated cytotrophoblast cells

4.3.1. The effects of CRF and ACTH on E1 and E2 production

There was no consistent significant effect of added CRF on the levels of E2 production as compared to control values in tissue explants incubated in DMEM alone from which aliquots were removed at timed intervals over a 24 hour period (results section 3.3.1). Similarly the addition of CRF to medium containing the precursor A did not significantly affect E1, E2 or TE levels as compared to control values produced by explants incubated with DMEM and A for 24 hours (results section 3.5).

Levels of E2 were not significantly different to those of the controls (incubated in DMEM alone) when tissue explants were incubated with ACTH in those studies in

which aliquots were obtained from three placentae at set time intervals over a period of 24 hours (results section 3.3.2). Similarly in those tissue explants incubated for 24 hours in medium containing A, E2 was not significantly affected by the addition of ACTH in any of the placentae studied although E1 and TE were significantly decreased in one of the three placentae (results section 3.5). These findings conflict with those presented by Barnea et al (1986) who found that ACTH stimulates the production of E2 by placental minces.

Overall there appears to be no consitent modulatory role for CRF or ACTH on E1 or E2 production in isolated placental cytotrophoblast cells. However the levels of these hormones were significantly different from those of control preparations in certain placentae. In particular CRF and ACTH seemed to have affected steroid synthesis in placentae B2 and B3 (see results section 3.4.) but why this occurred in these two placentae is unclear as all seven placentae were selected by the criteria described in the methods and treated in the same way. In both these placentae CRF inhibited E1 production but significantly increased E2 production in the same cell populations at the same time. The TE levels produced by these two placentae on incubation with CRF were not significantly different to those produced by controls from those cells isolated from placenta B2 but were increased in those from placenta B3. Why this is the case in only these two placentae is difficult to explain. The fact that more E2 was produced by these cells than E1 may indicate that in these individual placentae the 17β-HSD was activated in such a way as to increase the reductive pathway towards E2 perhaps by stimulating type I or inhibiting type II activity. It is difficult to determine whether or not this could be due to addition of CRF and ACTH.

4.3.2 The effects of CRF and ACTH on E3 production by the placenta

There was no consistent effect of either CRF or ACTH, compared to controls, on E3 production by tissue minces obtained from three placentae, incubated for 24 hours in

DMEM from which aliquots were obtained at fixed time intervals (see results section 3.6). Similarly in those tissue minces obatined from three placentae and incubated for 24 hours with 16α-OHA, with/without CRF, levels of E3 did not differ significantly from those of the controls. Those explants incubated with 16α-OHA with/without ACTH showed no significant differences from those of controls with the exception of one of the three placentae (B2) which produced significantly lower levels of E3 (see results section 3.9).

The isolated cell preparations again yielded more variable results. Of the seven placentae studied, cell preparations from three placentae, (B1, B2, B3, results section 3.7) produced sigificantly higher levels of E3 when CRF was present and two of these placentae (B2 and B3) had increased E3 levels when ACTH was present compared to controls.

The general finding however is that CRF and ACTH do not have a consistent significant effect on the aromatization of 16α-OHA in the human placenta as assessed in this experimental system.

4.3.3. The effects of CRF and ACTH on P production by the placenta

There was no consistent significant effect of either CRF or ACTH on P levels from placental tissue minces obtained from three placentae and incubated in DMEM alone with/without CRF/ACTH for 24 hours (results section 3.10). A further study utilising tissue minces that were incubated in DMEM and pregnenolone with/without CRF or ACTH indicated that P levels were not affected in two placentae but were raised in placenta B3 by both CRF and ACTH (see results section 3.12). These findings are not in accord with those documented by Barnea (1986) who found that ACTH increased P production in all three of the placentae that he studied.

Of those cytotrophoblast cell preparations (obtained from eight placentae), incubated with CRF only those from placenta B4 produced P levels which were significantly decreased compared to those produced by the controls (results section 3.11). On incubation with ACTH, inhibtion of P production was observed in the same placenta whereas two other placentae, B2 and B3, were observed to have significantly raised P levels. Those placentae which produced significantly higher levels of P were incubated at the lower concentration of pregnenolone. It may be that the higher concentration of pregnenolone or the high levels of P produced by the isolated cells inhibit 3β-HSD activity and over ride any stimulatory effect that ACTH might have. On addition of ACTH at the lower concentration of pregnenolone it may be that ACTH has stimulated synthesis of the enzyme which would account for the higher levels obtained in two of the three placentae studied.

These observations suggest that there is no consistent effect of CRF or ACTH on the P-450cscc or 3β-HSD enzyme system within the human placenta in these experimental models.

4.3.4. General summary on the action of CRF and ACTH on steroid synthesis in human placenta

The few examples in which any apparent modulatory effect was observed were mainly expressed by placentae B2 and B3 but there is no apparent reason for this as all seven placentae were collected and processed using identical protocols. However, different types of sera have been shown to effect the levels of P and E2 produced by trophoblast cultures (Henson et al 1996) and different batches of proteases have even affected the levels of HPL and HCG produced by human trophoblasts (Richards et al 1994). Although the cells in this study were not incubated with bovine sera they were in contact with it during the isolation procedure. It is possible that different batches of bovine sera contain variable levels of certain factors. These factors could "activate" or

"inhibit" the cellular enzymes in some way, which in combination with exposure to CRF and ACTH, may have resulted in the variable responses observed by isolated cells in the individual placenta studied. This highlights the difficulties in interpreting data from such experimental models.

The general conclusion from this study is that CRF and ACTH under the experimental conditions described do not have a modulatory role on the the aromatase system in the production of E1, E2, E3 or a regulatory role on 3β-HSD in placental tissue or isolated cytotrophoblast cells. The affect of CRF and ACTH on P-450cssc is more difficult to asses in this study, although the tissue studies suggest that there is no affect of either hormone on P production. It is not possible to determine from this study whether P production observed is release from an already discrete pool present within the cells of the tissue explants, or whether it is being synthesized from a supply of precursors present within the tissue. In order to explore this it would be necessary to selectively inhibit the enzyme system and observe whether or not the increase in P continues but even this would not rule out the possibility that the inhibitor affects the release of pre-formed hormone from the cell into the surrounding medium.

The addition of CRF to placental tissue and purified cells did not have any effect on steroid production by cells or tissue and this suggests that it does not play a role in modulating placental E1, E2, E3 or P synthesis. Attempts were made to measure ACTH in the media of isolated cytotrophoblast cells and tissue explants after incubation. No measurable levels of ACTH were obtained from media in which tissue had been incubated, but ACTH was measurable in the medium from cell preparations although at very low levels of 1-2 pg/ml. The addition of CRF to the medium did not increase the levels of ACTH present in these cell incubations.

In the adrenal ACTH stimulates the hydrolysis of cholesterol esters (Davis and Garren 1966). In addition, ACTH increases the number of lipoprotein receptors on the cell surface (Kovanen et al 1979) stimulating the uptake of LDL, which in turn is thought to

be enhance ACAT activity and suppress HMG COA reductase activity in a similar manner to that observed in human fibroblast cells (Brown et al 1973; Brown et al 1974; Goldstein et al 1974; Brown, Faust and Goldstein 1975; Brown Dana and Goldstein 1975), thereby increasing the amount of pregnenolone and P produced. *De novo* synthesis of cholesterol is low in the human placenta (Woolever et al 1961; van Leusden and Villee 1965; Zelewski and Villee 1966; Hellig et al 1970) which is thought to be due to inhibition of HMG COA reductase activity by LDL (Simpson, Porter et al 1978; Winkel et al 1981). In addition the levels of P produced by the placenta are thought to inhibit ACAT activity suggesting that the enzyme system in the placenta is regulated in a different manner to that of the adrenal (Winkel et al 1980; Simpson and Burkhart 1980a; Simpson and Burkhart 1980b). The level of pregnenolone production appears to be dependant on the uptake of LDL by the placenta, a process which is regulated by the number of LDL receptors on the cell surface (Simpson, Porter et al 1978; Simpson, Bilheimer et al 1979; Simpson and Burkhart 1980b; Winkel, Synder et al 1980; Winkel, Gilmore et al 1980; Winkel et al 1981; Simpson and MacDonald 1981). If the production of P from the tissue explants is due to utilization of endogenous cholesterol then the fact that there was no increase in P production on addition of ACTH in the first few hours of the study indicates that ACTH does not play a role in the stimulation of P production in the human trophoblast in contrast to its role in the adrenal. Indeed the StAR protein, involved in the acute regulation of steroidogenesis in the adrenal, has not been located in the placenta (Sugawara et al 1995).

The discrepancies between this study and that of Barnea et al (1986) are not easily explicable as this study failed to detect any modulatory role of ACTH on placental tissue explants or isolated cells. One experimental difference between the two studies is that the placentae utilized in the study by Barnea et al (1986) were obtained from women who had elective caesereans whilst those women in this study had gone into labour. It is possible, but unlikely, that changes occurring within the placenta during the process of parturition in some way alter the intrinsic properties of the cells such as those regulating the production of LDL receptors on the cell surface. It was noticeable that

there could be large variations within repeated groups of tissue incubates in the amount of steroid produced which was probably associated with the proportion of steroid producing cells within the tissue fragments. It is obvious that care must be taken in interpreting results from a small set of data. In this study a total of six placentae were used in the tissue explant experiments (and the number of flasks containing tissue explants for each individual placenta was also six at each time point in each experiment) to study the effect of CRF and ACTH. However the number of tissue preparations for each placentae used by Barnea (1986) was smaller (number of placentae studied=3, number in control group = 10, number exposed to ACTH 10 nmoles/l = 7).

The duration of experimental procedure in this study was relatively short term and does not preclude a modulatory role for CRF or ACTH on steroid synthesis in placental tissue that may take place after 24 hours has elapsed. It may be that if ACTH does act to stimulate the synthesis of enzymes involved in steroid production, which has been observed in adrenal steroid transforming enzymes systems such as P450cssc and 3β–HSD (Dubois et al 1981;Funkenstein et al 1983; Kramer et al 1984; Zuber et al 1985; Mason et al 1985; John et al 1986), then this would only be apparent on incubating cells or tissue preparations for longer periods of time. The roles of CRF and ACTH produced by the placenta during pregnancy may instead be directed towards the fetus increasing fetal adrenal activity and hence increasing the supply of precursors for oestrogen synthesis.

4.4.1. Is there a role for IGF-I and IGF-II in modulating steroid production in the placenta?

Research has indicated that both IGF-I and IGF-II have a role in modulating steroidogenic activity in numerous endocrine tissues, IGF-I stimulates P-450cscc activity in swine granulosa cells and enahances aromatase activity in human granulosa cells (Veldhuis et al 1986; Ercikson et al 1989). Stimulation of cortisol by ACTH in

bovine adrenal cells and that of 17α, 21α and 11β hydroxylase activities in human granulsoa cells have been shown to be augmented by IGF-I (Penhoat et al 1989; Pham-Huu Trung et al 1991). IGF-II has been shown to increase the abundance of ACTH stimulated mRNAs for P-450cssc, 3β-HSD and 17α hydroxylase/17, 20 lysase in human fetal adrenal cell cultures (Mesiano and Jaffe 1993). Both IGF-I and IGF-II have been shown to inhibit aromatase activity whilst stimulating P-450cscc and 3β-HSD activity in cytotrophoblast cells (Nestler and Williams 1987; Nestler 1989; Nestler 1990) The purpose of this part of the study was to assess the effects of these somatomedins on the production of E1, E2, E3 and P production in placental tissue explants and isolated cytotrophoblast cell cultures.

4.4.2. The effects of IGF-I on E1 and E2 production in placental tissue

There was no consistent effect of IGF-I on E2 production by placental tissue explants when compared to those explants incubated with DMEM alone (results section 13). There was no effect of IGF-I on E1, E2 or TE levels produced by those explants incubated in the presence of A.

The levels of E1 produced by cytotrophoblast cells were unaffected by the presence of IGF-I when compared to controls in isolated cell preparations obtained from six of the seven placentae. The two placentae which did appear to respond to IGF-I were placentae B2 and B3 in which E1 levels were significantly decreased at both concentrations of IGF-I (results section 3.14.1).

In comparison to E2 levels obtained from controls, three of the seven placentae studied showed a significant increase in E2 production when purified cells were incubated with IGF-I at 20 ng/ml but none were significantly different at the higher concentration of IGF-I (results section 3.14.2).

In contrast to the findings of Nestler (1990) that IGF-I inhibits TE production in cytotrophoblast cells, there were no significant differences in TE production by cells incubated with IGF-I at 400 ng/ml. TE levels were significantly different in cell preparations obtained from two placental cell preparations at the lower concentration of 20 ng/ml as compared to those obtained from controls, being lower in placenta B4 and higher in placenta B3 (results section 3.14.3).

4.4.3. The effects of IGF-II on E1 and E2 production

There was no significant effect of IGF-II on E1 or E2 production by tissue explants incubated with A at either concentration of IGF-II in any of the three placentae studied. However there was a significant reduction in TE levels compared to controls in placenta B2 when incubated with IGF-II at 400 ng/ml (results section 3.15).

Significant effects of IGF-II on E1 production by isolated cytotrophoblast cells were apparent in three of the seven placentae but these were variable. At both concentrations of IGF-II, levels of E1 were significantly decreased in placenta B2 and were also significantly lower in cell preparations from placenta B3 but only when incubated with IGF-II at 400 ng/ml. In contrast E1 levels were significantly higher than control values produced by isolated cells obtained from placenta B1 (results section 13.14.4).

Levels of E2 and TE were significantly raised compared to those of the controls in cytotrophoblast preparations from both B2 and B3 at both concentrations of IGF-II but no significant effect was observed in any of the other five placentae (result sections 13.14.5 and 13.14.6). The overall conclusion is that in contrast to the findings of Nestler (1990), who found that IGF-II inhibited aromatase activity in cytotrophoblast cells, this study suggests that IGF-II does not appear to have a modulatory role on TE production by these cells.

4.4.4. The effects of IGF-I on E3 production

There was no significant effect of IGF-I on E3 synthesis in any of the placental tissue explants studied whether or not 16α OHA was used as a precursor with the exception of one placenta A4 which produced significantly lower E3 levels at just one time point (results section 3.16 and 3.18).

The effects of IGF-I on E3 production by purified cytotrophoblast cells were inconsistent. There was a significant increase in E3 levels measured in three of the seven placentae studied compared to controls but a decrease in one on incubation with IGF-I at 20 ng/ml. At the higher concentration of IGF-I a significant increase was observed only in two of the placentae (results section 3.17).

4.4.5. The effects of IGF-II on E3 production

There was no consistent significant effect of IGF-II on E3 levels produced by placental explants incubated with/without 16α OHA as a precursor (results section 3.18).

There was a significant effect of IGF-II (at both concentrations) in cell preparations obtained from only two of the seven placentae studied suggesting that IGF-II has no regulatory role to play on E3 production by the human placenta.

4.4.6. The effects of IGF-I on P production

There was no overall consistent effect of IGF-I on either placental tissue explants obtained from three placentae or isolated cytotrophoblast cells obtained from seven placentae. Of those tissue explants showing any effect of IGF-I, all levels were significantly lower than those produced by controls (results section 3.19). This

reduction was also present in cells isolated from one placenta (B4) incubated with IGF-I at both concentrations used (results section 3.20.1). These findings fail to confirm those of Nestler (1989) who suggested that 3-βHSD activity in isolated cytotrophoblast cells is enhanced by IGF-I.

4.4.7. The effects of IGF-II on P production

There was no overall consistent effect of IGF-II on either placental explants or isolated cytotrophoblast cells although the levels of P produced by cells from two placentae were significantly different from controls. Whilst P production by isolated cells incubated with a pregnenolone concentration of 10 μmol/l from placenta B4 was significantly reduced at both concentrations of IGF-II used, P production was significantly decreased only at the higher concentration of IGF-II in placentae B7 (20 μmol/l pregnenolone) and B8 (10 μmol/l pregnenolone), and levels were significantly higher in placenta B6 (20 μmol/l pregnenolone), (results section 3.20.2) . These findings fail to confirm those of Nestler (1990) who suggested that 3-βHSD activity in isolated cytotrophoblast cells is enhanced by IGF-II.

4.4.8. Overall summary of the role of IGF-I and IGF-II on steroid production in human placenta

The presence of either insulin like growth factor had no consistent effect on steroid output in tissue explants. Although the collection of placentae and subsequent isolation of cytotrophoblast cells was conducted in a similar way to that of Nestler et al (1987, 1988) inhibition of aromatase and stimulation of 3-βHSD activity was not apparent in this study. The variation in responses by individual placentae in this study may be due to the differences in batches of bovine calf sera or the proteases used in the experiments. In purified cytotrophoblast cell preparations obtained from two of the placentae in this

study the overall effect of IGF-I and IGF-II on TE levels was actually to enhance its production, indicating that if anything, IGF-I and IGF-II stimulate aromatase activity rather than inhibiting it, as has been suggested by other researchers (Nestler and Williams 1987, Nestler 1990).

It appears that the length of time that the cells are in culture is important in determining any modulatory role of IGF-I on steroid synthesis. The inhibition of E2 production in choriocarcinoma cells by this somatomedin was observed after 24 hours (Ritvos 1988), whilst studies on swine granulosa cells have shown that IGF-I induces P-450cscc synthesis as early as 16 hours of culture (Veldhuis et al 1986). In a similar experiment to those in this study, cytotrophoblast cells incubated with A and IGF-I for 24 hours, showed up to 30% inhibtion of aromatase activity (Nestler et al 1987) and further time course studies indicated that the effect of IGF-II was apparent only after incubation with IGF-II for 20 hours (Nestler et al 1988). The cells in this study were incubated for 24 hours and as the collection of placental tissue and cell culture conditions were similar to those of Nestler (1987) it is difficult to determine why the modulatory effects observed by Nestler (1987, 1989, 1990) were not observed in this study. The number of cells plated by Nestler was between 500,000-1,000,000 cells/well whilst in this study 100,000 viable cells were plated per well. Dependant upon the size of the well it is possible that the cell densities in Nestlers study created an environment which was in some way necessary for the effects of the insulin like growth factors to become apparent. However the tissue studies reported above suggest that this is unlikely as there was no effect on steroid production in these experiments.

4.5. Tissue experiments with NADPH

The production of E1 and E3 were not generally affected by the addition of NADPH to DMEM in the experiments conducted on tissue explants. The experiments utilising NADPH suggest that there are more than sufficient endogenous steroid precursors

present within the tissue for P and E2 production, as the addition of NADPH created a large increase in output of these steroids (in some cases by 200%) when no additional precursor was present. That NADPH did not just stimulate the release of E2 and P from preformed stores cannot be excluded but it is unlikely. This suggests that as P production increases with the addition of NADPH that P-450cscc is active in the placental tissue explants as this enzyme utilises NADPH whilst 3β–HSD utilzes NAD+. The enzyme systems P-450cscc and aromatase both utilise NADPH and so the addition of this compound would be expected to stimulate the activites of both these enzyme systems if the amount of energy available to the cell is a limiting factor in the production of P and E2 rather than the amount of steroid precursor present. In the case of E3 the lack of affect of NADPH on aromatase activity may be because the concentration of precursors, or their uptake by the cells, might be rate limiting for the overall reaction. In the experiments utilising NADPH it was apparent that the effect varied over time with E2 levels falling and then rising throughout the 24 hours (see figure 3.22.2). As the aromatase system utilises NADPH it might have been expected that E1 would also be seen to increase. However this was not the case. The 17β-HSD enzyme system can also utilise NADPH when converting E1 to E2. Stimulation of aromatase by NADPH would increase the amount of E1 available to this enzyme system. The reductive pathway appears to be more active in cytotrophoblast cells than the oxidative pathway (Shepherd et al 1995). The addition of NADPH could stimulate E2 production via the reductive pathways that is, through the interconversion of E1 to E2 and also through the interconversion of A to T and its subsequent conversion to E2. If 17β-HSD is not saturated with substrate and is more active than aromatase in placental tissue then E1 would be converted to E2 as E1 becomes available so there would be no apparent increase in E1 levels produced.

4.6. General conclusions

The enzyme systems 3β–HSD, aromatase and 17β–HSD are active in cytotrophoblast cells and tissue explants from human placenta when maintained in *in vitro* culture. The isolated cell studies indicate that the activity of 17β–HSD is predominantly in the reductive direction whilst the converse is apparently true for the tissue explants. The enzyme system P-450cscc does not appear to utilise 25α-OH as a precursor in isolated cytotrophoblast cells.

The variable responses seen by the placental cell preparations illustrate the importance of repeating experiments with more than 3-4 placentae, and in having a sufficient number of replicate flasks or wells at each time point studied.

There does not appear to be a role for CRF or ACTH in the production of E1, E2, E3 or P in human placental tissue. This study failed to confirm the findings of Barnea (1986) that ACTH enhances P and E2 production in the human placenta. The experimental models used in this study are limited however, and a role for these compounds in modulating steroid production *in vivo* cannot be precluded.

There was no consistent effect of IGF-I or IGF-II on the production of E1, E2, E3 or P in isolated human placental tissue. This study failed to confirm the findings of Nestler (1987,1988,1989) that indicated IGF-I and IGF-II had an inhibitory effect on "TE" production and a stimulatory one on P production in isolated cytotrophoblast cells.

The addition of NADPH to tissue explants appears to stimulate the activity of aromatase, 17β–HSD and cytochrome P-450cscc with an increase in P and E2. It did not however stimulate E1 or E3 production. The lack of apparent stimulation of E1 may be due to an increase in reductive 17β–HSD activity which converts E1 to E2 as rapidly as E1 is formed. The lack of stimulation of E3 production by addition of NADPH may be because the supply of 16α-OHA is rate limiting for this steroid.

4.7. Recommendations for future work

As the isolated cytotrophoblast cells in this study produced very little P from 25-OHcholesterol it would be of interest to investigate the precursor cholesterol sulphate to determine whether this is a major substrate for P synthesis in the human placenta.

Although there was no consistent effect by CRF, ACTH, IGF-I or IGF-II on steroid production by isolated cytotrophoblast cells incubated for 24 hours it would be of interest to study long term cell cultures a) to see if any modulatory effects become apparent beyond 24 hours b) to see if any possible modulatory effects might depend on the cell type and the formation of syncytia. In addition it would be interesting to study 17β-HSD activity in cultured syncytiotrophoblasts to see if it differs from that in cyotrophoblast cells. Preliminary data (not presented here) suggests that 17β-HSD is more active in cells cultured for 96 hours compared to cells cultured for 24 hours.

In view of the finding that ACTH significantly increased P production in two of the three placentae incubated with lower concentrations of pregnenolone further studies utilising this substrate at more physiological concentrations might determine whether ACTH modulates the 3β-HSD enzyme under these experimental conditions.

It would also be interesting to see whether the addition of ACTH to the insulin like growth factors would enhance or decrease the production of P and E2 as has been reported in other cell systems (Penhoat et al 1989; Pham-Huu-Trung et al 1991; Mesiano and Jaffe 1993).

The addition of NADPH to cell culture sytems would perhaps help in answering the question of why it stimulates E2 production but not E1 or E3 in tissue explants.

Tissue explants and cell cultures prepared from preterm placentae and elective caesarean sections might clarify whether the discrepancies between this study and that

of Barnea et al (1986) were due to the fact that this study only used placentae from women who had gone into labour. It would also be of interest to assess whether the growth factors and CRF have any affect on placental P, E1, E2 and E3 production when the placenta is obtained from a preterm delivery or elective caesarean section.

References

Adamski J., Normand T., Iffnders F., Monté D., Begue A., Stehelin D., Jungslut P. W. and de Launoit Y Molecular cloning of a novel widely expressed human 80kDa 17β-hydroxysteroid dehydrogenase IV. *Biochemical Journal* 1995; **311**:437-443.

Akhtar M. and Skinner S. J. M. The intermediary role of a 19-oxoandrogen in the biosynthesis of oestrogen. *Biochemical Journal* 1968; **109**: 318-321.

Alexandrova M. and Soloff M. S. Oxytocin receptors and parturition. I. Control of oxytocin receptor concentration in the rat myometrium at term. *Endocrinology* 1980; **106**: 730-735.

Alfano J., Brownie A. C., Orme-Johnson W. H. and Beinert H. Adrenal mitochondrial cytochrome P-450 and cholesterol side chain cleavage activity. Differences in the response of the zona glomerulosa and zona fasticulata-reticularis to adrenocorticotropic hormone and its withdrawal. *The Journal of Biological Chemistry* 1973; **248**: 7860-7864.

Allen W. M. and Doisy E. A. An ovarian hormone. Preliminary report on its localization, extraction and partial purification, and action in test animals. *Journal of the American Medical Association* 1923; **81**: 819-821.

Allen W. M. and Corner G. W. Physiology of the corpus luteum III. Normal growth and implantation of embryos after very early ablation of the ovaries, under the influence of extracts of the corpus luteum. *American Journal of Physiology* 1929; **88**: 340-346.

Allen W. M. In discussion of Hunt A. B., McConahey W. and Minn R. Pregnancy associated with diseases of the adrenal gland. *American Journal of Obstetrics and Gynaecology* 1953; **66**: 970-987.

Allen J. P., Cook D. M., Kendall J. W. and McGilvra R. Maternal-fetal ACTH relationship in man. *Journal of Clinical Endocrinology and Metabolism* 1973; **37**; 230-234.

Alsat E., Boulai Y., Goldstein S., Malassiné A., Berthelier M., Mondon F. and Cedard L. Low-density lipoprotein binding sites in the microvillous membranes of human placenta at different stages of gestation. *Molecular and Cellular Endocrinology* 1984; **38**: 197-203.

Al-Timimi A. and Fox H. Immunohistochemical localization of follicle-stimulating hormone, luteinizing hormone, growth hormone, adrenocorticotrophic hormone and prolactin in the human placenta. *Placenta* 1986; **7**: 163-172.

Anderson N. G. and Lieberman S. C_{19} Steroidal precursors of estrogens. *Endocrinology* 1980; **106**: 13-18.

Anderson P. J. B., Hancock K. W. and Oakey R. E. Non-protein-bound oestradiol and progesterone in human peripheral plasma before labour and delivery. *Journal of Endocrinology* 1985; **104**: 7-15.

Anderson J., Grine E., Eng C. L. Y., Zhao K., Barbieri R. L., Chumas J. C. and Brink P. R. Expression of connexin-43 in human myometrium and leiomyoma. *American Journal of Obstetrics and Gynaecology* 1993; **169**: 1266-1276.

Andersson S., Geissler W. M., Patel S. and Wu L. The molecular biology of androgenic 17β-hydroxysteroid dehydrogenases. *Journal of Steroid Biochemistry and Molecular Biology* 1995; **53**: 37-39.

Appleby J. I. and Norymberski J. K. The urinary excretion of 17-hydroxycorticosteroids in human pregnancy. *Journal of Endocrinology* 1 957; **15**: 310-319.

Arthur J. R., Mason J. I. and Boyd G. S. The effect of calcium ions on the metabolism of exogenous cholesterol by rat adrenal mitochondria. *FEBS Letters* 1976; **66**: 206-209.

Ascheim S. and Zondek B. Hypophysenvorderlappenhomon und Ovarialhormon im Harn von Schwangeren. *Klinische Wochenschrift* 1927; **6**: 1322.

Assali N. S., and Hamermesz J. Adrenocorticotropic substances from human placenta. *Journal of Clinical Endocrinology* 1954; **14**: 781-782.

Bachman C., Leekley D. and Hirschmann H. Excretion of sodium pregnanediol glucuronidate in urine of normal human pregnancy. *Journal of Clinical Investigation* 1940; **19**: 801-807.

Balducci J., Risek B., Gilula N. B., Hand A., Egan J. F. X. and Vintzileos A. M. Gap junction formation in human myometrium: a key to preterm labour? *American Journal of Obstetrics and Gynaecology* 1993; **168**: 1609-1615.

Balieu E-E. Three sulfate esters of 17-ketosteroids in the plasma of normal subjects and after administration of ACTH. *Journal of Clinical Endocrinology and Metabolism* 1960; **20**: 900-904.

Balieu E-E. and Dray F. Conversion of H^3 -dehydroisoandrosterone (3β-hydroxy- Δ^5-androsten-17-one) sulfate to H^3-estrogens in normal pregnant women. *Journal Of Clinical Endocrinology and Metabolism* 1963; **23**: 1298-1301.

Barnea E. R., Lavy G., Fakih H. and Decherney A. H. The role of ACTH in placental steroidogenesis. *Placenta* 1986; **7**: 307-313.

Bassett J. M., Oxoborrow T. J., Smith I.D. and Thorburn G. D. The concentration of progesterone in the peripheral plasma of the pregnant ewe. *Journal of Enocrinology* 1969; **45**: 449-457.

Beaudoin C., Blomquist C. H. and Tremblay Y. Gene expression of 17β-hydroxysteroid dehydrogenase type 2 isozyme in primary cultures of human trophoblasts predicts different mechanisms regulating type 1 and type 2 enzymes. *Endocrinology* 1995; **136**: 3807-3814.

Beckett G. J. and Boyd G. S. Purification and control of bovine adrenal cortical cholesterol hydrolase and evidence for the activation of the enzyme by a phosphorylation. *European Journal of Biochemistry* 1977; **72**: 223-233.

Bellino F. L. and Osawa Y. Evidence of the direct aromatization of testosterone and different aromitization sites for testosterone and androstenedione in human placental microsomes. *Biochemistry* 1974; **13**:1925-1931.

Beyer K. F. and Samuels L. T. Distribution of steroid-3β-ol-dehydrogenase in cellular structures of the adrenal gland. *The Journal of Biological Chemistry* 1956; **219**: 69-76.

Beyer E. C., Kistler J., Paul D. L. and Goodenough D. A. Antisera directed against connexin 43 peptides react with 43-kD protein localized to gap junctions in myocardium and other tissues. *Journal of Cell Biology* 1989; **108**: 595-605.

Bhaumick B., Bala R. M. and Hollenberg M. D. Somatomedin receptor of human placenta: solubilization, photolabeling, partial purification, and comparison with insulin receptor. *Proceedings of the National Acadamy of Sciences* 1981; **78**: 4279-4283.

Bhaumick B. and Bala R. M. Basic somatomedin (B-SM) receptors in human term placenta explants. *Biochemical and Biophysical Research Communications* 1984; **122**: 583-592.

Bloch K. The biological conversion of cholesterol to pregnanediol. *The Journal of Biological Chemistry* 1945; **157**: 661-666.

Blomquist C. H., Linderman N. J. and Hakanson E. Y. 17β-Hydroxysteroid and 20α-hydroxysteroid dehydrogenase activities of human placental micrososmes: kinetic evidence for two enzymes differing in substrate specificity. *Archives of Biochemistry and Biophysics* 1985: **239**: 206-215.

Blomquist C. H., Linderman N. J. and Hakanson E. Y. Steroid modulation of 17β-hydroxysteroid oxidoreductase activities in human placental villi *in vitro. Journal Of Clinical Endocrinology and Metabolism* 1987; **65**: 647-652.

Blomquist C. H., Bealka D. G., Hensleigh H. C. and Tagatz G. E. A comparison of 17β-hydroxysteroid oxidoreductase type 1 and type 2 activity of cytosol and microsomes from human term placenta, ovarian stroma and granulosa-luteal cells. *Journal of Steroid and Molecular Biology* 1994; **49**: 183-189.

Bolté E., Mancuso S,. Eriksson G., Wiqvist N. and Diczfalusy E. Studies on the aromatization of neutral steroids in pregnant women. I. Aromatisation of C-19 steroids by placentas perfused *in situ. Acta Endocrinologica* 1964a; **45**: 535-559.

Bolte E., Mancuso S., Eriksson G., Wiqvist N. and Diczfalusky E. Studies on the aromatisation of neutral steroids in pregnant women. 2. Aromatisation of dehydroepiandrosterone and of its sulphate administered simultaneously into a uterine artery. *Acta Endocrinologica* 1964b; **45**: 560-575.

Bradshaw T. E. T. and Jessop W. J. E. The urinary excretion of oestrogens and pregnanediol at the end of pregnancy, during labour and during early puerperium. *Journal of Endocrinology* 1953; **9**: 427-439.

Brown J. B. Urinary excretion of oestrogens during pregnancy, lactation and the re-establishment of menstruation. *Lancet* 1956 ; **1**: 704-707.

Brown M. S., Dana S. E. and Goldstein J. L. Regulation of 3-hydroxy-methyglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. *Proceedings of the National Acadamy of Sciences USA* 1973; **70**: 2162-2166.

Brown M. S., Dana S. E. and Goldstein J. L. Regulation of 3-hydroxy-methyglutaryl coenzyme A reductase activity in cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *The Journal of Biological Chemistry* 1974; **249**: 789-796.

Brown M. S., Dana S. E. and Goldstein J. L. Cholesterol ester formation in cultured human fibroblasts. *The Journal of Biological Chemistry* 1975; **250**: 4025-4027.

Brown M. S., Faust J. R. and Goldstein J. L. Role of the low density lipoprotein receptor in regulating the content of free and esterified cholesterol in human fibroblasts. *The Journal of Clinical Investigation.* 1975; **55**: 783-793.

Brown M. S., Kovanen P. T. and Goldstein J. L. Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. *Recent Progress in Hormone Research* 1979; **35**: 215-257.

Browne J. S. H., Henry J. S. and Venning E. H. The corpus luteum hormone in pregnancy. *Journal of Clinical Investigation* 1937; **16**: 678.

Brownie A. C., Simpson E. R., Jefcoate C. R., Boyd G. S., Orme-Johnson W. H. and Beinert H. Effect of ACTH on cholesterol side-chain cleavage in rat adrenal mitochondria. *Biochemical and Biophysical Research Communications* 1972; **46**: 483-490.

Brownie A. C., Alfano J., Jefcoate C. R., Orme-Johnson W., Beinert H. and Simpson E. R. Effect of ACTH on adrenal mitochondrial cytochrome P-450 in the rat. *Annals of New York Acadamy of Sciences* 1973; **212**: 344-358.

Burstein S., Byon C. Y., Kimball H. L. and Gut M. Conversion of 20 (22)-dehydrocholesterol as an intermediate in the biosynthesis of pregnenolone in bovine adrenocortical mitochondrial acetone-dried powder preparations. *Steroids* 1976; **27**: 691-701.

Burstein S. and Gut M. Intermediates in the conversion of cholesterol to pregnenolone: kinetics and mechanism. *Steroids* 1976; **28**: 115-131.

Caldwell B. V., Tillson S. A., Brock W. A. and Speroff L. The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes. *Prostaglandins* 1972; **1**: 217-228.

Campbell E. A., Linton E. A., Wolfe D. A., Scraggs P. R., Jones M. T. and Lowry P. J. Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. *Journal Of Clinical Endocrinology and Metabolism* 1987; **64**: 1054-1059.

Canick J. A. and Ryan K. J. The inhibition of 16α-hydroxytestosterone aromatization by carbon monoxide. *Biochemical and Biophysical Research Communications* 1975; **63**: 496-501.

Canick J. A. and Ryan K. J. Cytochrome P-450 and the aromatization of 16α-hydroxytestosterone and androstenedione by human placental microsomes. *Molecular and Cellular Endocrinology.* 1976; **6**: 105-115.

Cantineau R., Kremers P., De Graeve J., Giellen J. E. and Lambotte R. Aromatization of 15α- and 16α- hydroxylated androgens in the human placenta using (1, 2-3H) substrates. *Journal of Steroid Biochemistr*y 1982; **16**: 157-163.

Casella S. J., Han V. K., D'Ercole A. J. Svoboda M. E. and Van-Wyk J. J. Insulin-like growth factor II binding to the type I somatomedin receptor. Evidence for two high affinity binding sites. *The Journal of Biological Chemistry* 1986; **261**: 9268-9273

Cassmer O. Hormone production of the human placenta. Studies on the role of the foetus in the endocrine functions of the placenta. *Acta Endocrinologica* 1959; **supplement 45** : 5-82.

Castracane V. D. and Jordan V. C. The effect of estrogen and progesterone on uterine prostaglandin biosynthesis in the ovariectomized rat. *Biology of Reproduction* 1975; **13**: 587-596.

Caufriez A., Frankenne F., Englert.Y., Golstein J., Cantraine F., Hennen G and Copinischi G. Placental growth hormone as a potential regulator of maternal IGF-I during human pregnancy. *American Journal of Physiology* 1990; **258**: E1014-E1019.

Challis J. R. G. Sharp increase in free circulating oestrogens immediately before parturition in sheep. *Nature* 1971; **229**: 208.

Chen S., Besman M. J., Sparkes R. S., Zollman S., Klisak I., Mohandas T., Hall P. F. and Shively J. E. Human aromatase: cDNA cloning, southern blot analysis, and the assignment of the gene to chromosome 15. *DNA* 1988; **7**: 27-38.

Chow L. and Lye S. T. Expression of the gap junction protein connexin-43 is increased in the human myometrium toward term and with the onset of labour. *American Journal of Obstetrics and Gynaecology* 1994; **170**: 788-795.

Clark B. J., Wells J., King S. R. and Stocco D. M. The purification, cloning, and expression of a novel luteinizing hormone- induced mitochondrial protein in MA-10 mouse leydig tumour cells. Characterization of the steroidogenic acute regulatory protein (StAR). *The Journal of Biological Chemistry* 1994; **269**: 28314-28322.

Cohen B. E., Marrian G. F. and Watson M. C. Excretion of oestrin during pregnancy. *Lancet* 1935; **1**: 674-676.

Cole P. A. and Robinson C. H. A peroxide reaction for placental aromatase. *Journal of the American Chemical Society* 1988; **110**: 1284-1285.

Constantopoulos G. and Tchen T. T. Conversion of 20-α-hydroxycholesterol to pregnenolone. *Biochemical and Biophysical Research Communications* 1961a; **4**: 460-463.

Constantopoulos G. and Tchen T. T. Cleavage of cholesterol side chain by adrenal cortex. I. Cofactor requirement and product of cleavage. *The Journal of Biological Chemistry* 1961b; **236**: 65-67.

Cooper E. S., Brooks A. N., Miller M. R. and Greer I. A. Corticotrophin-releasing factor immunostaining is present in placenta and fetal membranes from the first trimester onwards and is not affected by labour or administration of mifepristone. *Clinical Endocrinology* 1994; **41**: 677-683.

Corbin C. J., Graham-Lorence S., McPhaul M., Mason J. I., Mendelson C. R. and Simpson E. R. Isolation of a full-length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in nonsteroidogenic cells. *Proceedings of the National Acadamy of Sciences U. S. A* 1988; **85**: 8948-8952.

Corner G. W. and Allen W. M. Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. *American Journal of Physiology* 1929; **88**: 326-339.

Csapo A. I. and Wiest W. G. An examination of the quantitative relationship between progesterone and the maintenance of pregnancy. *Endocrinology* 1969; **85**: 735-746.

Currie W. B. and Jeremy J. Y. *In vitro* action of progesterone on myometrium: I. reversible modulation of the resistance of rabbit uterus to excitation-contraction uncoupling. *Biology of Reproduction* 1979; **21**: 945-952.

Darne J., McGarrigle H. H. G. and Lachelin G. C. L. Saliva oestriol, oestradiol, progesterone levels in pregnancy: spontaneous labour at term is prededed by a rise in the saliva oestriol:progesterone ratio. *British Journal of Obstetrics and Gynaecology* 1987; **94**: 227-235.

Davis M. E., Plotz E. J., LeRoy G. V., Gould R. G. and Werbin H. Hormones in human reproduction. Part I: Metabolism of progesterone. *American Journal of Obstetrics and Gynaecology* 1956; **72**: 739-755.

Davis W. W. and Garren L.D. Evidence for the stimulation by adrenocorticotropic hormone of the conversion of cholesterol esters to cholesterol in the adrenal, *in vivo*. *Biochemical and Biophysical Research Communications.* 1966; **24**: 805-810.

De Hertogh R., Thomas K., Bietlot Y., Vanderheyden I. and Ferin J. Plasma levels of unconjugated estrone, estradiol and estriol and of HCS throughout pregnancy in normal women. *Journal Of Clinical Endocrinology and Metabolism* 1975; **40**: 93-101.

Demers L. M., Yoshinaga K. and Greep R. O. Prostaglandin F in monkey uterine fluid during the menstrual cycle and following steroid treatment. *Prostaglandins* 1974; 5: 513-519.

Diczfalusy E. Progesterone in human placental tissue. I. Separation and determination of progesterone . *Acta Endocrinologica* 1952; **10**: 373-389.

Diczfalusy E. and Troen P. Endocrine functions of the human placenta. *Acta Endocrinologica* 1953; **supplementum 12 (XII)**: 229-311.

Doisy E. A., Veller C. D. and Thayer S. A. Folliculin from urine of pregnant women. *American Journal of physiology* 1929; **90**: 229-330.

Dubois R. N., Simpson E. R., Kramer R. E. and Waterman M. R. Induction of synthesis of cholesterol side chain cleavage cytochrome P-450 by adrenocorticotropin in cultured bovine adrenocortical cells. *The Journal of Biological Chemistry* 1981; **256**: 7000-7005.

Dupont E., Labrie F., Luu-The V. and Pelletier G. Localization of 17β-hydroxysteroid dehydrogenase throughout gestation in human placenta. *The Journal of Histochemistry and Cytochemistry.* 1991; **39**: 1403-1407.

Duque C., Morisaki M. and Ikekawa N. The enzyme activity of bovine adrenocortical cytochrome P-450 producing pregnenolone from cholesterol: kinetic and electrophoretic studies on the reactivity of hydroxycholesterol intermediates. *Biochemical and Biophysical Research Communications* 1978; **82**: 179-187.

Easterling W. E., Simmer H., Dignam W. J., Frankland M. V. and Naftolin F. Neutral C_{19} -steroids and steroid sulfates in human pregnancy. II. Dehydroepiandrosterone sulfate, 16α-hydroxydehydroepiandrosterone, and 16α-hydroxydehydroepiandrosterone sulfate in maternal and fetal blood of pregnancies with anencephalic and normal fetuses. *Steroids* 1966; **8**: 157-177.

Erickson G. F., Garzo V. G. and Magoffin D. A. Insulin-like growth factor-I regulates aromatase activity in human granulosa and granulosa luteal cells. *Journal Of Clinical Endocrinology and Metabolism* 1989; **69**: 716-724.

Estabrook R. W., Cooper D. Y. and Rosenthal O. The light reversible carbon monoxide inhibition of the steroid C21- hydroxylase system of the adrenal cortex. *Biochemische Zeitschrift* 1963; **338**: 741-755.

Fant M., Munro H. and Moses A. An autocrine/paracrine role for insulin-like growth factors in the regulation of human placental growth. *Journal Of Clinical Endocrinology and Metabolism* 1986; **63**: 449-505.

Faust J. R., Goldstein J. L. and Brown M. S. Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured mouse adrenal cells. *The Journal of Biological Chemistry* 1977; **252**: 4861-4871.

Fishman J. and Goto J. Mechanism of estrogen biosynthesis. *The Journal of Biological Chemistry* 1981; **256**: 4466-4471.

Fostis T. The multicomponent analysis of estrogens in urine by ion-exchange chromatography and GC-MS-II. Fractionation and quantitation of the main groups of estrogen conjugates. *Journal of Steroid Biochemistry* 1987; **28**: 215-226.

Fournet-Dulguerov N., MacLusky N. J., Leranth C. Z., Todd R., Mendleson C. R., Simpson E. R. and Naftolin F. Immunohistochemical localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta. *Journal Of Clinical Endocrinology and Metabolism* 1987; **65**: 757-764.

Fransden V. A. and Stakeman G. The site of production of oestrogenic hormones in human pregnancy. Hormone excretion in pregnancy with anencephalic foetus. *Acta Endocrinologica* 1961; **38**: 383-391.

Frienkel R. Of pregnancy and progeny. *Diabetes* 1980; **29**: 1023-1035.

Frim M., Emanuel R. L., Robinson B. G., Smas C. M., Adler G. K. and Majzoub J. A. Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. *Journal of Clinical Investigation* 1988; **82**: 287-292.

Froesch E. R., Bürgi H., Ramseier E. B., Bally P. and Labhart A. Antibody-suppressible and nonsupressible insulin-like activities in human serum and their physiological significance. An insulin assay with adipose tissue of increased precision and specificicty. *Journal of Clinical Investigation* 1963; **42**: 1816-1833.

Fuchs A. R., Fuchs F., Husslein P. and Soloff M. S. Oxytocin receptors in the human uterus during pregnancy and parturition. *American Journal of Obstetrics and Gynaecology* 1984; **150**: 734-741.

Fujita-Yamaguchi Y., LeBon T. R., Tsubokawa M., Henzel W., Kathuria S., Koyal D. and Ramachandran J. Comparison of insulin-like growth factor I receptor and insulin receptor purified from human placental membranes. *The Journal of Biological Chemistry* 1986; **261**: 16727-16731.

Funkenstein B., McCarthy J. L., Dus K. M., Simpson E. R. and Waterman M. R. Effect of adrenocorticotropin on steroid 21-hydroxylase synthesis and activity in cultured bovine adrenocortical cells. *The Journal of Biological Chemistry* 1983; **258**: 9398-9405.

Furlanetto R. W., Underwood L. E., Van Wyk J. J. and Handwerger S. Serum immunoreactive somatomedin-C is elevated late in pregnancy. *Journal Of Clinical Endocrinology and Metabolism* 1978; **47**: 695-696.

Garfield R. E., Sims S. M. and Daniel E. E. Gap junctions: their presence and necessity in myometrium during parturition. *Science* 1977; **198**: 958-960.

Garfield R. E., Sims S. M., Kannan M. S. and Daniel E. E. Possible role for gap junctions in activation of myometrium during parturition. *American Journal of Physiology* 1978; **235**: C168-C179.

Garfield R. E., Rabideau S., Challis J. R. G. and Daniel E. E. Hormonal control of GAP junction formation in sheep myometrium during parturition. *Biology of Reproduction* 1979a; **21**: 999-1007.

Garfield R. E., Rabideau S., Challis J. R. G. and Daniel E. E. Ultrastructural basis for maintenance and termination of pregnancy. *American Journal of Obstetrics and Gynaecology* 1979b; **133**: 308-315.

Garfield R. E., Kannan M. S. and Daniel E. E. Gap junction formation in myometrium: control by estrogens, progesterone, and prostaglandins. *American Journal of Physiology* 1980; **238**: C81-C86.

Garfield R. E. and Hayashi R. H. Appearance of gap junctions in the myometrium of women during labour. *American Journal of Obstetrics and Gynaecology* 1981; **140**: 254-260.

Garfinkel D. Isolation and properties of cytochrome b_5 from pig liver. *Archives of Biochemistry and Biophysics* 1957; **71**: 111-120.

Gargosky S. E., Moyse K. J., Walton P. E., Owens J. A., Wallace J. C., Robinson J. S. and Owens P. C. Circulating levels of insulin-like growth factors increase and molecular forms of their serum binding proteins change with human pregnancy. *Biochemical and Biophysical Research Communications* 1990; **170**: 1157-1163.

Garren L. D., Ney R. L. and Davis W. W. Studies on the role of protein synthesis in the regulation of corticosterone production by adrenocorticotropic hormone *in vivo*. *Proceedings of the National Acadamy of Sciences* 1965; **53**: 1443-1450.

Geissler W. M., Davis D. L., Wu L., Bradshaw K. D., Patel S., Mendonca B. B., Elliston K. O. Wilson J. D., Russell D. W. and Andersson S. Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. *Nature Genetics* 1994; **7**: 34-39.

Genazzani A. R., Fraioli F., Hurlimann J., Fioretti P. and Felber J. P. Immunoreactive ACTH and cortisol plasma levels during pregnancy. Detection and partial purification of corticotrophin-like placental hormone: The human chorionic corticotrophin (HCC). *Clinical Endocrinology* 1975; **4**: 1-14.

Gill G. N. and Garren L. D. On the mechanism of action of adrenocorticotropic hormone: the binding of cyclic-3',5'-adenosine monophosphate to an adrenal cortical protein. *Proceedings of the National Acadamy of Sciences USA* 1969; **63**: 512-519.

Gill G. N. and Garren L. D. Role of the receptor in the mechanism of action of adenosine 3':5'- cyclic monophosphate. *Proceedings of the National Acadamy of Sciences USA* 1971; **68**: 786-790.

Giudice L. C., Dsupin B. A., Jin I. H., Vu T. H. and Hoffman A. R. Differential expression of messenger ribonucleic acids encoding insulin-like growth factors and their receptors in human uterine endometrium and decidua. *Journal of Clinical Endocrinology and Metabolism* 1993; **76**: 1115-1122.

Goland R. S., Wardlow S. L., Stark R. I., Brown L. S. and Frantz A. G. High levels of corticotropin releasing-hormone immunoactivity in maternal and fetal plasma during pregnancy. *Journal Of Clinical Endocrinology and Metabolism* 1986; **63**: 1199-1203.

Goldstein J. L., Dana S. E. and Brown M. S. Esterification of low density lipoprotein in human fibroblasts and its absence in familial hypercholesterolemia. *Procedings of the National Acadamy of Sciences USA* 1974; **71**: 4288-4292.

Grino M., Chrousos G. P. and Margioris A. N. The corticotropin releasing hormone gene is expressed in human placenta. *Biochemical and Biophysical Research Communications* 1987; **148**: 1208-1214.

Hagerman D. D. Human placenta estrogen synthetase (aromatase) purified by affinity chromatography. *The Journal of Biological Chemistry* 1987; **262**: 2398-2399.

Halkerston I. D. K., Eichhorn J. and Hechter O. TPNH requirement for cholesterol side chain cleavage in adrenal cortex. *Archives of Biochemistry and Biophysics* 1959; **85**: 287-289.

Halkerston I. D. K., Eichhorn J. and Hechter O. A requirement for reduced triphsphopyridine nucleotide for cholesterol side-chain cleavage by mitochondrial fractions of bovine adrenal cortex. *The Journal of Biological Chemistry* 1961; **236**: 374-380.

Hall K., Enberg G., Hellem. E., Lundin G., Ottosson-Seeberger A., Sara V., Trygstad O. and Öfverholm U. Somatomedin levels in pregnancy: longitudinal study in healthy subjects and patients with growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* 1984; **59**: 587-594.

Ham E. A., Cirillo V. J., Zanetti M. E. and Kuehl F. A. Estrogen-directed synthesis of specific prostaglandins in uterus. *Proceedings of the National Acadamy of Sciences USA* 1975; **72**: 1420-1424.

Han V. K., Bassett N., Walton J. and Challis J. R. G. The expression of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) genes in the human placenta and membranes: evidence for IGF-IGFBP interactions at the feto-maternal interface. *Journal of Clinical Endocrinology and Metabolism* 1996; **81**: 2680-2693.

Harada N. Novel properties of human placental aromatase as cytochrome P450: purification and characterization of a unique form of aromatase. *Journal of Biochemistry* 1988; **103**: 106-113.

Harada N., Yamada K., Saito K., Kibe N,. Dohmae S. and Takagi Y. Structural characterization of the human estrogen synthetase (aromatase) gene. *Biochemical and Biophysical Research Communication* 1990; **166**: 365-372.

Harding B., Wong B. Y. and Nelson D. H. Carbon monoxide-combining substances in rat adrenal. *Biochemica et Biophysica Acta* 1964; **92**: 415-417.

Harding B. W., Wilson L. D., Wong S. H. and Nelson D. H. Electron carriers of the rat adrenal and the 11β-hydroxylating system. 1965 *Steroids* ; **6 Supplement 2** : 51-77.

Haynes R. C. The activation of adrenal phosphorylase by the adrenocorticotropic hormone. *The Journal of Biological Chemistry* 1958; **233**: 1220-1222.

Hellig H., Gattereau D., Lefebvre Y. and Bolté E. Steroid production from plasma cholesterol. I. Conversion of plasma cholesterol to progesterone in humans. *Journal of Clinical Endocrinology and Metabolism* 1970; **30**: 624-631.

Henson M. C., Shi W., Greene S. J. and Reggio B. C. Effects of pregnant human, nonpregnant human, and fetal bovine sera on human chorionic gonadotropin, estradiol, and progesterone release by cultured human trophoblast cells. *Endocrinology* 1996; **137**: 2067-2074.

Hosoda H. and Fishman J. Unusually facile aromatization of 2β-hydroxy-19-oxo-4-androstene-3,17-dione to estrone. Implications in estrogen biosynthesis. *Journal of the American Chemical Society* 1974; **96**: 7325-7329.

Huffman M. N., Thayer S. A. and Doisy E. A. The isolation of α-dihydrotheelin from human placenta. *The Journal of Biological Chemistry* 1940; **133**: 567-571.

Huffman M. N., MacCorquodale D. W., Thayer S. A., Doisy E. A., Smith G. V. and Smith O. W. The isolation of α-dihydrotheelin from human pregnancy urine. *The Journal of Biological Chemistry* 1940; **134**: 591-604.

Hume R. and Boyd G. S. Cholesterol metabolism and steroid hormone production. *Biochemical Society Transactions* 1978; **6**: 893-898.

Hume R., Kelly R. W., Taylor P. L. and Boyd G. S. The catalytic cycle of cytochrome P-450scc and intermediates in the conversion of cholesterol to pregnenolone. *European Journal of Biochemistry*. 1984; **140**: 583-591.

Ishii-Ohba H., Inano H. and Tamaoki B. I. Testicular and adrenal 3β-hydoxy-5-ene-steroid dehydrogenase and 5-ene-4-ene isomerase. *Journal of Steroid Biochemistry* 1987; **27**: 775-779.

Jaffe R. B., Pérez-Palacios G. and Lamont K. G. *De novo* steroid sulfate biosynthesis. *Journal Of Clinical Endocrinology and Metabolism* 1968; **28**: 1671-1674.

Jailer J. and Knowlton A. I. Stimulated adreno-cortical activity during pregnancy in an Addisonian patient. *Journal Of Clinical Investigation* 1950; **29**: 1430-1436.

Jefcoate C. R., Simpson E. R. and Boyd G. S. Spectral properties of rat adrenal-mitochondrial cytochrome P-450. *European Journal of Biochemistry* 1974; **42**: 539-551.

Jefcoate C. R. and Orme-Johnson W. Cytochrome P-450 of adrenal mitochondria. *In vitro* and *in vivo* changes in spin states. *The Journal of Biological Chemistry* 1975; **250**: 4671-4677.

Johansson E. D. B. Plasma levels of progesterone in pregnancy measured by a rapid competitive protein binding technique. *Acta Endocrinologoica* 1969; **61**: 607-617.

John M. E., John M. C., Ashley P., MacDonald R. J., Simpson E. R. and Waterman M. R. Identification and characterization of cDNA clones specific for cholesterol side-chain cleavage cytochrome P-450. *Proceedings of the National Acadamy of Sciences USA* 1984; **81**: 5628-5632.

John M. E., John M. C., Boggaram V., Simpson E. R. and Waterman M. R. Transcriptional regulation of steroid hydroxylase genes by corticotropin. *Proceedings of the National Acadamy of Sciences USA* 1986; **83**: 4715-4719.

Jones S. A., Brooks A. N. and Challis J. R. G. Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. *Journal of Clinical Endocrinology and Metabolism* 1989; **68**: 825-830.

Jones S. A. and Challis J. R. G. Local stimulation of prostaglandin production by corticotropin-releasing hormone in human fetal membranes and placenta. *Biochemical and Biophysical Research Communications* 1989; **159**: 192-199.

Jones S .A. and Challis J. R. G. Steroid, corticotrophin-releasing hormone, ACTH and prostaglandin interactions in the amnion and placenta of early pregnancy in man. *Journal of Endocrinology* 1990; **125**: 153-159.

Karaboyas G. C. and Koritz S. B. Identity of the site of action of 3', 5'-adenosine monophosphate and adrenocorticotropic hormone in corticosteroidogenesis in rat adrenal and beef adrenal cortex slices. *Biochemistry* 1965; **4**: 462-468.

Kellis J. T. and Vickery L. E. Purification and characterization of human placental aromatase cytochrome P-450. *The Journal of Biological Chemistry* 1987; **262**: 4413-4420.

Kelly W. G., Judd D. and Stolee A. Aromatisation of Δ^4-androstene-3,17-dione, 19-hydroxy-Δ^4-androstene-3, 17-dione, and 19-oxo-Δ^4-androstene-3,17-dione at a common catalytic site in human placental microsomes. *Biochemistry* 1977; **16**: 140-145.

Kimura T. Effects of hypophysectomy and ACTH administration on the level of adrenal cholesterol side-chain desmolase. *Endocrinology* 1969; **85**: 492-499.

Kirschner M. A., Wiqvist N. and Diczfalusy E., Studies on oestriol synthesis from dehydroepiandrosterone sulphate in human pregnancy. *Acta Endocrinologica* 1966; **53**: 584-597.

Kitawaki J., Inoue S., Tamura T., Yamamoto T., Noguchi T., Osawa Y. and Okada H. Increasing aromatase cytochrome P-450 level in human placenta during pregnancy: studied by immunohistochemistry and enzyme-linked immunosorbent assay. *Endocrinology* 1992; **130**: 2751-2757.

Kliman H. J., Nestler J. E., Sermasi E., Sanyer J. M. and Strauss J. F. III. Purification, characterization, and *in vitro* differentiation of cytotrophoblasts from human term placentae. *Endocrinology* 1986; **118**: 1567-1582.

Klingenberg M. Pigments of rat liver microsomes. *Archives of Biochemistry and Biophysics* 1958; **75**: 376-386.

Knowlton A. I., Gilbert H., Mudge G. H. and Jailer J. W. Pregnancy in Addinsons disease. Report on four patients. *Journal of Clinical Endocrinology* 1949; **9**: 514-528.

Koide S. S. and Torres M. T. Distribution of 3β-hydroxysteroid dehydrogenase and Δ^5-3-oxosteroid isomerase in homogenate fractions of human term placenta. *Biochimica et Biophysica Acta* 1965; **105**: 115-120.

Koritz S. B. and Kumar A. M. On the mechanism of action of the adrenocorticotrophic hormone. The stimulation of the activity of enzymes involved in pregnenolone synthesis. *The Journal of Biological Chemistry* 1970; **245**: 152-159.

Koritz S. B. and Moustafa A. M. Some characterisitics of adrenal steroidogenesis and their possible relationships to the action of the adrenocorticotropic hormone. *Archives of Biochemistry and Biophysics* 1976; **174**: 20-26.

Kovanen P. T., Faust J. R., Brown M. S. and Joseph L. Low density lipoprotein receptors in bovine adrenal cortex. I. Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured adrenocortical cells. *Endocrinology* 1979; **104**: 599-609.

Kramer R. E., Rainey W. E., Funkenstein B., Dee A., Simpson E. R. and Waterman M. R. Induction of synthesis of mitochondrial steroidogenic enzymes of bovine adrenocortical cells by analogs of cyclic AMP. *The Journal of Biological Chemistry.* 1984; **250**: 707-713.

Laatikainen T., Saijonmaa O., Salminen K. and Whalström T. Localization and concentrations of β–endorphin and β-lipotrophin in human placenta. *Placenta* 1987; **8**: 381-387.

Laatikainen T., Virtanen T., Räisänen I. and Salminen K. Immunoreactive corticotropin-releasing factor and corticotropin during pregnancy, labour and puerperium. *Neuropetides* 1987; **10**: 343-353.

Labrie F., Luu-The V., Lin S., Labrie C., Simard J., Breton R. and Bèlanger A. The key role of 17β-hydroxysteroid dehydrogenases in sex steroid biology. *Steroids* 1997; **62**: 148-158.

Lang L. M., Beyer E. C., Schwatlz A. L. and Gitlin J. D. Molecular cloning of a rat uterine gap junction protein and analysis of gene expression during gestation. *American Journal of Physiology* 1991; **260**: E787-793.

Langer L. J. and Engel L. L. Human placental estradiol-17β dehydrogenase I. concentration, characterization and assay. *The Journal of Biological Chemistry* 1958; **233**: 583-588.

LeBon T. R., Jacobs S., Cuatrecasas P., Kathuria S. and Fujita-Yamaguchi Y. Purification of insulin-like growth factor I receptor from human placental membranes. *The Journal of Biological Chemistry* 1986; **261**: 7685-7689.

Ledger W. L., Webster M. A., Anderson A. B. M. and Turnbull A. C. Effect of inhibition of prostaglandin synthesis on cervical softening and uterine activity during ovine parturition resulting from progesterone withdrawal induced by epostane. *Journal of Endocrinology* 1985; **105**: 227-233.

Leenders F., Adamski J., Husen B., Thole H. H. and Jungblut P. W. Molecular cloning and amino acid sequence of the porcine 17β-estradiol dehydrogenase. *European Journal of Biochemistry* 1994; **222**: 221-227.

Liggins G. C., Fairclough R. J., Grieves S. A., Kendall J. Z. and Knox B. S. The mechanism of initiation of parturition in the ewe. *Recent Progress in Hormone Research* 1973; **29**: 115-150.

Lind T., Billewicz W. Z. and Brown G. A serial study of changes occurring in the oral glucose tolerance test during pregnancy. *The Journal of Obstetrics and Gynaecology of the British Commonwealth* 1973; **80**: 1033-1039.

Linton E. A., Perkins A. V., Woods, R. J., Eken F., Wolfe C. D. A., Behan D. P., Potter E., Vale W. W. and Lowry P. J. Corticotropin releasing hormone-binding protein (CRH-BP): plasma levels decrease during the third trimester of normal human pregnancy. *Journal of Clinical Endocrinology and Metabolism* 1993; **76**: 260-262.

Liotta A. S. and Krieger D. T. *In vitro* synthesis and comparative posttranslational processing of immunoreactive precursor corticotropin β-endorphin by human placental and pituitary cells. *Endocrinology* 1980; **106**: 1054-1511.

Liotta A., Osathanondh R., Ryan K. J. and Krieger D. T. Presence of corticotropin in human placenta: demonstration of *in vitro* synthesis. *Endocrinology* 1977; **101**: 1552-1558.

Lorence M. C., Murry B. A., Trant J. M. and Mason J. I. Human 3β-hydoxysteroid dehydrogenase/ Δ^{5}-> ^{4} isomerase from placenta: expression in nonsteroidogenic cells of a protein that catalyzes the dehydrogenation/isomerization of C21 and C19 steroids. *Endocrinology* 1990; **126**: 2493-2498.

Luu The V., Labrie C., Zhao H. F., Couët J., Lachance Y., Simard J., Leblanc G., Côté J., Bérubé D., Gagné R. and Labrie F. Characterization of cDNAs for human estradiol 17β-dehydrogenase and assignment of the gene to chromosome 17: evidence for two mRNA species with distinct 5'-termini in human placenta. *Molecular Endocrinology* 1989; **3**: 1301-1309.

Luu-The V., Lachance Y., Labrie C., Leblanc G., Thomas J. L., Strickler R. C. and Labrie F. Full length cDNA structure and deduced amino acid sequence of human 3β-hydroxy-5-ene steroid dehydrogenase. *Molecular Endocrinology* 1989; **3**: 1310-1312.

Luu-The V., Labrie C., Simard J., Lachance Y., Zhao H. F., Couët J., Leblanc G. and Labrie F. Structure of two tandem human 17β-hydroxysteroid dehydrogenase genes. *Molecular Endocrinology* 1990; **4**: 268-275.

Luu-The V., Takahashi M. and Labrie F. Purification of microsomal and mitochondrial 3β-hydroxysteroid dehyrdogenase/Δ^{4}-Δ^{5} isomerase from human placenta. *Annals of the New York Acadamy of Sciences* 1990; **595**: 386-388.

Luu-The V., Takahashi M., de Launoit Y., Dumont M., Lachance Y. and Labrie F. Evidence for distinct dehydrogenase and isomerase sites within a single 3β-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase protein. *Biochemistry* 1991; **30**: 8861-8865.

Lye S. J., Nicholson B. J., Mascarenhas M., MacKenzie L. and Petrocelli T. Increased expression of connexin-43 in the rat myometrium during labor is associated with an increase in the plasma estrogen:progesterone ratio. *Endocrinology* 1993; **132**: 2380-2386.

Lynn W. S., Staple E. and Gurin S. The degradation of cholesterol by mammalian tissue extracts. *Journal of the American Chemical Society* 1954; **76**: 4048.

MacCorquodale D. W., Thayer S. A. and Doisy E. A. Crystalline ovarian follicular hormone. *Proceedings of the Society of Experimental Biology New York* 1935; **32**: 1182.

MacDonald P. C. and Sitteri P. K. Origin of estrogen in women pregnant with an anencephalic fetus. *Journal of Clinical Investigations* 1965; **44**: 465-474.

MacDonald P. C. and Sitteri P. Placenta estrogen biosynthesis during human pregnancy. *Journal Of Clinical Endocrinology and Metabolism* 1966 ; **26**: 751-761.

Mackenzie L. W. and Garfield R. E. Hormonal control of gap junctions in the myometrium. *American Journal of Physiology* 1985; **248**: C296-C308.

Magendantz H. G. and Ryan K. J. Isolation of an estriol precursor, 16α-hydroxydehydroepiandrosterone, from human umbilical sera. *Journal of Clinical Endocrinology* 1964; **24**: 1155-1162.

Margioris A. N., Grino M., Protos P., Gold P. W. and Chrousos G. P. Corticotropin-releasing hormone and oxytocin stimulate the release of placental proopiomelanocortin peptides. *Journal of Clinical Endocrinology and Metabolism* 1988; **66**: 922-926.

Marrian G. F. The chemistry of oestrin. I. Preparation from urine and separation from an unidentified solid alcohol. *Biochemical Journal* 1929; **23**: 1090-1098.

Marrian G. F. The chemistry of oestrin. III. An improved method of preparation and the isolation of active crystalline material. *Biochemical Journal* 1930; **24**: 435-445.

Marshall R. N., Underwood L. E., Voina S. J., Foushee D. B. and Van Wyk J. J. Characterization of the insulin and somatomedin-C receptors in human placental cell membranes. *Journal of Clinical Endocrinology and Metabolism* 1974; **39**: 283-292.

Martel C., Rhéaume E., Takahashi M., Trudel C., Couet J., Luu-The V., Simard J. and Labrie F. Distribution of 17β-hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. *Journal of Steroid Biochemistry and Molecular Biology* 1992; **41**: 597-603.

Mason J. I. and Boyd G. S. The cholesterol side-chain cleavage enzyme system in mitochondria of human term placenta. *European Journal of Biochemistry* 1971; **21**: 308-321.

Mason J. I., Ushijima K., Doody K. M., Nagai K., Naville D., Head J. R., Milewich L., Rainey W. E. and Ralph M. M. Regulation of expression of the 3β-hydroxysteroid dehydrogenases of human placenta and fetal adrenal. *Journal of Steroid Biochemistry and Molecular Biology* 1993; **47**: 151-159.

Massague J. and Czech M. P. The subunit structure of two distinct receptors for insulin-like growth factoors I and II and their relationship to the insulin receptor. *The Journal of Biological Chemistry* 1982; **257**: 5038-5045.

Mathur R. S., Landgrebe S. and Williamson H. O. Progesterone, 17-hydroxyprogesterone, estradiol, and estriol in late pregnancy and labour. *American Journal of Obstetrics and Gynaecology* 1980; **136**: 25-27.

McGarrigle H. H. G. and Lachelin G. C. L. Increasing saliva (free) oestriol to progesterone ratio in late pregnancy: a role for oestriol in initiating spontaneous labour in man? *British Medical Journal* 1984; **289**: 457-459.

McLean M., Bisits A., Davis J., Woods R., Lowry P. and Smith R. A placental clock controlling the length of human pregnancy. *Nature Medicine* 1995; **1**: 461-463.

Means G. D., Mahendroo M. S., Corbin C. J., Mathis J. M., Powell F. E,. Mendelson C. R. and Simpson E. R. Structural analysis of the gene encoding human aromatase cytochrome P-450, the enzyme responsible for estrogen biosynthesis. *The Journal of Biological Chemistry* 1989; **264**: 1385-19391.

Meigs R. A. and Ryan K. J. Mixed function oxidase reactions in biological oxidations: human placenta. *Science* 1967; **158**: 532.

Meigs R. A. and Ryan K. J. Cytochrome P-450 and steroid biosynthesis in the human placenta. *Biochimica et Biophysica Acta* 1968; **165**: 476-482.

Meigs R. A. and Ryan K. J. Enzymatic aromatization of steroids I. Effects of oxygen and carbon monoxide on the intermediate steps of estrogen biosynthesis. *The Journal of Biological Chemistry* 1971; **246**: 83-87.

Mendelson C. R., Wright E. E., Evans C. T., Porter J. C. and Simpson E. R. Preparation and characterization of polyclonal and monoclonal antibodies against human aromatase cytochrome P-450 (P-450arom), and their use in its purification. *Archives of Biochemistry and Biophysics* 1985; **243**: 480-491.

Mesiano S. and Jaffe R. B. Interaction of insulin-like growth factor-II and estradiol directs steroidogenesis in the human fetal adrenal toward dehydroepiandrosterone sulfate production. *Journal Of Clinical Endocrinology and Metabolism* 1993; **77**: 754-758.

Meyer A. S. Conversion of 19-hydroxy-Δ^4-androstene-3,17-dione to estrone by endocrine tissue. *Biochimica et Biophysica Acta* 1955; **17**: 441-442.

Migeon C. J. and Plager J. E. Identification and isolation of dehydroiosandrosterone from human peripheral plasma. *Journal of Biological Chemistry* 1954; **209**: 767-772.

Miller W. L., Johnson L. K., Baxter J. D. and Roberts J. L. Processing of the precursor to corticotropin and β-lipotropin in humans. *Proceedings of the National Acadamy of Sciences USA* 1980; **77**: 5211-5215.

Mills N. C., D'Ercole A. J., Underwood L. E. and Ilan J. Synthesis of somatomedin C/insulin-like growth factor I by human placenta. *Molecular Biology of Reproduction.* 1986; **11**: 231-236.

Mitchell F. L. and Davies R. E. The isolation and estimation of the steroid oestrogens in placental tissue. *Biochemical Journal* 1954; **56**: 690-698.

Miyakawa I and Maeyama M. Transport of ACTH across human placenta. *Journal Of Clinical Endocrinology and Metabolism* 1974; **39**: 440-442.

Morohashi K., Sogawa K., Omura T. and Fujii-Kuriyama Y. Gene structure of human cytochrome P-450(scc), cholesterol desmolase. *Journal of Biochemistry* 1987; **101**: 879-887.

Mujaji W., Toumba K. J. and Oakey R. E. Urinary excretion of oestrone, oestradiol-17β and oestriol in pregnancies complicated by steroid sulphatase deficiency. *Journal of Endocrinology* 1979; **83**: 95-100.

Naghshineh S., Treadwell C. R., Gallo L. and Vahouny G. V. Activation of adrenal sterol ester hydrolase by dibutyryl cAMP and protein kinase. *Biochemical and Biophysical Research Communications* 1974; **61**: 1076-1082.

Naghshineh S., Treadwell C. R., Gallo L. L. and Vahouny G. V. Protein kinase-mediated phosphorylation of a purified sterol ester hydrolase from bovine adrenal cortex. *Journal of Lipid Research* 1978; **19**: 561-569.

Nakai Y., Nakao K., Oki S. and Imura H. Presence of immunoreactive β-lipotropin and β-endorphin in human placenta. *Life Science* 1978; **23**: 2013-2018.

Nakajin S., Shinoda M. and Hall P. F. Purification to homogeneity of aromatase from human placenta. *Biochemical and Biophysical Research Communications* 1986; **134**: 704-710.

Nestler J . E. and Wiliams T. Modulation of aromatase and P450 cholesterol side-chain cleavage enzyme activities of human placental cytotrophoblasts by insulin and insulin-like growth factor I. *Endocrinology* 1987; **121**: 1845-1852.

Nestler J. E. Insulin and insulin-like growth factor-I stimulate the 3β-hdroxysteroid dehydrogenase activity of human placental cytotrophoblasts. *Endocrinology* 1989; **125**: 2127-2133.

Nestler J. E. Insulin-like growth factor II is a potent inhibitor of the aromatase activity of human placental cytotrophoblasts. *Endocrinology* 1990; **127**: 2064-2070.

Numazawa M., Osada R., Tsuji M. and Osawa Y. High performance liquid chromatographic determination of aromatization of 16α-hydroxylated androgens with human placental microsomes. *Analytical Biochemistry* 1985; **146**: 75-81.

Odigari E., Sherrell B. J., Mount C. D., Nicholson W. E., and Orth D. N. Human placental immunoreactive corticotropin, lipotropin, and β-endorphin: evidence for a common precursor. *Proceedings of the National Acadamy of Sciences USA* 1979; **76**: 2027-2031.

Okamoto E., Takagi T., Makino T., Sata H., Iwata E., Nishino N., Mitsuda N., Sugita N., Otsuki Y. and Tanizawa O. Immunoreactive corticotrophin-releasing hormone, adrenocorticotropin, and cortisol in human plasma during pregnancy and delivery and postpartum. *Hormone and Metabolic Research* 1989; **21**: 566-572.

Omura T. and Sato R. A new cytochrome in liver micrososmes. *Journal of Biological Chemistry* 1962; **237**: PC1375-1376.

Omura T., Sato R., Cooper D. Y., Rosenthal O. and Estabrook R. W. Function of cytochrome P-450 of microsomes. *Federation Proceedings* 1965; **24**: 1181-1189.

Orth D. N. and Mount C. D. Specific high-affinity binding protein for human corticotropin-releasing hormone in normal human plasma. *Biochemical and Biophysical Research Communications* 1987; **143**: 411-417.

Osawa Y., Tochigi B., Higashiyama T., Yarborough C., Nakamura T. and Yamamoto T. Multilple forms of aromatase and response of breast cancer aromatase to antiplacental aromatase II antibodies. *Cancer Research* 1982; **42**: 3299s-3306s.

Paul S., Das C., Jailkhani B. L. and Talwar G. P. Progesterone synthesis by human placental syncytiotrophoblast *in vitro* -preferred precursor and effect of human chorionic gonadotropin. *Journal of Steroid Biochemistry* 1981; **14**: 311-313.

Pearlman W. H. and Cerceo E. The isolation of progesterone from human placenta. *Journal of Biological Chemistry* 1952; **198**: 79 -83.

Pearlman W. H., Cerceo E. and Thomas M. The conversion of Δ^5-pregnen-3β-ol-20-one to progesterone by human placental tissue. *Journal of Biological Chemistry* 1953; **208**: 231-239.

Penhoat A., Jaillard C. and Saez J. M. Synergistic effects of corticotropin and insulin like-growth factor I on corticotopin receptors and corticotropin responsiveness in cultured bovine adrenocortical cells. *Biochemical and Biophysical Research Communications* 1989; **165**: 355-359.

Peterson G. L. A simplification of the protein assay method of Lowry *et al* which is more generally applicable. *Analytical Biochemistry* 1977; **83**: 346-356.

Petraglia F., Sawchenko P. E., Rivier J. and Vale W. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature* 1987; **328**: 717-719.

Petraglia F., Potter E., Cameron V. A., Sutton S., Behan D. P., Woods R. J., Sawchenko P. E., Lowry P. J. and Vale W. Corticotropin-releasing factor-binding protein is produced by human placenta and intrauterine tissues. *Journal of Clinical Endocrinology and Metabolism* 1993; **77**: 919-924.

Petrocelli T. and Lye S. Regulation of transcripts encoding the myometrial gap junction protein, connexin-43, by estrogen and progesterone. *Endocrinology* 1993; **133**: 284-290.

Pham-Huu-Trung M. T., Villette J. M., Bogyo A., Duclos J. M., Fiet J. and Binoux M. Effects of insulin-like growth factor (IGF-I) on enzymatic activity in human adrenocortical cells. Interactions with ACTH. *Journal of Steroid Biochemistry and Molecular Biology* 1991; **39**: 903-909.

Potter E. A., Behan D. P., Fischer W. H., Linton E. A., Lowry P. J. and Vale W. W. Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. *Nature* 1991; **349**: 423-426.

Privalle C. T., Crivallo J. F. and Jefcoate C. R. Regulation of intramitochondrial cholesterol transfer to side-chain cleavage cytochrome P-450 in rat adrenal gland. *Proceedings of the National Acadamey of Sciences USA* 1983; **80**: 702-706.

Pulkkinen M. Arylsulphatase and the hydrolysis of some steroid sulphates in developing organism and placenta. *Acta Physiologica Scandinavia* 1961; **52** suplementum 180 :9-92.

Purohit A. and Oakey R. E. Evidence for separate sites for aromatisation of androstenedione and 16 alpha-hydroxyandrostenedione in human placental microsomes. *Journal of Steroid Biochemistry* 1989; **33**: 439-448.

Purvis J. L., Canick J. A., Mason J. I., Estabrook R. W. and McCarthy J. L. Lifetime of adrenal cytochrome P-450 as influenced by ACTH. *Annals New York Academy of Sciences* 1973; **212**: 319-343.

Quatero H. W. P. and Fry C. H. Placental corticotrophin releasing factor may modulate human parturition. *Placenta* 1989; **10**: 439-443.

Rees L. H., Burke C. W., Chard T., Evans S. W. and Letchworth A. T. Possible placental origin of ACTH in normal human pregnancy. *Nature* 1975; **254**: 620-622.

Renwick A. G. C. and Oliver J. F. The aromatization of (7α-^{3}H) androstenedione by human placental mitochondria. *Steroids* 1973; **22**: 123-131.

Rhéaume E., Lachance Y., Zhao H. F., Breton N., Dumont M., de Launoit Y., Trudel C., Luu-The V., Simard J. and Labrie F. Structure and expression of a new complementary DNA encoding the almost exclusive 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4- isomerase in human adrenals and gonads. *Molecular Endocrinology* 1991; **5**: 1147-1157.

Richards R. G., Hartman S. M. and Handwerger S. Human cytotrophoblast cells cultured in maternal serum progress to a differentiated syncytial phenotype expressing both human chorionic gonadotropin and human placental lactogen. *Endocrinology* 1994; **135**: 321-329.

Riley S. C., Walton J. C., Herlick J. M. and Challis J. R. G. The localization and distribution of corticotropin-releasing hormone in the human placenta and fetal membranes throughout gestation. *The Journal of Clinical Endocrinology and Metabolism* 1991; **72**: 1001-1007.

Rinderknecht E. and Humbel R. E. Polypeptides with nonsuppressible insulin-like and cell growth promoting activities in human serum: Isolation, chemical characterization, and some biological properties of forms I and II. *Proceedings of the National Acadamy of Sciences USA* 1976a; **73**: 2365-2369.

Rinderknecht E. and Humbel R. E. Amino-terminal sequences of two polypeptides from human serum with nonsuppressible insulin-like and cell-growth-promoting activities: evidence for structural homology with insulin B chain. *Proceedings of the National Acadamy of Sciences USA* 1976b; **73**: 4379-4381.

Rinderknecht E. and Humbel R. E. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *The Journal of Biological Chemistry* 1978a; **253**: 2769-2776.

Rinderknecht E. and Humbel R. E. Primary structure of humn-like growth factor II. *FEBS Letters* 1978b; **89**: 283-286.

Risek B., Guthrie S., Kumar N. and Gilula N. B. Modulation of gap junction transcript and protein expression during pregnancy in the rat. *The Journal of Cell Biology* 1991; **110**: 269-282.

Ritvos O. Modulation of steroidogenesis in choriocarcinoma cells by cholera toxin, phorbol ester, epidermal growth factor and insulin-like growth factor I. *Molecular and Cellular Endocrinology* 1988; **59**: 125-33.

Roberts J. S. and Share L. Effects of progesterone and estrogen on blood levels of oxytocin during vaginal distention. *Endocrinology* 1969; **84**: 1076-1081.

Roberts K. D., Bandy L. and Lieberman S. The occurrence and metabolism of 20α-hydroxycholesterol in bovine adrenal preparations. *Biochemistry* 1969; **8**: 1259-1270.

Robinson B. G., Emanuel R. L., Frim D. M. and Majzoub J. A. Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proceedings of the National Acadamy of Sciences USA* 1988; **85**: 5244-5248.

Rutherford K. J., Chen S. and Shivley J. E. Isolation and amino acid sequence analysis of bovine adrenal 3β-hydroxysteroid dehydrogenase/steroid isomerase. *Biochemistry* 1991; **30**: 8108-8115.

Ryan K. J. and Engel L. L. The interconversion of estrone and estradiol by human tissue slices. *Endocrinology* 1953; **52**: 286-291.

Ryan K. J. and Engel L. L. Hydroxylation of steroids at carbon 21. *The Journal of Biological Chemistry* 1957; **225**: 103-114 .

Ryan K. J. Conversion of androstenedione to estrone by placental microsomes. *Biochimica et Biophysica Acta* 1958; **27**: 658-659.

Ryan K. Biological aromatization of steroids. *The Journal of Biological Chemistry* 1959a; **234**: 268-272.

Ryan K. J. Metabolism of C-16-oxygenated steroids by human placenta: the formation of estriol. *The Journal of Biological Chemistry* 1959b; **234**: 2006-2008.

Ryan M. J., Clark K. E., Van Orden D. E., Farley D., Edvinsson L., Sjoberg N. O., Van Orden L. S. and Brody M. J. Role of prostaglandins in estrogen-induced uterine hyperemia. *Prostaglandins* 1974; **5**: 257-268.

Saba N., Hechter O. and Stone D. The conversion of cholesterol to pregnenolone in bovine adrenal homogenates. *Journal of the American Chemical Society* 1954; **76**: 3862-3865.

Saijonmaa O., Laatikainen T. and Wahlström T. Corticotrophin-releasing factor in human placenta: localization, concentration and release in vitro. *Placent*a 1988; **9**: 373-385.

Salhanick H. A., Noall M. W., Zarrow M . X. and Samuels L. T. The isolation of progesterone from human placentae. *Science* 1952; **115**: 708-709.

Salido E. C., Yen P. H., Barajas L. and Shapiro L. J. Steroid sulfatase expression in human placenta: immunocytochemistry and *in situ* hybridization study. *Journal Of Clinical Endocrinology and Metabolism* 1990; **70**: 1564-1567.

Samuels L. T., Evans G. T. and McKelvery J. L. Ovarian and placental function in Addison's disease. *Endocrinology* 1943; **32**: 422-428.

Samuels L .T., Helmreich M. L., Lasater M. B. and Reich H. An enzyme in endocrine tissues which oxidizes Δ^5-3 hydroxy steroids to α, β unsaturated ketones. *Science* 1951; **113**: 490-491.

Sasaki A., Liotta A. S., Luckey M. M., Margioris A. N., Suda T. and Krieger D. T. Immunoreactive corticotropin-releasing hormone in human plasma during the third trimester of pregnancy. *Journal of Clinical Endocrinology and Metabolism.* 1984; **59**: 812-814.

Sasaki A., Tempst P., Liotta A. S., Margioris A. N., Hood L. E., Kent S. B. H., Sato S., Shinkawa O., Yoshinaga K. and Krieger D. T. Isolation and characterization of a corticotropin-releasing hormone-like peptide from human placenta. *Journal Of Clinical Endocrinology and Metabolism* 1988; **67**: 768-773.

Sasaski A., Shinkawa O., Margioris A. N., Liotta A. S., Sato S., Murakami O., Go M., Shimizu Y., Hanew K. and Yoshinaga K. Immunoreactive corticotropin-releasing hormone in human plasma during pregnancy, labour, and delivery. *Journal Of Clinical Endocrinology and Metabolism* 1987; **64**: 224-229.

Scott J., Cowell J., Robertson M. E., Priestly L. M., Wadey R., Hopkins B., Pritchard J., Bell G. I., Rall L. B., Graham C. F. and Knott T. J. Insulin-like growth factor II gene expression in Wilms' tumor and embryonic tissues. *Nature* 1985; **317**: 260-262.

Shaw D. A., Dalziel E. M. and O'Donnell V. J. Biogenesis of oestrogens from (^{14}C) testosterone in the mitochondrial fraction of the human placenta. *Biochemical Journal* 1969; **113**: 737- 739.

Shen S. J., Wang C. Y., Nelson K. K., Jansen M. and Ilan J. Expression of insulin-like growth factor II in human placentas from normal and diabetic pregnancies. *Proceedings of the National Acadamy of Sciences USA* 1986; **83**: 9179-9182.

Shen S. J., Daimon M., Wang C. Y., Jansen M. and Ilan J. Isolation of an insulin-like growth fatcor II cDNA with a unique 5' untranslated region from human placenta. *Proceedings of the National Acadamy of Science*s 1988; **85**: 1947-1951.

Shepherd L. A., Beckett V. A., Marchant S., Serhal P. F. and McGarrigle H. H. G. The oxidative and reductive activities of 11β hydroxysteroid dehydrogenase (11β HSD) and 17β hydroxysteroid dehydrogenase (17β HSD) in five human cell types. *Journal of Endocrinology* 1995; **147 Supplement**: P108.

Shibasaki T, Odagiri E, Shizume K. and Ling N. Corticotropin-releasing factor-like activity in human placental extracts. *Journal of Clinical Endocrinology and Metabolism* 1982; **55**: 384-386.

Shikita M. and Hall P. F. The stoichiometry of the conversion of cholesterol and hydroxycholesterols to pregnenolone (3β-hydroxypregn-5-en-20-one) catalysed by adrenal cytochrome P-450. *Proceedings of the National Acadamy of Sciences USA* 1974; **71**: 1441-1445.

Siiteri P. K. and MacDonald P. C. The utilization of circulating dehydrisoandrosterone sulfate for estrogen synthesis during human pregnancy. *Steroids* 1963; **2**: 713-730.

Siiteri P. K. and MacDonald P. C. Placental estrogen biosynthesis during human pregnancy. *Journal of Clinical Endocrinology and Metabolism* 1966; **26**: 751-761.

Siiteri P. K. and Thompson E. A. Studies on human placental aromatase. *Journal of Steroid Biochemistry* 1975; **6**: 317-322.

Simpson E. R. and Boyd G. S. The cholesterol side-chain cleavage system of the adrenal cortex: a mixed-function oxidase. *Biochemical and Biophysical Research Communications* 1966; **24**: 8-17.

Simpson E. R. and Boyd G. S. The cholesterol side-chain cleavage system of bovine adrenal cortex. *European Journal of Biochemistry* 1967; **2**: 275-285.

Simpson E. R., Jefcoate C. R., Brownie A. C. and Boyd G. S. The effect of ether anaesthesia stress on cholesterol-side-chain cleavage and cytochrome P450 in rat-adrenal mitochondria. *European Journal of Biochemistry* 1972; **28**: 442-450.

Simpson E. R., McCarthy J. L. and Peterson J. A. Evidence that the cylcoheximide-sensitive site of adrenocorticotropic hormone action is in the mitochondrion. Changes in pregnenolone formation, cholesterol content, and the electron paramagnetic resonance spectra of cytochrome P-450. *The Journal of Biological Chemistry* 1978; **253**: 3135-3139.

Simpson E. R., Porter J. C., Milewich L., Wilheimer D. W. and MacDonald P. C. Regulation by plasma lipoproteins of progesterone biosynthesis and 3-hydroxy-3-methyl glutaryl coenzyme A reductase activity in cultured human choriocarcinoma cells. *Journal of Clinical Endocrinology and Metabolism* 1978; **47**: 1099-1105.

Simpson E. R., Bilheimer D. W., MacDonald P. C. and Porter J. C. Uptake and degradation of plasma lipoproteins by human choriocarcinoma cells in culture. *Endocrinology* 1979; **104**: 8- 16.

Simpson E. R. and Burkhart M. F. Acyl CoA:cholesterol acyl transferase activity in human placental microsomes: inhibition by progesterone. *Archives of Biochemistry and Biophysics* 1980a; **200**: 79-85.

Simpson E. R. and Burkhart M. F. Regulation of cholesterol metabolism by human choriocarcinoma cells in culture: effect of lipoproteins and progesterone on cholesteryl ester synthesis. *Archives of Biochemistry and Biophysics* 1980b; **200**: 86-92.

Simpson E. R. and MacDonald P. C. Endocrine physiology of the placenta. *Annual Review of Physiology* 1981; **43**: 163-188.

Sinensky M. A comparison of solution properties of cholesterol and 25-hydroxycholesterol. *Archives of Biochemistry and Biophysics* 1981; **209**: 321-324.

Skinner S. J. M. and Akhtar M. The stereospecific removal of a C-19 hydrogen atom in oestrogen biosynthesis. *Biochemical Journal* 1969; **114**: 75-81.

Sobrevilla L., Hagerman D. and Villee C. The metabolism of pregnenolone and 17α -hydroxyprogesterone by homogenates of human term placentas. *Biochimica et Biophysica Acta* 1964; **93**: 665-667.

Soloff M. S., Fernstrom M. A., Periyasamy S., Soloff S., Baldwin S. and Wieder M. Regulation of oxytocin receptor concentration in rat uterine explants by estrogen and progesterone. *Canadian Journal of Biochemistry and Cell Biology* 1983; **61**: 625-630.

Solomon S. Formation and metabolism of neutral steroids in the human placenta and fetus. *Journal Of Clinical Endocrinology and Metabolism* 1966; **26**: 762-777.

Staple E., Lynn W. S. and Gurin S. An enzymatic cleavage of the cholesterol side chain. *The Journal of Biological Chemistry* 1956; **219**: 845-851.

Stimmel B. F. The fractionation and photometric estimation of the oestrogens in human pregnancy urine. *Journal of Biological Chemistry* 1946; **162**: 99-109.

Stocco M. and Clark B. J. The role of the steroidogenic acute regulatory protein in steroidogenesis. *Steroids* 1997; **62**: 29-36.

Stone D. and Hechter O. Studies on ACTH action in perfused bovine adrenals: the site of action of ACTH in corticosteroidogenesis. *Archives of Biochemistry and Biophysics Acta* 1954; **51**: 457-469.

Strickler R. C. and Thomas J. L. Affinity labeling identifies histidine at the active site of human placental 3β-hydroxysteroid dehydrogenase and steroid 5->4-ene-isomerase. *American Journal of Obstetrics and Gynaecology* 1993; **168**: 1216-1222.

Sugawara T., Holt J. A., Driscoll D., Strauss J. F III., Lin D., Miller W. L., Patterson D., Clancy K. P., Hart I. M., Clark B. J. and Stocco D. Human steroidogenic acute regulatory protein: functional activity in COS-I cells, tissue expression, mapping of the structural gene to 8p11.2 and a pseudogene to chromosome 13. *Proceedings of the National Acadamy of Sciences USA* 1995; **92**: 4778-4782.

Talay P. and Wang V. S. Enzymatic isomerization of Δ^5-3-ketosteroids. *Biochimica et Biochemica Acta* 1955; **18**: 300-301.

Tan L. and Muto N. Purification and reconstitution properties of human placental aromatase. A cytochrome P-450-type monooxygenase. *European Journal of Biochemistry* 1986; **156**: 243-250.

Thomas J. L., Berko E. A., Faustino A., Myers R. P. and Strickler R. C. Human placental 3β-hydroxy-5-ene-steroid dehydrogenase and steroid 5->4-ene-isomerase: purification from microsomes, substrates kinetics, and inhibition by product steroids. *Journal of Steroid Biochemistry* 1988; **31**: 785-793.

Thomas J. L., Myers R. P. and Strickler R. C. Human placental 3β-hydroxy-5-ene-steroid dehydrogenase and steroid 5->4-ene-isomerase: purification from mitochondria and kinetic profiles, biophysical characterization of the purified mitochondrial and microsomal enzymes. *Journal of Steroid Biochemistry* 1989; **33**: 209-217.

Thompson E. A. and Siiteri P. K. Studies on the aromatization of C-19 androgens. *Annals of New York Acadamy of Sciences USA* 1973; **212**: 378-391.

Thompson E. A. and Siiteri P. K. Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. *The Journal of Biological Chemistry* 1974a; **249**: 5364-5372.

Thompson E. A. and Siiteri P. K. The involvement of human placental microsomal cytochrome P-450 in aromatization. *The Journal of Biological Chemistry* 1974b; **249**: 5373-5378.

Thompson E. A. and Siiteri P. K. Partial resolution of the placental microsomal aromatase complex. *Journal of Steroid Biochemistry* 1976; **7**: 635-639.

Toaff M. E., Schleyer H. and Strauss J. F. III. Metabolism of 25-hydroxycholesterol by rat luteal mitochondria and dispersed cells. *Endocrinology* 1982; **111**: 1785-1790.

Townsley J. D. and Brodie H. J. Studies on the mechanism of estrogen biosynthesis. III. The stereochemistry of aromatization of C19 and C18 steroids. *Biochemistry* 1968; **7**: 33-40.

Trzeciak W. H. and Boyd G. S. The effect of stress induced by ether anaesthesia on cholesterol content and cholesteryl-esterase activity in rat-adrenal cortex. *European Journal of Biochemistry* 1973; **37**: 327-333.

Tseng L., Stolee A. and Gurpide E. *In vitro* measurements in human placenta and fetal membranes of rates of entry, metabolism and release of steroids. *Endocrinology* 1972; **90**: 405-414.

Tuckey R. C. Cholesterol side-chain cleavage by mitochondria from the human placenta. Studies using hydroxycholesterols as substrates. *Journal of Steroid Biochemistry*. 1992; **42**: 883-890.

Tuckey R. C., Kostadinovic Z. and Cameron K. J. Cytochrome P-450scc activity and substrate supply in human placental trophoblasts. *Molecular and Cellular Endocrinology* 1994; **105**: 103-109.

Tulchinsky D., Hobel C. J., Yeager E. and Marshall J. R. Plasma estrone, estradiol, estriol, progesterone and 17-hydroxyprogesterone in human pregnancy. I normal pregnancy. *American Journal of Obstetrics and Gynaecology* 1972; **112**: 1095-1100.

Tulsky A. S. and Koff A. K. Some observations on the role of the corpus luteum in early human pregnacy. *Fertility and Sterility* 1957; **8**: 118-130.

van Leusden H. and Villee C. A. The *de novo* synthesis of sterols and steroids from acetate by preparations of human term placenta. *Steroids* 1965; **6**: 31-45.

Veldhuis J. D., Rodgers R. J., Dee A. and Simpson E. R. The insulin-like growth factor, somatomedin-C, induces the synthesis of cholesterol side-chain cleavage cytochrome P-450 and adrenodoxin in ovarian cells. *The Journal of Biological Chemistry* 1986; **261**: 2499-2502.

Venning E. H. and Browne J. S. L. Isolation of a water-soluble pregnandiol complex from human pregnancy urine. *Proceedings of the Society of Experimental Biology and Medicine* 1936; **34**: 792-793.

Venning E. H. and Browne J. S. L. A study of the metabolism of crystalline progesterone. *Endocrinology* 1940; **27**: 707-720.

Venning E. H., Sybulski S., Pollak V. E. and Ryan R. J. Aldosterone excretion in two adrenalectomized pregnant women. *Journal of Clinical Endocrinology and Metabolism* 1959; **19**: 1486-1491.

Vickery L. E. and Kellis T. K. Aromatase cytochrome P-450. Purification and characterization of the enzyme from human placenta. *Steroids* 1987; **50**: 29-36.

Voutilainen R., Miller W. L. Coordinate tropic hormone regulation of mRNAs for insulin-like growth factor II and the cholesterol side-chain-cleavage enzyme, P450ssc, in human steroidogenic tissues. *Proceedings National Acadamy of Sciences USA* 1987; **84**: 1590-1594.

Waddell B. J. and Burton P. J. Release of bioactive ACTH by perifused human placenta at early and late gestation. *Journal of Endocrinology* 1993; **136**: 345-353.

Warren J. C. and Timbelake C. E. Steroid sulfatase in the human placenta. *Journal of Clinical Endocrinology* 1962; **22**: 1148-1151.

Warren J. C. and Timbelake C. E. Biosynthesis of estrogens in pregnancy: precursor role of plasma dehydroisoandrosterone. *Obstetrics and Gynaecology* 1964; **23**: 689-698.

Warren J. C. and Crist R. D. Site specificity and mechanism of human placental 17β-hydroxysteroid dehydrogenase. *Archives of Biochemistry and Biophysics* 1967; **118**: 577-584.

Warren W. B. and Silverman A. J. Cellular localization of corticotrophin releasing hormone in the human placenta, fetal membranes and decidua. *Placenta* 1995; **16**: 147-156.

Warren W. B., Patrick S. L. and Goland R. S. Elevated maternal corticotropin-releasing hormone levels in pregnancies complicated by preterm labour. *American Journal of Obstetrics and Gynaecology* 1992; **166**: 1198-1207.

Westerfeld W. W., MacCorquodale D. W., Thayer S. A. and Doisy E. A. The isolation of theelin from human placenta. *Journal of Biological Chemistry* 1938; **126**: 195-200.

Williams-Smith D. L., Simpson E. R., Barlow S. M. and Morrison P. J. Electron paramagnetic resonance studies of cytochrome P-450 and adrenal ferredoxin in single whole rat adrenal glands. Effect of corticotropin. *Biochimica et Biophysica Acta* 1976; **449**: 72-83.

Wilson D. M., Bennett A., Adamson. G. D., Nagashima R. J., Lui F., DeNatale M. L., Hintz R. L. and Rosenfeld R. G. Somatomedins in pregnancy: a cross-sectional study of insulin-like growth factors I and II and somatomedin peptide content in normal human pregnancies. *Journal of Clinical Endocrinology and Metabolism* 1982; **55**: 858-861.

Winkel C. A., Synder J. M., MacDonald P. C. and Simpson E. R. Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. *Endocrinology* 1980; **106**: 1054-1060.

Winkel C. A., Gilmore J., MaDonald P. C. and Simpson E. R. Uptake and degradation of lipoproteins by human trophoblastic cells in primary culture. *Endocrinology* 1980; **107**: 1892-1898.

Winkel C. A., MacDonald P., Hemsell P. G. and Simpson E. R. Regulation of cholesterol metabolism by human trophoblastic cells in primary culture. *Endocrinology* 1981; **109**: 1084-1090.

Winters A. J., Oliver C., Colston C., MacDonald P. C. and Porter J. C. Plasma ACTH levels in the human fetus and neonate as related to age and parturition. *Journal of Clinical Endocrinology and Metabolism* 1974; **39**: 269-273.

Woolever C. A., Goldfien A. and Page E. W. Studies of the isolated perfused human placenta. II. Progesterone content of perfusates. *American Journal of Obstetrics and Gynaecology* 1961; **81**: 1137-1139.

Wu L., Einstein M., Geissler H., Chan H. K., Elliston K. O. and Andersson S. Expression cloning and characterization of human 17β-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20α-hydroxysteroid dehydrogenase activity. *The Journal of Biological Chemistry* 1993; **268**: 12964-12969.

Yago N. and Ichii S. Submitochondrial distribution of components of the steroid 11β-hydroxylase and cholesterol sidechain-cleaving enzyme systems in hog adrenal cortex. *Journal of Biochemistry* 1969; **65**: 215-224.

Yago N., Kobayashi S., Sekiyama S., Kurokawa H., Iwai Y., Suzuki I. and Ichii S. Further studies on the submitochondrial localization of cholesterol side chain-cleaving enzyme system in hog adrenal cortex by sonic treatment. *Journal of Biochemistry* 1970; **68**: 775-783.

Yam L. T., Li C. Y. and Crosby W. H. Cytochemical identification of monocytes and granulocytes. *American Journal of Cellular Pathology* 1971; **55**: 283-290.

Yoshida N. and Osawa Y. Purification of human placental aromatase cytochrome P-450 with monoclonal antibody and its characterization. *Biochemistry* 1991; **30**: 3003-3010.

Yoshinaga K., Hawkins R. A. and Stocker J. F. Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. *Endocrinology* 1969; **85**: 103-112.

Zelewski L. and Villee C. The biosynthesis of squalene, lanosterol, and cholesterol by minced human placenta. *Biochemistry* 1966; **5**: 1805-1814.

Zhou J. and Bondy C. Insulin-like growth factor-II and its binding proteins in placental development. *Endocrinology* 1992; **131**: 1230-1240.

Zuber M. X., Simpson E. R., Hall P. F. and Waterman M. R. Effects of adrenocorticotropin on 17α-hydoxlase activity and cytochrome P-$450_{17\alpha}$ synthesis in bovine adrenocortical cells. *The Journal of Biological Chemistry*. 1985; **260**: 1842-1848.

Appendix

For all the Appendix tables listed below the symbol: ~ indicates that the level of steroid produced by the tissue explants or isolated cells was below the sensitivity of the assay. The concentrations of CRF and ACTH used in this study are 100 nmol/l and 10 nmol/l respectively.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	447.9	503.7	550.7	552.1	505.9	721.3	629.6	456.7	461.8
Control	3410.0	581.1	821.2	556.2	439.0	469.9	689.0	649.0	614.5
Control	579.3	600.6	355.7	504.0	567.9	540.0	761.0	790.4	761.0
Control	1433.2	705.9	636.2	624.1	534.5	576.3	652.7	671.4	660.0
Control	1015.0	1410.8	749.2	1013.9	879.2	789.6	1042.6	921.8	1153.8
Control	1663.3	552.1	489.3	3151.9	941.2	646.1	589.9	881.8	709.6
ACTH	367.8	434.6	781.2	542.6	698.6	533.8	772.8	560.9	526.8
ACTH	819.0	422.9	556.2	588.5	456.7	379.6	794.8	638.4	512.8
ACTH	392.4	1038.2	478.0	578.9	536.3	498.1	852.0	674.0	616.4
ACTH	470.6	558.4	475.8	588.1	908.2	562.0	751.8	1696.0	612.3
ACTH	700.1	1176.6	824.5	777.5	1043.3	762.8	1116.0	1645.7	1121.9
ACTH	725.0	923.6	817.5	859.8	600.6	1036.0	1069.0	1705.9	1113.4
NADPH	3269.4	350.2	2349.1	4688.2	8505.0	1733.1	2663.7	1113.1	1718.0
NADPH	1471.7	602.4	4969.8	5149.7	1987.1	1280.1	1544.4	1395.0	1285.2
NADPH	3769.8	3907.8	4462.1	3796.6	5737.4	1566.1	2581.4	1331.1	1332.6
NADPH	2121.8	2199.3	3310.9	1572.3	8770.4	1729.4	1769.8	1835.9	1567.2
NADPH	5326.2	1138.7	6213.9	7749.5	10179.3	1293.7	2719.1	4560.1	2006.2
NADPH	4542.1	886.9	6845.3	2732.3	7306.0	4584.0	1911.1	1893.1	2283.0
ACTH + NADPH	2865.2	740.8	4597.6	4798.4	4631.3	1691.6	1823.4	1311.3	1797.7
ACTH + NADPH	1467.3	458.1	4550.9	4070.0	2631.0	891.0	1501.8	1872.2	1219.5
ACTH + NADPH	2324.8	441.6	4806.8	2932.8	3157.8	812.4	1205.2	905.6	979.8
ACTH + NADPH	3545.8	1028.2	5611.5	1079.3	3018.3	1071.6	2093.9	1962.9	979.4
ACTH + NADPH	6447.0	1011.0	7835.4	5139.4	10186.3	2400.1	2681.3	2569.7	2785.6
ACTH + NADPH	5180.5	1928.7	7179.0	4331.0	1465.5	2273.1	3179.4	4022.3	2678.4

Appendix table 6.1.1. The values for E2 levels (fmol/mg protein) produced by tissue explants obtained from placenta A1 and incubated for a total period of 24 hours.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	0.62	0.60	0.87	0.82	0.96	0.93	0.92	0.76	1.05
Control	0.64	0.55	0.59	0.69	0.70	0.59	0.60	0.64	0.81
Control	0.52	0.56	0.63	0.70	0.90	0.48	0.88	0.69	0.75
Control	0.73	0.64	0.68	0.60	0.70	0.79	0.74	1.11	1.26
Control	1.00	0.84	0.87	0.78	0.89	0.87	0.85	1.15	1.26
Control	0.57	0.44	0.45	0.49	0.78	0.75	0.78	0.75	0.96
ACTH	0.61	0.59	0.86	0.81	0.94	0.92	0.90	0.75	1.00
ACTH	0.82	0.42	0.57	0.59	0.71	0.43	0.62	0.52	0.75
ACTH	0.61	0.47	0.64	0.65	0.88	0.66	0.68	0.64	0.74
ACTH	0.72	0.68	0.73	0.62	0.64	0.91	0.77	1.05	1.07
ACTH	1.16	0.94	1.08	1.16	1.15	1.21	1.21	1.83	1.95
ACTH	0.88	0.73	1.19	0.97	1.24	0.72	0.85	1.33	1.42
NADPH	0.46	0.61	0.89	0.81	1.03	0.72	0.81	0.91	1.02
NADPH	0.61	0.57	0.74	0.75	0.80	0.59	0.77	0.73	0.75
NADPH	0.47	0.49	0.58	0.67	0.82	0.62	0.67	0.68	0.97
NADPH	0.65	0.69	0.72	0.74	0.65	0.74	0.80	0.87	0.93
NADPH	1.19	1.11	1.16	0.98	0.95	1.01	1.13	1.71	1.64
NADPH	0.82	0.75	0.90	0.74	0.90	0.83	0.81	1.18	1.13
ACTH + NADPH	0.62	0.60	0.65	0.65	0.65	0.67	0.85	0.79	0.77
ACTH + NADPH	0.59	0.52	0.78	0.79	0.77	0.71	0.75	0.67	0.77
ACTH + NADPH	0.34	0.26	0.41	0.43	0.60	0.43	0.46	0.39	0.74
ACTH + NADPH	0.74	0.71	0.77	0.83	0.90	1.02	1.01	1.14	1.22
ACTH + NADPH	1.20	0.92	1.01	0.90	0.98	1.39	1.30	1.37	1.49
ACTH + NADPH	0.82	0.90	0.73	0.78	1.02	0.98	1.04	1.49	1.50

Appendix table 6.1.2. The values for E3 levels (pmol/mg protein) produced by tissue explants obtained from placenta A1 and incubated for a total period of 24 hours.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	17.30	24.74	26.90	21.02	27.54	27.19	38.22	40.96	38.29
Control	16.38	22.90	23.21	22.93	23.06	31.99	23.66	43.25	48.40
Control	22.48	27.86	24.99	26.11	22.48	36.25	33.68	24.99	50.69
Control	28.97	43.03	38.19	41.24	35.78	50.59	46.17	66.84	56.86
Control	36.51	48.65	39.69	64.59	60.13	87.55	60.01	67.38	72.56
Control	42.96	27.25	19.78	42.39	27.22	38.70	35.65	40.45	48.65
ACTH	20.22	24.30	24.93	23.72	23.95	38.45	45.22	51.48	56.76
ACTH	12.34	28.24	28.78	26.84	28.02	32.53	29.42	24.20	40.39
ACTH	20.07	19.10	20.96	18.98	24.30	25.54	23.12	27.22	38.22
ACTH	26.11	33.93	36.12	34.60	37.37	42.39	36.47	42.20	52.76
ACTH	40.61	43.60	46.33	60.29	45.06	47.76	47.75	46.27	62.55
ACTH	22.93	45.76	36.86	65.54	40.64	47.38	50.28	50.31	62.42
NADPH	31.04	63.06	69.48	48.15	88.94	125.55	117.06	120.81	166.25
NADPH	35.74	41.66	64.84	58.64	62.11	100.17	98.36	122.37	159.99
NADPH	28.40	43.76	56.57	67.77	67.77	78.51	83.92	150.83	145.77
NADPH	34.53	64.11	67.99	83.47	84.49	125.96	114.70	156.65	161.61
NADPH	83.57	130.19	143.64	188.64	242.32	224.99	230.33	257.45	283.15
NADPH	50.43	107.13	107.77	114.16	206.92	187.05	218.47	177.76	230.61
ACTH + NADPH	26.68	22.55	24.04	30.85	34.31	35.27	39.97	48.05	65.54
ACTH + NADPH	22.61	35.49	44.07	46.75	41.50	60.90	76.54	82.87	83.13
ACTH + NADPH	29.89	35.01	40.89	41.88	38.76	51.96	44.87	49.42	72.12
ACTH + NADPH	35.74	46.01	56.67	64.90	88.75	95.11	76.92	91.52	105.29
ACTH + NADPH	0.54	75.94	82.93	102.68	125.16	117.82	123.19	143.29	175.89
ACTH + NADPH	35.62	77.85	91.74	113.56	167.75	133.59	158.01	150.00	174.11

Appendix table 6.1.3. The values for P levels (pmol/mg protein) produced by tissue explants obtained from placenta A1 and incubated for a total period of 24 hours.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	136.6	262.1	269.1	252.9	241.2	315.0	294.8	263.2	359.4
Control	425.5	259.9	298.8	292.2	326.4	301.8	305.8	291.1	320.1
Control	258.1	186.9	315.7	236.1	294.4	415.2	328.9	354.6	360.5
Control	356.1	454.5	379.6	220.6	341.4	359.0	385.5	352.8	348.4
Control	242.6	200.4	244.1	209.2	241.6	266.9	265.4	266.1	279.7
Control	273.9	219.5	286.7	301.4	262.5	627.0	305.1	309.8	273.9
CRF	481.3	375.9	475.0	244.1	412.2	349.1	360.9	396.1	370.0
CRF	170.3	261.0	301.4	208.5	215.1	279.7	271.6	263.2	384.7
CRF	222.1	290.0	296.6	226.5	250.7	318.6	218.1	272.8	288.9
CRF	223.2	577.5	267.6	249.6	342.1	291.1	291.5	357.6	365.3
CRF	306.9	561.3	545.5	297.7	317.5	678.4	146.9	340.3	418.5
CRF	347.6	480.9	575.6	371.5	405.3	675.5	574.5	442.7	1717.7
ACTH	364.5	225.4	283.4	333.3	334.4	299.9	322.3	287.1	372.2
ACTH	244.9	240.1	281.2	270.5	355.0	340.7	357.6	352.8	352.8
ACTH	208.9	190.2	259.5	193.1	270.2	274.6	346.2	271.3	502.9
ACTH	214.0	228.0	156.8	182.4	260.3	304.7	207.0	257.7	291.8
ACTH	273.1	221.7	237.9	290.4	278.3	349.1	309.8	314.2	278.3
ACTH	257.0	196.4	292.6	254.8	291.1	288.5	283.0	316.8	364.9
IGF-I	157.9	225.4	212.9	342.1	328.5	379.6	315.7	281.9	333.7
IGF-I	233.5	196.8	316.8	323.8	326.0	254.4	304.7	347.6	313.1
IGF-I	185.4	218.8	304.7	233.5	227.6	563.9	283.8	277.5	490.8
IGF-I	324.1	283.0	263.2	277.9	406.0	367.1	271.3	375.5	641.7
IGF-I	279.4	272.4	173.3	172.5	287.4	281.9	270.2	240.4	413.7
IGF-I	168.5	222.1	251.5	233.8	421.8	276.4	271.6	263.9	315.3
NADPH	1265.4	996.3	752.9	846.9	1221.7	1023.8	732.0	445.7	494.9
NADPH	1375.9	1236.4	973.9	1018.0	593.6	455.6	443.1	426.2	532.3
NADPH	743.4	757.0	676.6	557.3	1010.6	1398.3	389.9	326.7	421.1
NADPH	1481.2	1285.6	1008.1	1200.4	1050.3	975.0	422.2	380.3	559.1
NADPH	1579.3	1336.6	844.7	911.1	1121.1	1565.3	527.2	881.8	547.0
NADPH	547.3	842.1	684.6	697.1	677.3	1211.8	384.7	442.0	419.6
CRF + NADPH	936.1	745.2	575.2	696.4	931.7	1487.5	418.5	470.3	411.1
CRF + NADPH	1673.6	1314.6	993.7	1095.1	1632.1	535.6	785.6	781.9	628.1
CRF + NADPH	1008.4	1136.5	634.3	507.7	901.6	1003.3	384.4	473.9	445.7
CRF + NADPH	998.1	1341.0	857.5	728.7	972.1	1234.6	440.9	465.5	510.3
CRF + NADPH	676.2	885.8	708.5	732.0	811.3	1855.3	377.0	391.3	429.9
CRF + NADPH	658.2	712.9	606.8	595.4	1065.3	1710.3	443.1	497.8	426.9
ACTH + NADPH	639.1	637.3	625.9	908.2	987.1	1290.4	351.3	431.7	391.0
ACTH + NADPH	427.3	912.6	639.9	447.9	875.2	1389.5	405.3	569.7	421.1
ACTH + NADPH	909.3	868.2	643.2	849.1	800.3	1386.5	414.5	306.9	468.4
ACTH + NADPH	1077.1	1651.6	1370.8	1231.6	1164.8	1312.4	509.2	1084.1	643.5
ACTH + NADPH	670.3	844.3	665.5	811.3	981.3	1109.7	544.8	443.5	449.3
ACTH + NADPH	994.1	1160.8	526.8	560.9	324.1	747.0	383.2	361.6	455.9
IGF-I + NADPH	1169.9	1096.2	737.9	664.8	1214.4	1530.1	609.8	835.2	498.9
IGF-I + NADPH	885.1	682.4	721.7	914.5	1070.5	688.0	330.8	397.2	426.9
IGF-I + NADPH	1443.8	942.7	797.3	884.7	1074.5	1479.8	601.3	500.4	505.5
IGF-I + NADPH	1357.9	1245.9	976.8	1163.3	1018.0	944.9	409.0	368.6	541.8
IGF-I + NADPH	1413.0	899.8	567.5	586.3	1205.6	591.4	440.1	761.4	449.7
IGF-I + NADPH	1317.5	1240.4	1124.8	980.5	1158.2	1507.7	660.4	1040.0	617.5

Appendix table 6.2.1. The values for E2 levels (fmol/mg protein) produced by placental tissue explants obtained from placenta A2 and incubated for 24 hours. The concentration of IGF-I used in this experiment was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	2.01	2.59	2.07	2.99	2.43	2.64	2.82	2.46	2.66
Control	2.47	2.34	2.59	2.01	2.14	2.33	2.87	2.48	2.23
Control	2.35	1.31	2.66	2.90	2.07	2.16	2.57	2.25	2.44
Control	2.32	2.11	2.30	2.04	2.90	2.65	4.85	3.47	4.22
Control	2.14	2.34	1.69	2.58	2.39	2.52	2.56	2.39	3.39
Control	2.39	1.65	2.24	2.53	3.34	3.18	2.24	2.17	3.91
CRF	4.37	4.55	4.84	3.13	4.48	4.85	4.49	4.73	4.37
CRF	2.09	2.02	2.68	2.03	2.57	1.80	2.28	2.15	2.31
CRF	1.98	1.87	2.50	1.90	2.17	2.47	2.65	2.20	2.27
CRF	1.83	1.76	1.89	2.05	1.81	3.97	5.63	2.64	3.42
CRF	2.27	1.43	1.83	2.36	2.63	2.68	2.14	2.60	3.81
CRF	2.15	1.82	2.05	2.48	3.35	2.70	2.46	2.81	3.27
ACTH	2.07	2.42	2.32	2.49	2.39	2.46	2.52	2.24	2.20
ACTH	2.47	2.33	2.13	2.16	2.39	2.58	2.93	2.60	2.11
ACTH	1.80	1.67	2.22	2.30	2.26	2.63	2.43	2.45	3.14
ACTH	2.43	1.91	2.13	2.72	3.33	3.14	2.92	2.85	4.43
ACTH	2.06	2.03	2.77	1.62	2.62	2.51	2.67	2.68	2.10
ACTH	2.02	1.73	1.62	2.40	1.26	2.34	2.39	2.42	3.40
IGF-I	2.19	2.04	2.13	2.52	2.22	1.93	2.71	2.20	2.59
IGF-I	2.37	2.00	2.90	3.21	3.01	2.91	3.14	3.01	1.40
IGF-I	1.89	1.85	2.68	1.81	2.21	1.19	2.12	2.23	2.50
IGF-I	2.21	1.70	2.15	2.69	2.55	2.66	2.71	3.01	3.72
IGF-I	2.15	1.69	2.10	2.65	2.59	2.94	2.75	2.83	3.65
IGF-I	2.23	1.61	2.00	2.76	2.46	2.23	3.07	2.63	4.16
NADPH	2.36	2.47	2.14	2.11	2.57	2.53	3.04	2.56	2.00
NADPH	2.91	2.51	2.29	2.94	2.66	2.74	2.93	3.09	2.58
NADPH	1.93	1.89	2.38	2.22	2.27	2.20	2.57	2.40	2.24
NADPH	2.75	2.39	1.99	3.01	2.85	3.25	3.42	3.34	3.66
NADPH	1.80	1.77	1.74	2.43	2.35	2.59	2.36	2.49	3.61
NADPH	2.65	2.39	1.95	2.85	3.23	3.34	2.90	3.19	4.08
CRF + NADPH	2.12	1.99	2.21	2.48	2.16	2.44	2.87	2.43	2.20
CRF + NADPH	2.83	2.85	3.61	3.01	3.20	3.19	3.61	3.38	2.55
CRF + NADPH	2.37	2.32	3.19	2.96	2.61	2.89	2.71	2.97	2.92
CRF + NADPH	2.19	1.54	1.95	2.83	2.91	3.24	3.29	3.22	4.38
CRF + NADPH	1.65	1.78	2.05	2.65	2.38	2.47	2.54	2.56	4.12
CRF + NADPH	2.50	2.04	1.99	2.75	2.61	2.74	2.99	2.19	3.95
ACTH + NADPH	2.60	2.45	2.76	3.22	2.74	3.07	3.12	2.50	2.68
ACTH + NADPH	2.04	1.96	2.41	2.64	2.51	2.54	2.63	2.57	2.34
ACTH + NADPH	1.77	2.13	1.90	2.19	2.35	2.42	2.95	2.96	3.33
ACTH + NADPH	2.21	1.77	1.83	2.26	2.65	2.80	3.11	2.87	3.66
ACTH + NADPH	2.49	2.27	3.17	3.05	3.63	3.74	3.50	3.92	4.95
ACTH + NADPH	2.00	1.71	2.33	2.75	2.36	2.28	2.98	2.39	2.08
IGF-I + NADPH	2.78	2.37	2.44	2.38	2.54	2.39	2.96	2.65	2.49
IGF-I + NADPH	2.14	2.13	2.37	3.15	2.69	3.00	3.02	2.64	2.57
IGF-I + NADPH	1.99	1.98	2.33	2.79	2.45	2.59	2.86	2.71	2.78
IGF-I + NADPH	2.48	2.16	2.26	2.88	2.95	3.03	3.38	3.46	5.84
IGF-I + NADPH	3.28	2.78	2.57	3.53	2.85	3.58	3.78	3.66	5.23
IGF-I + NADPH	2.39	1.85	2.50	2.69	3.61	3.30	2.86	2.87	4.10

Appendix table 6.2.2. The values for E3 levels (pmol/mg protein) produced by tissue explants obtained from placenta A2 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	54.85	59.62	39.05	56.67	73.65	61.85	64.74	66.90	91.87
Control	54.38	55.43	84.40	48.27	46.97	84.02	53.84	69.17	66.59
Control	67.73	77.59	83.64	72.19	77.91	87.13	85.86	92.54	102.71
Control	54.38	73.43	60.96	55.94	64.49	74.03	69.55	76.35	73.71
Control	60.39	65.51	57.56	68.75	75.40	72.57	70.25	84.49	75.97
Control	59.78	84.91	71.55	78.55	88.40	80.45	83.95	96.35	79.82
CRF	106.82	124.31	93.56	89.23	135.63	137.09	100.65	131.43	280.38
CRF	57.27	78.26	75.94	56.29	76.77	92.32	87.67	96.96	77.05
CRF	51.96	63.92	41.66	58.93	61.53	61.47	67.03	70.21	68.31
CRF	59.88	63.00	53.42	61.18	74.32	45.60	61.02	73.11	67.48
CRF	59.88	68.05	81.15	79.75	72.92	94.54	89.01	91.04	80.58
CRF	47.38	68.37	65.83	73.78	50.24	63.28	73.46	62.96	78.23
ACTH	62.61	64.55	51.64	71.55	89.20	81.60	83.67	74.51	95.65
ACTH	72.22	68.02	49.96	66.33	73.62	82.55	85.26	90.31	76.22
ACTH	48.72	61.15	49.74	57.69	62.17	80.93	74.06	63.06	73.43
ACTH	45.79	68.59	57.27	64.97	68.08	69.86	64.65	66.18	84.33
ACTH	58.13	78.64	69.55	75.87	66.21	79.09	78.48	75.30	87.51
ACTH	61.98	70.15	61.15	63.38	58.10	69.58	67.64	75.18	86.31
IGF-I	53.74	65.64	56.89	64.59	74.48	63.19	65.79	70.79	95.24
IGF-I	58.26	77.05	72.28	95.65	99.18	93.78	99.28	102.33	111.62
IGF-I	46.43	49.61	55.94	56.03	62.42	61.47	55.40	66.88	69.32
IGF-I	61.06	72.50	48.02	60.74	63.60	59.78	82.68	72.50	87.45
IGF-I	62.90	75.72	59.82	67.70	57.88	66.18	69.55	87.07	86.18
IGF-I	52.53	69.99	70.21	61.60	71.07	73.94	64.74	64.68	81.98
NADPH	58.00	84.02	106.66	150.41	116.96	130.76	147.71	149.46	151.23
NADPH	64.62	104.94	75.97	153.98	172.29	138.27	127.36	186.03	163.39
NADPH	48.05	61.85	76.03	93.68	93.36	95.53	102.97	117.50	127.17
NADPH	82.08	106.75	106.91	141.61	139.22	151.75	140.56	196.71	219.93
NADPH	45.95	75.43	76.48	85.80	103.89	90.66	98.96	114.89	136.61
NADPH	72.41	104.78	85.96	113.02	131.24	156.04	128.69	199.35	171.69
CRF + NADPH	40.32	73.01	70.12	98.20	116.39	97.09	92.60	86.88	94.70
CRF + NADPH	73.97	93.97	144.85	146.63	202.95	211.95	202.98	203.20	141.64
CRF + NADPH	49.32	79.69	92.79	108.82	100.90	91.71	124.50	144.37	117.63
CRF + NADPH	45.57	88.05	100.33	103.03	131.78	106.88	131.56	154.68	198.05
CRF + NADPH	51.83	86.81	65.51	88.72	97.63	78.55	96.99	112.89	136.42
CRF + NADPH	55.81	71.36	86.05	108.76	110.73	95.53	108.34	76.19	100.36
ACTH + NADPH	58.58	97.56	95.27	114.61	151.91	109.01	132.70	157.82	137.38
ACTH + NADPH	56.10	101.95	71.84	47.45	135.69	141.06	127.20	133.15	129.14
ACTH + NADPH	48.27	74.19	67.54	81.09	99.66	95.15	104.97	92.89	109.20
ACTH + NADPH	21.94	72.50	69.64	77.91	86.81	103.99	114.16	137.69	136.42
ACTH + NADPH	53.14	91.43	90.44	107.58	110.38	104.69	118.90	134.80	153.53
ACTH + NADPH	70.15	138.74	97.21	143.77	155.37	186.13	194.90	207.91	219.80
IGF-I + NADPH	53.42	78.99	118.61	131.49	146.28	131.78	145.77	158.81	163.99
IGF-I + NADPH	54.73	90.25	70.56	112.10	160.49	138.49	126.44	127.23	125.20
IGF-I + NADPH	54.35	69.64	62.46	84.68	100.90	100.58	108.15	104.65	118.49
IGF-I + NADPH	61.82	98.20	102.24	126.72	108.57	130.16	135.47	134.61	207.08
IGF-I + NADPH	80.71	112.57	111.87	142.69	151.59	171.40	170.73	200.44	212.71
IGF-I + NADPH	64.74	82.78	83.54	116.42	161.32	131.78	159.99	151.05	189.02

Appendix table 6.2.3. The values for P levels (pmol/mg protein) produced by tissue explants obtained from placenta A2 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	1.41	1.88	2.02	2.41	2.90	1.26	2.47	2.11	2.29
Control	3.02	2.15	2.26	1.93	2.45	1.85	2.41	2.14	2.57
Control	1.70	1.65	1.98	4.40	3.83	2.64	1.79	2.39	2.99
Control	2.62	0.91	1.99	2.25	2.81	1.84	1.92	2.13	2.46
Control	1.44	1.48	2.04	1.03	2.27	2.11	2.25	2.41	2.96
Control	2.05	1.59	2.98	2.53	2.70	2.32	2.49	2.58	2.66
CRF	1.47	1.01	0.74	1.24	1.26	0.74	0.61	1.11	1.27
CRF	1.78	1.13	1.32	1.64	1.29	1.70	1.50	1.51	1.87
CRF	2.30	1.41	1.85	1.75	3.05	1.91	2.06	2.18	2.15
CRF	2.77	2.03	2.19	1.92	2.57	2.52	1.52	2.39	2.99
CRF	2.45	1.73	2.71	2.22	2.83	2.30	2.33	2.92	2.70
CRF	1.65	1.17	1.96	1.30	1.98	2.28	1.82	2.09	1.75
ACTH	3.14	1.50	1.74	2.39	2.87	1.35	2.32	1.51	2.35
ACTH	1.70	1.59	2.38	1.80	2.70	2.20	2.16	2.81	2.93
ACTH	2.24	1.89	2.13	2.72	3.88	2.47	3.56	2.78	3.24
ACTH	2.40	1.68	2.35	2.40	2.92	2.34	2.46	3.04	2.80
ACTH	1.61	1.52	1.68	2.05	2.11	1.96	1.78	2.10	2.19
ACTH	1.53	1.36	1.79	2.11	2.20	2.16	2.55	1.80	2.43
IGF-I	1.98	1.24	1.71	2.07	1.99	2.05	1.72	1.79	1.87
IGF-I	2.12	1.54	1.99	2.19	2.45	1.78	2.37	2.75	2.49
IGF-I	2.32	1.31	1.75	1.62	2.98	2.00	1.93	2.27	2.34
IGF-I	2.45	1.45	2.08	1.60	2.15	2.00	2.30	2.16	2.49
IGF-I	3.29	1.73	2.17	0.98	2.47	2.38	2.30	2.49	2.49
IGF-I	2.38	1.64	2.07	2.33	2.29	2.10	2.29	2.31	2.56
NADPH	1.82	1.33	1.70	1.85	2.60	2.69	2.50	2.57	2.57
NADPH	2.58	1.48	1.97	1.91	2.58	1.62	2.73	2.76	2.92
NADPH	2.80	1.79	2.12	4.64	3.35	3.20	2.12	3.35	3.00
NADPH	1.76	1.42	2.04	1.65	3.72	2.64	2.76	2.64	3.23
NADPH	2.68	1.61	2.52	1.38	2.59	2.75	2.91	3.12	4.98
NADPH	0.99	0.90	1.64	1.84	2.28	1.89	2.96	2.54	2.60
CRF + NADPH	1.96	1.20	2.10	1.85	2.40	1.69	2.00	2.16	2.26
CRF + NADPH	1.77	1.06	1.38	1.72	2.31	1.90	2.00	1.99	2.52
CRF + NADPH	1.60	1.17	1.39	1.44	2.88	2.24	1.95	2.37	2.52
CRF + NADPH	1.13	1.17	1.53	1.35	1.79	1.59	1.82	2.15	2.26
CRF + NADPH	2.52	1.40	1.88	2.15	2.62	2.32	2.64	2.62	2.89
CRF + NADPH	3.15	2.42	3.14	3.33	3.68	3.80	3.46	4.10	4.14
ACTH + NADPH	0.47	1.79	2.42	1.82	2.78	1.63	2.18	2.25	2.69
ACTH + NADPH	3.20	2.00	2.20	2.74	3.41	2.05	3.32	3.43	3.29
ACTH + NADPH	1.27	1.01	1.39	2.39	2.40	1.46	1.71	1.65	1.84
ACTH + NADPH	1.40	0.91	1.30	1.29	2.46	1.61	1.79	1.84	1.86
ACTH + NADPH	2.00	1.58	2.06	2.04	1.23	2.08	2.40	2.06	2.95
ACTH + NADPH	2.07	0.88	1.97	1.00	2.41	2.41	2.21	2.46	2.76
IGF-I + NADPH	0.27	1.06	1.52	1.74	1.92	1.55	2.10	2.14	2.17
IGF-I + NADPH	1.60	1.01	1.35	1.42	2.07	1.45	1.65	2.10	2.08
IGF-I + NADPH	1.09	0.90	1.48	1.63	2.76	1.77	2.15	2.08	2.45
IGF-I + NADPH	2.23	1.07	1.49	1.55	1.44	1.90	1.82	2.22	2.56
IGF-I + NADPH	1.92	1.57	1.92	1.56	2.16	2.09	2.36	2.34	2.52
IGF-I + NADPH	1.43	0.98	1.62	1.57	1.99	2.06	1.85	2.53	2.21

Appendix table 6.3.1. The values for E1 levels (pmol/mg protein) produced by tissue explants obtained from placenta A3 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	1061.3	584.2	573.2	643.2	567.2	583.2	571.2	479.1	815.2
Control	1219.3	726.2	594.2	607.2	508.1	713.2	635.2	535.2	853.2
Control	1051.3	559.2	517.1	662.2	582.2	670.2	585.2	633.2	968.3
Control	1397.4	395.1	482.1	1390.4	616.2	618.2	515.2	534.1	795.2
Control	631.2	542.1	549.1	491.1	537.1	587.2	539.2	508.1	647.2
Control	1508.4	685.2	652.2	665.2	746.2	700.2	605.2	695.2	841.2
CRF	165.1	280.1	351.1	416.1	340.1	351.1	384.1	312.1	677.2
CRF	1045.3	357.1	386.1	485.1	320.1	440.1	433.1	527.2	744.2
CRF	1203.3	547.2	542.1	456.1	464.1	571.2	490.1	466.1	646.2
CRF	1184.3	419.1	546.1	480.1	494.1	528.2	437.1	446.1	799.2
CRF	1692.5	505.1	627.2	640.2	603.2	869.3	637.2	438.1	753.2
CRF	1589.4	804.2	737.2	806.2	753.2	772.2	638.2	645.2	1021.3
ACTH	1919.5	1019.3	1060.3	762.2	626.2	772.2	442.1	561.2	799.2
ACTH	948.3	548.2	582.2	572.2	696.2	784.2	703.2	621.2	973.3
ACTH	536.2	439.1	422.1	400.1	415.1	475.1	1181.3	498.1	735.2
ACTH	486.1	438.1	469.1	487.1	464.1	550.1	542.1	388.1	739.2
ACTH	499.1	494.1	608.2	575.2	740.2	582.2	599.1	538.1	849.2
ACTH	260.1	374.1	486.1	604.2	449.1	628.2	586.2	507.1	851.2
IGF-I	252.1	344.1	441.1	529.1	419.1	513.1	540.2	456.1	571.2
IGF-I	808.2	445.1	547.2	688.2	461.1	600.2	381.1	508.1	792.2
IGF-I	495.1	450.1	371.1	623.2	517.1	523.2	506.2	450.1	576.2
IGF-I	363.1	428.1	415.1	446.1	408.1	477.1	320.1	420.1	658.2
IGF-I	324.1	450.1	570.1	637.2	512.1	557.2	713.2	478.1	746.2
IGF-I	326.1	353.1	516.1	528.2	507.1	554.1	505.1	622.2	881.2
NADPH	1294.4	1585.4	1593.4	2103.6	1550.4	1887.5	1312.3	939.3	997.3
NADPH	1447.4	1651.4	1578.4	1653.5	1653.5	1586.4	1146.3	1566.4	958.3
NADPH	1437.4	2237.6	2136.6	1805.5	1886.5	2125.6	1759.5	1024.3	1104.3
NADPH	1425.4	1021.3	1484.4	1579.4	1830.5	1675.4	895.2	885.2	823.2
NADPH	1984.5	1659.4	2397.6	2185.6	2407.7	2226.6	1787.5	1342.4	1142.3
NADPH	1441.4	1530.4	1631.4	1332.3	1144.3	1682.5	836.2	721.2	904.2
CRF + NADPH	1428.4	1509.4	1713.5	2044.6	1679.4	1471.4	1129.3	990.3	744.2
CRF + NADPH	1142.3	1187.3	986.3	964.3	1282.3	1443.4	842.2	403.1	474.1
CRF + NADPH	1179.3	1726.5	1259.3	2755.8	1542.4	1039.3	1398.4	830.2	832.2
CRF + NADPH	1177.3	1372.4	1369.4	1450.4	880.2	1536.4	814.2	676.2	835.2
CRF + NADPH	1558.4	1781.5	1828.5	1480.4	1787.5	1462.4	979.3	803.2	926.3
CRF + NADPH	3266.9	2986.8	1747.5	2318.6	2297.6	2356.6	1506.4	1136.3	1420.4
ACTH + NADPH	1650.4	1867.5	1723.5	2109.6	1693.5	1945.5	1314.4	947.3	1139.3
ACTH + NADPH	438.1	1965.5	1807.5	710.2	1521.4	2376.6	1325.4	1080.3	1100.3
ACTH + NADPH	813.2	976.3	1041.3	1143.3	1295.3	1397.4	483.1	712.2	580.2
ACTH + NADPH	903.2	940.2	1159.3	1186.3	1170.3	1129.3	673.2	578.2	699.2
ACTH + NADPH	1476.4	1743.5	1774.5	1593.4	1061.3	1569.4	684.2	816.2	811.2
ACTH + NADPH	1507.4	1631.4	1825.5	1446.4	1690.5	1360.4	1001.3	824.2	849.2
IGF-I + NADPH	1357.4	1347.4	1382.4	1774.5	1036.3	1840.5	1292.3	737.2	818.2
IGF-I + NADPH	1019.3	1197.3	1507.4	1583.4	1447.4	1402.4	1009.3	798.2	819.2
IGF-I + NADPH	1019.3	1471.4	1259.3	1429.4	966.2	1532.4	770.2	753.2	878.2
IGF-I + NADPH	1135.3	1574.4	1643.4	1581.4	870.2	2170.6	1260.3	862.2	859.2
IGF-I + NADPH	1451.4	1149.3	1426.4	1227.3	1323.4	1225.3	613.2	653.2	903.2
IGF-I + NADPH	1267.3	722.2	1359.4	1461.4	1185.3	1222.3	667.2	639.2	734.2

Appendix table 6.3.2. The values for E2 levels (fmol/mg protein) produced by tissue explants obtained from placenta A3 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	1.12	0.86	0.99	1.13	1.12	1.13	1.06	1.48	1.76
Control	0.71	0.72	0.89	0.95	0.70	0.79	0.80	1.17	1.46
Control	0.28	1.32	0.88	0.97	1.07	0.91	0.97	1.24	1.63
Control	0.64	0.74	0.90	0.87	0.86	0.95	0.90	1.25	1.17
Control	0.64	0.79	0.82	0.79	0.77	1.05	0.77	1.23	1.32
Control	0.90	0.88	1.04	0.93	0.97	1.05	0.90	1.36	1.34
CRF	0.55	0.79	0.71	0.74	0.70	1.27	0.61	0.82	0.98
CRF	0.55	0.70	0.74	0.76	0.85	0.88	0.79	1.05	1.32
CRF	0.79	0.53	0.81	0.76	0.68	0.72	0.71	0.98	1.23
CRF	0.29	0.72	0.84	0.78	0.66	0.91	0.92	1.28	1.95
CRF	0.90	0.86	1.18	0.82	0.88	1.14	0.92	1.34	1.49
CRF	0.92	1.01	1.00	1.02	1.20	1.00	1.00	1.55	2.27
ACTH	0.01	0.92	1.16	0.99	1.01	0.84	0.80	1.32	1.72
ACTH	0.83	0.75	1.23	0.89	0.92	0.95	1.17	1.50	1.51
ACTH	0.28	0.69	0.81	0.74	0.66	0.87	0.76	1.14	1.05
ACTH	0.49	0.77	0.85	0.89	0.75	0.94	0.80	0.99	1.25
ACTH	0.75	1.00	1.18	1.06	0.87	1.10	0.93	1.36	2.01
ACTH	0.61	0.59	0.83	0.65	0.62	0.83	0.82	1.37	1.49
IGF-I	0.71	0.82	0.92	0.84	1.00	0.94	0.85	1.11	1.31
IGF-I	0.67	0.85	1.04	0.70	1.00	0.81	0.85	0.92	1.26
IGF-I	0.58	0.57	0.65	1.01	0.77	0.83	0.79	1.11	1.13
IGF-I	0.65	0.98	0.85	0.96	1.00	1.10	0.96	1.26	1.41
IGF-I	0.50	0.58	0.90	0.77	0.85	0.92	0.80	1.32	1.16
IGF-I	0.73	0.83	0.99	0.97	0.88	0.84	0.85	1.14	1.38
NADPH	2.20	2.41	2.57	2.76	3.51	2.74	2.75	3.34	3.33
NADPH	1.00	1.03	1.17	1.00	1.05	1.11	0.96	1.41	1.71
NADPH	1.23	1.27	1.06	1.28	1.27	1.33	1.50	1.67	2.07
NADPH	0.78	0.74	1.02	1.20	0.97	1.45	1.35	1.61	1.44
NADPH	1.09	1.25	1.57	1.83	1.04	1.79	2.51	2.17	2.23
NADPH	0.88	0.85	1.05	0.92	0.75	1.03	0.86	1.23	1.17
CRF + NADPH	0.73	0.68	0.86	0.81	0.84	0.83	0.72	0.93	1.28
CRF + NADPH	0.85	0.69	0.83	0.86	0.77	0.81	1.04	1.14	1.33
CRF + NADPH	0.44	0.69	1.10	1.12	0.82	0.91	0.73	1.12	1.27
CRF + NADPH	0.22	0.92	1.10	0.89	0.85	1.16	0.81	1.25	1.27
CRF + NADPH	0.45	0.86	1.15	1.19	0.89	1.08	0.98	1.47	1.34
CRF + NADPH	1.53	2.17	1.93	1.75	1.74	1.73	1.73	2.49	2.51
ACTH + NADPH	1.38	1.08	1.37	1.37	1.31	1.54	1.14	1.83	1.77
ACTH + NADPH	0.84	0.96	1.49	1.01	1.06	0.81	1.17	1.42	1.79
ACTH + NADPH	0.62	0.54	0.73	0.59	0.53	0.60	0.66	0.93	1.12
ACTH + NADPH	0.42	0.80	0.77	0.78	0.64	0.83	0.75	1.02	0.93
ACTH + NADPH	0.73	1.04	1.22	1.14	0.59	1.23	0.98	1.41	1.59
ACTH + NADPH	0.84	1.02	1.21	0.97	1.11	1.05	1.02	1.12	1.48
IGF-I + NADPH	0.92	0.71	0.89	0.95	0.91	0.99	0.75	1.01	1.25
IGF-I + NADPH	0.66	0.73	0.93	0.93	0.72	0.83	0.69	1.07	1.23
IGF-I + NADPH	0.64	0.73	0.79	0.69	0.75	0.72	0.75	0.99	1.20
IGF-I + NADPH	0.17	0.61	0.97	0.91	0.64	1.02	1.06	2.52	1.22
IGF-I + NADPH	0.86	1.07	1.11	1.01	0.94	1.06	0.98	1.35	1.49
IGF-I + NADPH	0.67	0.75	0.90	0.79	0.65	0.67	0.65	1.15	1.08

Appendix table 6.3.3. The values for E3 levels (pmol/mg protein) produced by tissue explants obtained from placenta A3 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	25.33	18.25	22.22	21.83	23.90	25.70	26.70	29.66	34.17
Control	18.58	10.45	18.81	25.24	20.33	22.65	25.89	38.83	24.65
Control	23.66	14.45	17.71	24.60	24.19	33.27	30.82	55.34	46.40
Control	25.14	16.18	31.14	29.62	34.02	41.63	38.10	30.94	30.68
Control	25.49	19.68	26.31	23.96	26.36	33.78	29.00	28.73	35.21
Control	24.37	19.40	28.95	24.86	34.53	30.31	33.86	40.11	28.22
CRF	19.34	14.11	16.39	22.38	17.14	21.35	20.04	25.89	27.10
CRF	17.70	21.84	14.51	16.98	12.76	17.53	19.65	19.01	33.76
CRF	19.42	14.56	14.63	22.50	15.07	19.70	23.23	26.31	24.02
CRF	20.02	22.82	26.39	29.56	34.78	60.39	62.44	43.59	38.62
CRF	21.84	18.67	15.19	22.12	39.64	31.26	34.22	31.85	45.02
CRF	28.97	14.37	29.11	24.08	28.49	29.00	24.19	28.02	32.30
ACTH	22.16	19.59	19.88	30.16	21.49	26.21	25.81	34.85	-
ACTH	28.72	21.93	24.82	28.08	29.27	28.33	32.83	40.58	36.45
ACTH	11.65	15.37	28.77	16.71	26.20	26.00	28.40	36.97	33.76
ACTH	13.25	19.44	23.68	21.83	24.75	27.68	20.14	24.73	26.96
ACTH	16.97	13.86	20.50	22.31	28.82	26.89	27.93	29.95	26.59
ACTH	26.88	16.76	22.51	25.66	26.23	29.23	28.81	27.07	27.98
IGF-I	22.61	15.07	18.73	28.16	24.02	25.00	24.71	36.27	34.23
IGF-I	21.12	16.37	9.66	21.23	20.57	23.39	22.04	24.97	27.89
IGF-I	20.10	13.97	8.88	23.61	17.52	19.58	23.87	49.91	21.67
IGF-I	16.55	15.04	23.88	24.30	26.43	30.26	35.71	27.94	32.96
IGF-I	13.51	17.03	25.06	14.89	31.04	32.56	31.82	31.32	30.59
IGF-I	18.77	15.29	28.99	27.62	32.39	32.08	28.03	28.46	30.59
NADPH	28.38	23.25	28.89	35.51	48.92	44.57	4.78	74.24	57.48
NADPH	29.68	26.87	30.59	46.58	46.21	48.62	46.38	79.90	66.51
NADPH	28.48	23.21	31.66	36.93	51.53	54.36	52.63	47.82	62.44
NADPH	32.23	31.36	49.11	47.47	47.38	77.72	59.39	57.77	62.82
NADPH	28.75	21.37	38.55	45.06	70.46	80.88	64.55	85.25	112.18
NADPH	20.60	17.33	31.36	37.82	48.19	54.10	50.72	54.49	57.30
CRF + NADPH	27.21	22.02	29.87	33.95	36.78	38.59	55.62	54.31	56.61
CRF + NADPH	20.81	19.08	24.61	33.88	33.79	41.82	37.82	46.67	47.82
CRF + NADPH	25.43	15.16	20.57	34.75	34.98	36.31	32.77	48.30	53.86
CRF + NADPH	22.60	18.38	35.40	35.22	42.74	49.74	50.38	53.49	60.86
CRF + NADPH	22.06	24.05	22.65	39.64	53.11	55.89	60.29	65.50	73.23
CRF + NADPH	44.93	30.81	69.25	56.04	78.27	78.84	93.14	105.12	115.38
ACTH + NADPH	38.31	29.19	39.42	51.81	43.11	61.89	66.39	78.58	75.15
ACTH + NADPH	9.84	30.11	36.74	45.80	51.81	60.17	59.68	59.57	81.98
ACTH + NADPH	10.26	13.43	21.09	23.73	24.89	21.82	29.11	40.03	38.35
ACTH + NADPH	21.97	12.30	26.20	33.32	27.28	38.79	40.76	56.51	60.98
ACTH + NADPH	22.88	22.16	26.58	37.60	31.41	59.88	43.77	58.51	63.95
ACTH + NADPH	21.09	19.39	33.70	35.01	58.60	49.92	45.14	55.04	55.82
IGF-I + NADPH	16.82	21.99	27.03	27.23	33.36	39.13	39.74	51.45	55.22
IGF-I + NADPH	21.42	20.80	23.49	30.73	33.81	34.28	35.63	49.19	44.39
IGF-I + NADPH	16.05	16.46	25.91	30.38	30.01	33.71	39.35	46.06	41.64
IGF-I + NADPH	20.42	18.54	39.87	47.44	42.83	61.78	56.23	58.79	73.58
IGF-I + NADPH	33.74	21.49	37.68	43.25	55.70	47.60	49.10	60.94	73.57
IGF-I + NADPH	19.62	18.37	33.22	33.70	27.21	28.07	52.19	36.84	52.34

Appendix table 6.3.4. The values for P levels (pmol/mg protein) produced by tissue explants obtained from placenta A3 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 20 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	1037.1	1400.1	956.7	1034.1	1348.0	1490.4	1149.4	954.8	1070.8
Control	654.5	609.4	520.5	684.3	553.2	825.2	854.2	442.0	662.2
Control	750.7	656.4	681.0	790.0	906.0	1166.3	512.5	723.2	852.0
Control	1237.1	1161.5	1375.5	1127.0	1212.5	1590.6	1410.8	1082.2	1192.3
Control	781.2	705.6	729.8	725.4	646.1	1156.4	2147.5	693.8	928.8
Control	1064.2	494.1	455.6	600.6	533.4	706.7	759.9	660.8	996.7
CRF	617.5	904.2	783.8	639.9	711.1	899.0	701.2	557.6	590.7
CRF	547.7	552.1	950.0	801.8	880.3	846.9	852.0	606.5	1101.7
CRF	1108.6	984.9	1134.3	1095.1	1097.6	1354.2	1297.7	1041.5	1231.6
CRF	545.5	574.5	746.0	617.8	679.5	667.8	501.8	589.9	1102.8
CRF	447.9	454.1	623.3	471.7	530.1	675.1	646.8	540.4	884.0
CRF	454.8	435.0	492.3	588.5	564.2	677.7	609.8	711.8	-
IGF-1 20	625.9	613.8	567.2	501.8	672.5	783.8	1171.1	561.7	675.5
IGF-1 20	663.3	819.0	946.4	690.9	894.6	1005.8	996.7	794.8	979.8
IGF-1 20	431.0	369.3	597.6	585.9	585.2	783.0	768.0	604.2	604.2
IGF-1 20	515.8	591.0	684.6	686.8	624.4	826.3	824.1	643.5	761.0
IGF-1 20	734.2	739.3	842.1	955.9	872.2	1198.9	854.2	840.3	1111.2
IGF-1 20	480.5	532.3	658.2	741.5	490.8	805.0	733.5	615.3	705.2
IGF-II 20	494.5	533.0	461.1	516.5	710.7	699.3	608.7	545.9	681.0
IGF-II 20	1168.5	524.2	511.0	587.7	580.8	837.0	754.4	538.5	675.1
IGF-II 20	392.4	448.2	498.9	442.7	533.4	676.6	553.6	439.8	627.4
IGF-II 20	504.8	625.5	784.9	831.8	621.1	943.5	725.0	578.2	845.4
IGF-II 20	814.6	867.5	936.1	958.9	991.2	1138.0	861.2	802.8	966.9
IGF-II 20	664.5	656.7	926.2	823.0	735.7	905.3	712.9	1004.8	845.4
IGF-I 400	589.2	686.8	616.4	652.0	562.0	784.5	710.0	478.0	279.0
IGF-I 400	504.4	523.5	503.3	518.0	608.7	656.7	518.0	406.8	534.5
IGF-I 400	655.6	532.3	510.3	438.7	566.8	766.5	688.0	433.2	641.0
IGF-I 400	723.5	764.7	1087.7	839.6	804.3	1203.7	1042.2	922.5	1063.1
IGF-I 400	524.6	643.2	602.8	776.4	460.7	867.5	847.6	545.1	746.0
IGF-I 400	515.8	591.0	684.6	686.8	624.4	826.3	824.1	643.5	761.0
CRF + NADPH	2412.2	2232.0	1550.6	1987.1	1078.2	1280.1	1399.4	981.6	1199.7
CRF + NADPH	4384.6	4453.7	2594.3	2698.2	2087.0	2425.4	2397.2	1956.3	2071.2
CRF + NADPH	964.0	1009.2	594.0	623.3	583.3	526.0	637.3	603.5	540.4
CRF + NADPH	2950.4	3589.5	2656.7	2051.4	1412.6	1482.7	1345.4	899.0	1261.0
CRF + NADPH	2063.8	806.2	1073.0	1006.2	896.1	915.5	910.0	756.2	913.3
CRF + NADPH	1761.7	1819.0	1616.3	1024.9	967.3	1091.8	958.9	864.2	1174.0
IGF-I 20 +NADPH	1234.6	1544.4	1041.8	1148.7	964.7	1100.6	1120.8	906.4	810.6
IGF-I 20 +NADPH	1362.3	1367.8	960.3	985.3	682.4	853.1	742.3	646.8	689.4
IGF-I 20 +NADPH	915.2	1496.7	896.5	657.5	645.4	712.2	752.2	772.8	724.7
IGF-I 20 +NADPH	3493.3	5194.1	2761.0	1689.8	2109.7	3300.6	3004.7	2241.1	2567.1
IGF-I 20 +NADPH	1158.6	1791.1	557.6	571.6	616.4	907.1	2034.1	816.8	822.3
IGF-I 20 +NADPH	1677.3	1729.4	1332.6	1065.0	890.6	1063.1	1114.5	1022.7	848.4

IGF-I 20:- IGF-I at a concentration of 20 ng/ml.

IGF-II 20:- IGF-II at a concentration of 20 ng/ml.

IGF-I 400:- IGF-I at a concentration of 400 ng/ml.

Appendix table 6.4.1 The values for E2 levels (fmol/mg protein) produced by tissue explants obtained from placenta A4 and incubated for 24 hours.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	2.03	2.12	2.17	2.09	2.36	2.88	2.15	2.65	2.47
Control	1.66	1.52	1.56	1.45	1.96	1.67	2.33	1.93	2.10
Control	1.56	7.47	8.41	7.15	8.42	7.89	8.10	6.96	9.20
Control	12.14	12.16	10.15	11.60	10.69	12.85	11.27	14.05	11.91
Control	6.90	6.79	6.53	7.10	7.53	9.07	7.22	8.84	9.11
Control	6.39	7.22	5.96	7.19	12.79	7.65	6.64	8.61	8.88
CRF	6.24	9.66	6.85	7.03	6.69	9.71	6.87	8.03	8.31
CRF	4.73	7.24	7.34	6.23	11.06	6.88	7.30	7.94	8.46
CRF	10.36	10.29	13.23	11.11	13.51	13.26	12.96	9.81	14.06
CRF	10.36	4.60	7.26	7.26	7.19	7.66	6.56	7.84	7.54
CRF	7.11	6.02	6.20	6.54	5.53	7.45	7.45	7.14	6.83
CRF	4.96	6.18	6.07	5.68	5.72	6.54	5.53	6.55	6.67
IGF-1 20	5.26	5.45	5.61	5.73	6.43	6.34	6.39	6.62	7.54
IGF-1 20	7.29	6.96	7.37	7.19	8.25	8.50	8.52	7.64	9.48
IGF-1 20	1.32	4.84	5.51	5.01	6.65	5.76	6.35	5.14	6.74
IGF-1 20	5.90	6.75	5.29	5.74	5.21	6.69	5.95	8.09	7.58
IGF-1 20	7.43	8.34	8.64	9.65	8.70	8.50	8.30	10.13	10.44
IGF-1 20	5.41	5.37	5.14	5.46	4.60	5.77	5.10	6.12	6.44
IGF-II 20	4.99	6.05	5.47	6.54	6.59	7.05	6.60	7.08	8.25
IGF-II 20	4.93	5.72	5.38	5.60	6.92	6.33	6.52	6.62	7.58
IGF-II 20	10.79	4.99	3.20	4.00	5.47	5.19	5.46	4.64	6.05
IGF-II 20	7.80	7.87	6.81	7.03	7.12	8.73	6.92	8.78	8.87
IGF-II20	8.84	10.80	11.19	10.53	9.14	10.77	10.04	12.16	11.74
IGF-II 20	7.52	7.90	6.90	7.22	7.11	7.41	7.31	9.17	9.26
IGF-I 400	5.26	5.45	5.61	5.73	6.43	6.34	6.39	6.46	6.62
IGF-I 400	3.75	4.25	5.31	4.06	5.00	5.05	6.12	5.81	6.13
IGF-I 400	0.71	4.78	4.57	4.58	3.98	4.52	5.17	5.36	6.15
IGF-I 400	7.43	8.96	7.57	7.96	6.94	9.88	7.71	9.18	9.20
IGF-I 400	9.40	9.63	8.71	8.85	7.89	8.62	8.43	10.17	10.66
IGF-I 400	5.49	6.13	4.73	6.48	5.65	6.39	5.78	6.64	7.41
CRF + NADPH	5.45	7.43	7.80	7.20	9.04	8.79	8.41	7.20	8.57
CRF + NADPH	13.51	12.98	12.48	12.84	15.13	14.08	14.19	12.60	16.08
CRF + NADPH	3.34	3.87	4.47	4.74	5.23	4.84	4.80	4.75	5.17
CRF + NADPH	10.69	11.67	8.57	8.72	9.54	11.15	10.01	12.18	11.34
CRF + NADPH	5.47	6.47	6.20	7.30	10.96	7.62	7.07	9.04	8.05
CRF + NADPH	6.53	6.69	5.40	6.46	7.67	7.05	6.17	9.22	8.18
IGF-I 20 +NADPH	5.83	6.38	7.10	7.41	7.50	7.64	7.41	8.88	9.55
IGF-I 20 +NADPH	3.81	4.12	4.94	4.84	6.01	5.16	6.41	6.43	6.07
IGF-I 20 +NADPH	1.10	5.89	5.40	5.62	11.37	7.18	6.99	7.97	7.14
IGF-I 20 +NADPH	17.74	18.61	13.44	16.16	16.30	17.17	15.52	18.24	15.16
IGF-I 20 +NADPH	5.34	6.26	5.02	5.77	5.97	6.19	5.41	6.79	7.46
IGF-I 20 +NADPH	7.47	8.47	5.40	8.17	7.70	9.02	7.37	8.74	9.06

IGF-I 20:- IGF-I at a concentration of 20 ng/ml.

IGF-II 20:- IGF-II at a concentration of 20 ng/ml.

IGF-I 400:- IGF-I at a concentration of 400 ng/ml.

Appendix table 6.4.2. The values for E3 levels (pmol/mg protein) produced by tissue explants obtained from placenta A4 and incubated for 24 hours.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	43.15	46.56	44.07	60.93	61.09	66.56	59.53	69.48	73.27
Control	22.61	22.74	29.61	40.55	43.03	44.33	40.86	47.73	42.77
Control	32.66	53.01	40.86	49.54	54.95	62.23	74.63	62.49	61.63
Control	52.98	57.65	65.67	66.65	73.71	76.16	89.45	90.73	90.73
Control	19.05	44.27	49.10	49.26	58.89	52.12	56.86	52.06	57.75
Control	25.98	37.59	33.20	46.33	45.89	35.58	52.02	47.45	46.52
CRF	27.89	38.22	38.67	49.80	50.28	44.97	53.58	48.59	58.13
CRF	27.63	41.18	35.46	39.88	50.15	54.41	46.46	53.46	54.57
CRF	43.34	53.49	66.84	88.66	67.77	76.19	80.68	91.55	85.19
CRF	32.37	47.10	51.64	60.52	64.84	61.69	57.78	84.75	81.73
CRF	24.10	27.22	31.10	36.82	46.21	32.53	39.11	40.00	41.09
CRF	17.04	19.14	22.32	25.25	25.06	29.64	28.33	40.48	34.98
IGF-1 20	23.72	32.34	40.26	45.28	40.23	40.35	38.89	43.85	58.64
IGF-1 20	29.07	31.39	37.46	57.11	60.39	60.17	53.04	62.90	52.79
IGF-1 20	21.56	20.89	23.06	32.69	34.53	36.57	26.36	33.87	39.50
IGF-1 20	27.76	30.85	37.43	39.02	48.11	39.56	44.11	41.75	47.80
IGF-1 20	31.61	30.56	38.32	51.77	70.09	43.66	60.36	57.46	62.26
IGF-1 20	20.00	17.81	25.47	33.29	31.55	31.35	32.47	35.39	31.74
IGF-II 20	25.89	27.35	41.75	34.69	36.09	38.48	40.48	34.25	57.69
IGF-II 20	27.03	26.14	27.92	41.02	30.56	41.50	36.60	56.57	51.36
IGF-II 20	23.95	21.15	25.89	31.74	27.67	33.58	31.48	38.13	29.32
IGF-II 20	31.96	40.16	37.78	46.36	50.85	37.78	52.34	53.93	47.57
IGF-II 20	38.86	49.35	38.16	45.76	77.50	52.22	68.24	62.84	63.06
IGF-II 20	12.24	34.34	34.98	35.46	52.66	45.00	43.73	50.94	42.45
IGF-I 400	24.07	19.18	32.12	36.95	41.79	34.47	41.24	39.85	38.57
IGF-I 400	18.95	17.55	24.68	28.24	29.96	35.36	24.58	35.74	39.91
IGF-I 400	18.89	20.70	22.23	27.79	29.54	31.39	34.28	23.69	36.60
IGF-I 400	27.60	23.79	35.33	42.61	27.95	42.80	54.66	62.11	67.35
IGF-I 400	39.81	40.26	38.45	48.02	68.56	59.97	70.37	60.74	59.85
IGF-I 400	23.02	27.35	24.77	35.55	41.50	37.37	36.86	40.10	49.26
CRF + NADPH	39.78	47.54	47.70	68.05	76.32	71.71	86.56	81.85	121.32
CRF + NADPH	56.95	91.14	104.49	97.18	127.84	141.32	125.80	134.32	178.62
CRF + NADPH	16.92	20.73	19.84	32.31	34.38	32.25	33.26	34.92	37.81
CRF + NADPH	59.50	61.95	59.02	78.45	99.41	89.52	85.35	115.12	115.88
CRF + NADPH	31.64	32.44	42.55	44.65	77.97	59.08	61.15	61.37	75.72
CRF + NADPH	29.96	30.56	33.20	34.25	64.97	44.87	63.22	69.58	92.38
IGF-I 20 +NADPH	34.25	36.57	52.15	75.02	69.23	71.36	67.45	80.20	108.15
IGF-I 20 +NADPH	16.66	19.37	35.84	37.81	34.98	42.77	31.61	41.72	58.86
IGF-I 20 +NADPH	32.85	27.35	32.31	48.37	48.88	55.46	43.44	64.71	49.89
IGF-I 20 +NADPH	62.20	178.46	106.43	134.26	178.88	134.13	156.42	197.64	141.80
IGF-I 20 +NADPH	27.44	28.62	41.98	42.80	62.49	42.29	63.19	53.42	60.77
IGF-I 20 +NADPH	29.73	28.46	41.82	61.63	87.04	58.16	62.81	82.08	72.69

IGF-I 20:- IGF-I at a concentration of 20 ng/ml.

IGF-II 20:- IGF-II at a concentration of 20 ng/ml.

IGF-I 400:- IGF-I at a concentration of 400 ng/ml.

Appendix table 6.4.3. The values for P levels (pmol/mg protein) produced by tissue explants obtained from placenta A4 and incubated for 24 hours.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	2.30	2.45	2.47	2.59	2.80	2.97	3.15	3.26	3.99
Control	2.40	1.98	3.37	2.82	3.09	3.25	3.54	3.33	3.98
Control	2.29	2.39	2.76	2.97	2.59	3.10	3.07	3.41	4.08
Control	2.28	1.59	4.21	3.72	4.37	3.95	4.46	4.43	5.13
Control	2.90	2.78	3.30	3.27	3.92	3.82	3.79	3.97	4.42
Control	4.56	5.59	5.86	4.91	5.99	5.65	6.93	6.19	6.96
IGF-I	1.99	2.28	2.50	2.53	2.62	2.61	2.67	2.91	3.42
IGF-I	2.33	2.62	2.91	2.69	2.95	3.22	3.61	3.65	4.01
IGF-I	2.71	2.79	2.42	3.51	3.21	3.43	3.95	4.21	4.04
IGF-I	2.91	3.06	3.04	3.55	3.48	3.76	4.01	4.59	4.74
IGF-I	3.70	3.68	4.60	4.42	4.74	4.20	4.80	5.32	5.41
IGF-I	6.91	5.79	6.51	6.96	6.79	6.19	7.44	7.67	7.92
NADPH	1.64	1.76	1.38	1.29	1.85	2.08	2.24	2.18	3.49
NADPH	2.92	2.14	1.58	1.92	2.24	2.79	3.36	4.05	4.54
NADPH	2.73	2.06	2.67	1.96	2.24	2.63	3.24	3.33	3.68
NADPH	3.37	3.38	3.63	3.01	2.94	2.93	4.15	4.37	4.65
NADPH	2.78	2.91	2.33	2.53	2.77	2.45	3.48	3.46	3.86
NADPH	4.88	6.20	6.00	4.67	6.39	5.84	5.42	5.95	6.46
IGF-I + NADPH	2.48	2.06	2.25	1.88	2.33	2.64	2.82	3.56	3.73
IGF-I + NADPH	2.37	1.66	1.59	1.74	1.90	2.58	2.84	2.62	2.87
IGF-I + NADPH	1.90	2.05	2.14	2.28	2.14	2.48	3.12	1.98	4.16
IGF-I + NADPH	2.81	3.28	3.51	3.46	2.74	3.17	3.62	5.04	3.01
IGF-I + NADPH	5.83	6.63	5.62	4.78	5.37	5.39	5.92	6.79	6.66
IGF-I + NADPH	5.21	6.86	5.58	5.40	6.48	3.90	7.38	7.82	7.49

Appendix table 6.5.1. The values for E1 levels (pmol/mg protein) produced by tissue explants obtained from placenta A5 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	1.26	1.97	0.85	0.74	0.63	0.66	0.86	0.85	1.13
Control	1.90	1.71	0.66	0.72	0.57	0.65	0.77	0.69	1.25
Control	2.04	1.28	0.81	0.72	0.54	0.95	0.80	0.94	1.09
Control	2.80	1.91	1.72	0.91	1.02	1.18	0.98	1.54	1.54
Control	1.58	0.81	1.05	0.80	0.87	0.97	1.22	1.22	1.09
Control	1.92	3.37	1.58	1.44	1.64	1.89	1.71	2.29	1.82
IGF-I	0.88	0.83	0.40	0.57	0.55	0.53	0.64	0.61	0.91
IGF-I	1.13	1.96	0.88	0.88	0.63	0.66	0.73	0.70	1.05
IGF-I	1.65	0.77	0.73	0.66	0.71	0.81	1.07	1.08	1.21
IGF-I	1.31	1.70	1.03	0.86	0.78	0.74	1.06	1.16	1.22
IGF-I	1.38	1.30	1.12	1.36	1.22	0.97	1.40	1.34	1.39
IGF-I	2.34	2.51	2.45	2.02	2.27	2.37	2.58	3.14	2.40
NADPH	1.38	1.62	0.83	0.51	0.54	0.66	0.75	0.84	0.74
NADPH	1.71	1.68	1.33	1.74	0.61	1.50	1.19	0.97	1.25
NADPH	2.14	1.55	1.16	0.86	1.09	0.92	0.72	0.83	0.95
NADPH	2.29	2.23	2.31	2.03	2.55	2.38	2.40	2.25	1.45
NADPH	2.44	1.67	1.36	1.19	1.94	1.81	1.11	1.15	1.03
NADPH	4.97	4.19	3.45	2.67	3.41	3.92	2.56	3.15	1.88
IGF-I + NADPH	1.97	2.25	1.45	1.38	1.39	1.43	1.04	1.01	1.03
IGF-I + NADPH	1.95	1.31	0.99	1.06	0.79	1.06	0.91	0.72	0.89
IGF-I + NADPH	1.45	1.20	0.94	0.88	1.37	1.53	0.91	1.05	1.07
IGF-I + NADPH	1.88	1.55	1.39	1.21	1.80	1.11	1.38	1.42	1.34
IGF-I + NADPH	3.81	0.35	2.56	1.37	3.13	2.78	3.13	2.25	1.76
IGF-I + NADPH	5.51	5.30	2.52	3.71	3.32	3.64	4.14	3.17	2.15

Appendix table 6.5.2. The values for E2 levels (pmol/mg protein) produced by tissue explants obtained from placenta A5 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	2.74	3.62	7.37	4.44	4.53	7.50	4.36	5.03	5.96
Control	4.57	4.28	7.26	1.01	4.46	8.03	5.14	4.07	6.21
Control	4.31	4.29	7.05	4.71	5.02	9.53	4.04	5.54	5.20
Control	5.55	6.01	9.66	6.34	6.14	12.37	5.10	6.27	6.67
Control	4.44	4.88	7.98	4.66	5.76	9.94	5.14	4.90	6.02
Control	8.53	9.07	11.28	5.76	9.30	16.41	7.61	7.99	9.52
IGF-I	3.52	3.58	6.30	3.45	3.82	3.45	4.00	3.87	5.06
IGF-I	4.44	4.07	7.05	3.91	4.42	3.91	4.30	4.79	5.78
IGF-I	4.91	4.56	8.72	4.67	5.47	4.67	5.07	5.52	6.02
IGF-I	5.06	5.32	7.40	5.10	5.33	5.10	4.90	5.80	6.49
IGF-I	6.40	6.42	9.33	4.95	7.45	8.40	5.50	5.73	7.00
IGF-I	9.11	10.14	14.32	8.40	9.85	18.34	8.78	9.97	12.29
NADPH	2.96	3.14	3.53	3.40	3.70	6.59	3.73	4.14	5.55
NADPH	4.28	3.78	4.63	4.28	5.20	9.48	4.89	4.55	5.45
NADPH	3.41	3.75	3.95	4.51	4.80	8.59	4.37	4.53	5.92
NADPH	5.38	5.93	5.25	6.10	5.54	11.73	6.02	6.73	7.67
NADPH	4.68	4.63	4.26	5.08	4.74	9.93	4.56	4.69	5.60
NADPH	10.40	8.37	8.82	8.03	9.10	17.72	8.31	7.94	10.80
IGF-I + NADPH	3.55	4.10	8.33	5.14	5.50	9.11	5.64	5.03	5.52
IGF-I + NADPH	3.15	3.73	6.67	3.93	4.32	7.72	4.05	4.94	5.60
IGF-I + NADPH	3.24	3.78	6.34	4.25	4.64	8.91	3.91	4.87	5.25
IGF-I + NADPH	4.94	5.30	9.08	5.49	6.88	13.03	5.37	6.51	6.70
IGF-I + NADPH	8.64	8.45	14.90	7.87	10.19	16.89	7.79	8.32	11.40
IGF-I + NADPH	10.79	11.95	19.13	10.19	9.48	15.49	10.50	11.72	11.48

Appendix table 6.5.3. The values for E3 levels (pmol/mg protein) produced by tissue explants obtained from placenta A5 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	16.76	42.36	48.37	45.70	44.39	60.96	65.79	58.45	64.43
Control	33.84	38.57	42.10	45.44	46.81	48.75	66.84	50.69	69.99
Control	27.16	101.79	38.00	43.25	58.73	59.43	64.55	54.98	68.97
Control	43.63	99.22	123.00	148.79	93.24	116.83	85.73	119.41	114.77
Control	24.36	80.61	101.70	109.84	95.81	75.40	73.08	88.18	88.02
Control	60.13	155.72	194.17	270.55	198.46	143.23	156.33	168.73	165.04
IGF-I	24.80	42.80	46.08	58.96	51.93	55.11	57.08	50.02	62.49
IGF-I	34.63	40.86	61.22	67.32	58.83	58.32	67.23	58.77	68.40
IGF-I	30.05	36.76	51.26	56.38	62.17	52.79	72.44	80.26	84.68
IGF-I	38.13	65.44	102.30	109.74	80.36	77.94	82.11	130.54	88.18
IGF-I	37.02	105.54	99.22	108.69	92.35	73.08	102.87	113.97	108.09
IGF-I	55.97	162.34	202.53	225.59	124.56	135.28	183.14	167.11	191.63
NADPH	24.17	39.59	45.76	51.80	61.41	78.83	67.23	89.99	92.38
NADPH	34.66	46.65	55.65	65.22	83.60	77.85	94.45	84.68	100.30
NADPH	27.41	23.88	30.53	35.93	68.66	75.27	84.24	74.54	79.02
NADPH	50.05	102.90	132.26	163.52	122.56	135.82	142.31	180.66	175.41
NADPH	40.58	51.71	83.67	81.38	110.70	95.59	109.42	112.41	128.38
NADPH	83.09	155.37	243.02	304.96	146.25	201.90	223.52	240.06	261.05
IGF-I + NADPH	33.39	43.66	58.19	67.83	86.56	91.49	89.58	92.25	95.46
IGF-I + NADPH	27.60	31.51	30.08	35.65	88.18	67.29	74.51	61.69	77.88
IGF-I + NADPH	32.56	44.42	64.52	74.83	84.33	88.53	75.02	76.67	111.94
IGF-I + NADPH	40.55	147.77	86.46	127.55	134.16	147.97	147.14	206.89	167.14
IGF-I + NADPH	65.95	132.89	147.04	227.43	211.02	185.74	214.49	183.26	207.69
IGF-I + NADPH	82.68	193.82	281.59	241.97	167.40	136.26	141.32	180.12	196.68

Appendix table 6.5.4. The values for P levels (pmol/mg protein) produced by tissue explants obtained from placenta A5 and incubated for a total period of 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	2.15	2.80	3.20	3.15	3.26	4.01	3.94	3.96	4.33
Control	1.47	2.50	2.72	2.75	3.18	3.64	3.44	3.46	3.41
Control	2.38	3.61	4.22	4.45	5.09	5.40	5.14	5.34	5.41
Control	2.74	3.38	3.36	4.55	3.87	3.72	3.64	4.54	6.06
Control	5.04	7.29	6.18	8.23	7.29	6.51	7.69	7.37	9.84
Control	3.39	5.21	4.54	5.88	4.58	5.75	5.87	5.88	6.64
IGF-I	1.38	3.30	2.95	3.06	3.35	3.96	4.21	3.46	3.88
IGF-I	<0.01	2.07	2.41	2.19	2.50	1.59	0.66	3.01	2.32
IGF-I	2.29	3.13	3.52	3.50	3.96	4.47	4.63	4.62	5.05
IGF-I	2.24	3.36	3.47	3.69	3.70	3.71	4.05	4.02	4.79
IGF-I	1.93	3.34	3.24	3.65	3.63	3.89	3.40	4.09	4.98
IGF-I	3.57	4.27	4.27	5.27	4.28	4.52	5.29	5.53	6.92
NADPH	1.21	2.01	2.19	2.17	2.13	2.58	3.17	3.16	3.60
NADPH	3.38	5.80	4.87	5.72	4.45	4.88	5.13	5.07	8.28
NADPH	1.83	0.38	2.81	2.54	3.02	2.88	3.50	3.41	4.56
NADPH	2.44	3.26	3.63	3.39	3.98	4.22	4.31	4.28	5.83
NADPH	3.24	3.12	2.74	3.43	3.02	3.19	3.30	4.35	5.17
NADPH	2.40	3.11	3.04	3.46	2.92	3.39	3.14	3.78	5.56
IGF-I + NADPH	1.35	2.11	2.15	2.28	2.39	2.74	3.14	3.01	3.07
IGF-I + NADPH	2.41	3.40	3.48	3.45	3.43	3.52	3.89	3.77	6.14
IGF-I + NADPH	1.62	2.31	2.89	2.21	3.00	3.31	3.26	3.77	4.36
IGF-I + NADPH	2.45	2.88	2.63	3.64	2.61	2.80	3.35	3.69	4.38
IGF-I + NADPH	1.78	2.53	2.20	2.67	2.34	2.31	2.79	3.35	4.49
IGF-I + NADPH	7.95	8.73	8.15	7.26	5.64	8.04	9.85	8.20	11.89

Appendix table 6.6.1. The values for E1 levels (pmol/mg protein) produced by tissue explants obtained from placenta A6 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	4.44	4.89	3.29	2.02	1.77	1.76	1.66	1.64	1.49
Control	3.26	2.89	3.19	1.37	1.41	1.54	1.36	1.37	1.22
Control	1.26	1.81	2.59	1.54	1.92	1.88	1.74	1.83	1.64
Control	1.49	5.28	1.76	1.59	1.80	1.88	1.72	1.53	1.74
Control	6.31	5.03	2.70	2.84	3.51	3.55	3.01	3.48	3.01
Control	6.49	6.24	5.75	2.44	4.26	3.28	2.67	2.64	2.57
IGF-I	0.94	0.88	1.23	1.32	1.46	1.43	1.50	1.47	1.42
IGF-I	3.13	2.74	1.43	0.96	1.08	1.01	1.12	1.15	1.19
IGF-I	2.48	1.64	1.71	1.91	1.67	1.61	1.61	1.76	1.62
IGF-I	4.15	5.92	4.40	3.01	2.30	1.99	1.85	2.01	1.80
IGF-I	3.47	2.78	3.36	1.41	1.49	1.67	1.52	1.92	1.47
IGF-I	5.23	3.21	3.74	2.23	2.39	2.20	2.22	2.19	2.05
NADPH	3.40	3.98	4.96	4.68	5.87	5.72	4.74	4.89	2.31
NADPH	6.67	6.50	8.32	7.17	9.88	9.47	10.95	10.60	4.13
NADPH	2.56	5.25	5.95	5.10	5.89	6.53	5.62	7.64	2.81
NADPH	5.11	4.95	4.84	4.87	5.93	6.20	5.58	7.46	2.60
NADPH	4.92	5.61	5.28	4.97	4.95	5.64	5.66	6.72	2.55
NADPH	7.14	7.30	8.34	7.75	8.46	8.60	8.97	9.74	4.08
IGF-I + NADPH	3.58	3.07	3.48	2.27	4.36	4.39	2.00	2.38	1.84
IGF-I + NADPH	4.64	4.65	6.39	6.33	5.70	7.37	7.76	7.64	3.13
IGF-I + NADPH	3.00	2.44	3.75	3.40	4.69	5.27	3.34	4.08	2.02
IGF-I + NADPH	3.87	5.13	2.65	4.93	5.07	6.15	5.35	7.57	2.64
IGF-I + NADPH	3.70	4.26	3.84	3.71	4.09	4.35	4.07	4.48	2.12
IGF-I + NADPH	12.16	15.28	16.22	12.36	14.19	14.24	15.13	16.69	9.05

Appendix table 6.6.2. The values for E2 levels (pmol/mg protein) produced by tissue explants obtained from placenta A6 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	8.52	10.00	10.05	12.53	12.98	9.49	11.91	12.10	11.09
Control	6.79	9.75	8.53	12.58	13.28	11.10	10.38	10.69	8.29
Control	9.54	13.62	11.77	9.93	14.31	11.82	9.08	13.95	12.59
Control	9.70	12.17	12.37	11.99	12.29	8.54	12.59	14.76	14.26
Control	11.55	19.50	20.81	20.43	20.41	13.68	18.87	22.82	23.31
Control	13.66	15.72	15.71	16.27	14.79	15.18	13.67	18.37	18.38
IGF-I	6.99	8.78	9.25	9.77	11.32	12.43	10.41	12.36	11.45
IGF-I	7.76	8.25	8.14	9.95	11.30	8.40	8.53	9.56	9.56
IGF-I	8.16	10.18	10.13	11.25	12.26	11.42	10.35	12.44	12.36
IGF-I	9.09	10.92	10.57	11.57	11.17	7.84	10.26	13.54	12.01
IGF-I	7.42	9.96	9.62	11.48	9.21	8.46	10.04	12.18	10.66
IGF-I	9.96	11.83	11.81	13.06	12.74	10.64	12.82	15.34	18.69
NADPH	13.90	16.60	16.04	15.94	18.62	12.12	15.87	20.03	19.84
NADPH	7.14	10.91	10.05	11.93	11.72	7.72	11.47	12.55	12.73
NADPH	6.92	8.92	7.95	11.20	11.76	13.02	8.86	10.25	10.37
NADPH	10.46	12.03	10.32	11.71	13.58	11.20	11.00	13.99	12.52
NADPH	10.23	10.60	10.58	13.29	12.08	9.85	11.82	14.67	13.76
NADPH	9.26	11.27	11.37	13.36	13.33	12.70	11.61	13.51	13.50
IGF-I + NADPH	6.99	8.97	8.12	10.80	9.77	10.42	10.15	10.28	8.86
IGF-I + NADPH	8.70	13.51	13.01	15.48	17.13	15.17	14.13	14.58	15.38
IGF-I + NADPH	7.16	8.69	7.65	9.20	10.81	8.59	8.56	10.46	9.81
IGF-I + NADPH	9.23	12.47	12.19	15.04	13.01	11.50	9.62	14.06	14.70
IGF-I + NADPH	7.24	8.59	9.21	9.85	8.51	8.39	8.69	11.37	11.94
IGF-I + NADPH	23.67	28.63	28.72	31.20	33.21	27.00	31.47	39.12	35.14

Appendix table 6.6.3. The values for E3 levels (pmol/mg protein) produced by tissue explants obtained from placenta A6 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	20 m	40 m	60 m	80 m	100 m	120 m	150 m	180 m	1440 m
Control	66.75	82.65	112.22	113.05	92.79	159.16	117.82	125.42	135.28
Control	54.89	62.65	123.67	95.27	99.76	106.69	98.39	93.62	113.14
Control	87.00	91.77	107.64	80.39	131.37	143.93	147.07	150.03	168.44
Control	78.04	134.96	143.93	126.91	180.40	171.66	160.34	187.91	212.90
Control	94.83	168.25	231.92	224.51	200.21	279.59	222.35	241.68	220.63
Control	60.67	120.97	130.54	157.06	120.05	144.09	153.72	159.99	182.06
IGF-I	58.99	62.93	130.09	141.70	119.22	131.81	112.60	134.99	124.53
IGF-I	60.17	58.16	77.78	89.74	80.17	96.26	94.41	86.34	102.97
IGF-I	71.49	77.31	109.77	117.34	109.42	177.92	123.54	147.04	150.06
IGF-I	85.80	103.57	90.53	135.79	93.97	137.92	124.82	157.82	139.86
IGF-I	72.69	89.74	101.51	108.57	86.05	109.04	112.10	106.24	117.88
IGF-I	90.69	116.07	103.06	147.17	138.23	156.46	151.75	156.11	174.93
NADPH	82.23	92.82	115.47	168.19	167.49	217.58	190.99	181.86	221.33
NADPH	68.72	87.20	114.83	146.06	127.23	145.23	161.80	153.82	172.26
NADPH	77.02	134.42	207.34	225.72	222.12	242.70	262.76	263.18	309.95
NADPH	92.51	119.57	151.11	194.90	162.37	239.80	206.57	229.60	253.38
NADPH	99.76	126.12	160.81	182.31	209.56	213.06	230.36	229.21	272.27
NADPH	233.35	189.37	206.80	226.29	231.85	315.33	341.98	366.46	397.18
IGF-I + NADPH	56.35	68.75	114.67	106.63	102.17	168.57	133.66	132.19	105.58
IGF-I + NADPH	90.03	112.73	187.11	212.77	212.04	273.10	272.02	305.22	318.54
IGF-I + NADPH	74.92	88.66	117.82	141.96	143.74	150.57	161.70	188.54	160.08
IGF-I + NADPH	63.92	108.60	136.07	155.44	125.04	225.84	179.35	219.90	223.33
IGF-I + NADPH	73.20	106.08	143.55	149.05	147.58	187.24	168.22	171.88	238.31
IGF-I + NADPH	215.67	141.92	378.52	419.06	447.59	478.40	520.95	520.73	532.55

Appendix table 6.6.4. The values for P levels (pmol/mg protein) produced by tissue explants obtained from placenta A6 and incubated for 24 hours. The concentration of IGF-I used in these experiments was 400 ng/ml.

	HCG (mIU/100,000 viable cells)		HPL (ng/100,000 viable cells)	
	24 hours	**96 hours**	**24 hours**	**96 hours**
Experiment 1	19.82	4460.0	429.5	1059.0
	23.38	5664.8	375.4	1052.7
	18.85	7848.5	345.8	970.4
	19.71	6937.8	373.1	1132.2
Experiment 2	8.03	450.8	~	79.08
	7.32	233.3	~	84.50
	6.71	207.0	~	75.10
	~	195.7	~	79.14

Table 6.7.1 The values for HCG and HPL levels produced by isolated cytotrophoblast cells incubated for a total period of 96 hours.

	Experiment 1		Experiment 2	
	E1	**E2**	**E1**	**E2**
DHEA	163.5	321.5	33.58	139.7
DHEA	153.9	392.6	36.46	148.7
DHEA	148.5	378.4	53.23	195.2
DHEA	180.2	415.3	53.36	170.3
DHEAS	280.8	497.1	40.91	129.2
DHEAS	149.8	405.8	33.71	161.8
DHEAS	157.9	316.8	49.55	164.0
DHEAS	210.7	447.3	41.58	146.4
A	236.1	494.7	56.72	181.5
A	198.5	503.0	26.00	114.4
A	168.6	537.3	38.26	125.8
A	210.7	595.9	43.25	164.6
T	238.3	513.5	32.53	132.2
T	179.0	426.8	31.76	146.3
T	197.0	477.4	25.83	108.4
T	112.2	399.7	32.76	124.5

Table 6.7.2. The values for E1 and E2 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with a precursor at a concentration of 1 μmol/l for 24 hours.

Precursor	Experiment 1	Experiment 2
16α-OHDHEA	331.6	48.3
16α-OHDHEA	312.9	54.7
16α-OHDHEA	400.1	62.1
16α-OHDHEA	369.9	57.0
16α-OHA	372.9	61.5
16α-OHA	161.8	47.8
16α-OHA	268.7	46.8
16α-OHA	247.9	57.1
16α-OHT	106.2	37.7
16α-OHT	122.7	39.6
16α-OHT	124.5	24.9
16α-OHT	114.5	28.6
16α-OHE1	925.7	1141.9
16α-OHE1	995.0	1309.2
16α-OHE1	992.7	1271.2
16α-OHE1	975.9	1431.4

Table 6.7.3. The values for E3 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells obtained from two placentae incubated with precursors at a concentartion of (1 μmo/l) for 24 hours.

Experiment 1		Experiment 2	
25-OHcholesterol	**cells only**	**25-OHcholesterol**	**cells only**
104.4	126.8	712.2	760.4
197.6	224.4	758.4	577.8
114.4	116.0	493.0	550.0
94.0	202.0	592.4	432.0
122.0	158.0	412.8	227.6
119.2	231.0		

Table 6.7.4. The values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells obtained from two placentae, incubated with/without 25-OHcholesterol (5μmol/l) for 24 hours.

LDL (1.5 mg/ml)	Pregnenolone (1 μmol/l)	Cells only
1.18	4.44	1.10
1.56	3.46	0.78
1.42	4.46	0.63
1.53	3.72	0.79

Table 6.7.5. Values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells obtained from one placenta, incubated with/without LDL or pregnenolone for 24 hours.

A (concentration used nmol/l)	E1 Placenta 1	E1 Placenta 2	E2 Placenta 1	E2 Placenta 2
25	1.83	2.45	3.87	1.49
25	2.11	2.07	4.12	1.74
25	2.05	3.46	4.09	2.00
50	2.83	3.49	3.88	2.19
50	2.04	3.78	5.21	2.19
50	2.73	3.53	5.08	2.56
100	2.47	4.08	5.22	3.26
100	2.64	4.45	6.24	2.68
100	2.63	3.12	7.09	2.94
200	5.44	7.15	6.67	4.03
200	3.07	4.80	8.91	3.08
200	4.49	5.96	5.04	3.72
400	4.59	4.50	7.90	2.41
400	4.83	5.05	9.94	3.00
400	4.51	5.37	7.78	2.62
800	4.90	5.26	8.16	3.25
800	3.34	5.25	5.44	3.53
800	4.15	4.92	5.77	3.48
1600	3.83	5.07	6.85	3.54
1600	3.76	3.10	5.65	2.84
1600	5.73	4.58	5.87	3.23
3200	5.14	5.76	8.87	2.79
3200	3.92	6.30	5.10	2.98
3200	4.63	4.25	5.68	2.94

Appendix table 6.7.6. The values for E1 and E2 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with increasing concentrations of A.

16α–OHA (concentration used nmol/l)	Placenta 1	Placenta 2
25	1.18	0.96
25	1.14	0.85
25	1.03	0.34
50	2.35	0.55
50	1.49	0.44
50	2.50	0.47
100	3.51	0.91
100	4.18	0.95
100	1.21	0.49
200	5.79	1.11
200	6.76	1.49
200	6.94	1.07
400	-	1.87
400	6.60	2.36
400	6.59	2.25
800	9.56	1.81
800	8.46	2.31
800	7.18	3.36
1600	4.53	1.57
1600	7.66	1.70
1600	5.00	3.50
3200	6.60	2.11
3200	9.13	1.60
3200	6.92	2.29

Appendix table 6.7.7. The values for E3 levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with increasing concentrations of 16α–OHA.

Pregnenolone (concentratiuon used μmol/l)	Placenta 1	Placenta 2
0.5	93.1	114.9
0.5	92.0	116.7
0.5	84.6	88.9
1.0	155.8	196.0
1.0	212.7	168.5
1.0	98.9	217.0
5.0	220.2	333.5
5.0	269.3	387.9
5.0	244.9	375.2
10.0	295.4	430.1
10.0	342.3	312.8
10.0	149.4	529.1
20.0	335.0	496.7
20.0	302.7	475.5
20.0	351.4	602.2
40.0	263.2	616.5
40.0	351.2	699.0
40.0	339.8	579.3
80.0	299.8	630.5
80.0	285.1	1199.6
80.0		715.1

Appendix table 6.7.8. The values for P levels (pmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various pregnenolone concentrations.

	E1	E1	E2	E2	E3	E3	P	P
	24 hrs	**96 hrs**	**24 hrs**	**96 hrs**	**24 hrs**	**96 hrs**	**24 hrs**	**96 hrs**
Experiment 1	16.55	12.22	18.25	32.64	133.89	148.60	0.89	3.01
	8.95	19.83	18.44	75.26	85.66	194.94	0.96	3.12
	6.57	16.04	17.63	74.93	45.70	185.27	1.12	2.61
	6.37	17.17	17.27	62.87	48.37	58.37	1.09	1.72
	8.79	16.25	15.97	61.26			1.28	2.89
	8.62	22.24	10.64	57.25			1.39	2.91
Experiment 2	343.13	306.43	432.02	475.91	343.59	427.34	8.46	15.63
	223.02	220.07	429.03	479.00	353.05	519.28	8.84	16.07
	262.76	231.93	485.58	461.93	250.72	348.87	8.84	14.84
	252.77	210.28	450.85	433.35	325.62	325.62	8.46	13.74
Experiment 3	31.50	159.04	164.06	377.23	63.60	278.12	3.74	11.26
	41.64	153.41	153.24	413.54	62.90	227.89	3.16	10.20
	34.44	72.21	157.74	275.03	61.00	224.89	5.35	10.44
	36.16	122.06	144.25	355.41	49.80	175.39	5.15	9.97

Table 6.7.9. The values for levels of E1, E2, E3 (all pmol/100,000 viable cells/24 hours) and P (nmol/100,000 viable cells/24 hours) produced by isolated cells incubated with 20% calf serum.

	Placenta 3	Placenta 4	Placenta 5
16-αOHA	93.56	182.22	284.95
16-αOHA	84.62	127.73	531.92
16-αOHA	77.85	177.32	335.37
16-αOHA	83.11	162.76	188.66
16-αOHA	79.36	189.43	263.02
16-αOHA	82.68	165.35	416.15
16-αOHA + A 70 nmol/l	79.06	95.00	221.76
16-αOHA + A 70 nmol/l	63.88	76.84	194.32
16-αOHA + A 70 nmol/l	80.50	84.62	202.81
16-αOHA + A 70 nmol/l	72.81	88.51	129.56
16-αOHA + A 70 nmol/l	76.46	104.08	167.43
16-αOHA + A 70 nmol/l	69.69	101.79	114.30
16-αOHA + A 175 nmol/l	56.83		
16-αOHA + A 175 nmol/l	60.28		
16-αOHA + A 175 nmol/l	70.39		
16-αOHA + A 175 nmol/l	69.11		
16-αOHA + A 175 nmol/l	65.09		
16-αOHA + A 175 nmol/l	57.17		
16-αOHA + A 350 nmol/l	48.39		
16-αOHA + A 350 nmol/l	56.06		
16-αOHA + A 350 nmol/l	55.23		
16-αOHA + A 350 nmol/l	48.16		
16-αOHA + A 350 nmol/l	54.00		
16-αOHA + A 350 nmol/l	58.36		
16-αOHA + A 700 nmol/l	48.78	74.39	45.5
16-αOHA + A 700 nmol/l	52.46	72.36	23.0
16-αOHA + A 700 nmol/l	52.78	71.50	116.1
16-αOHA + A 700 nmol/l	47.56	18.70	21.9
16-αOHA + A 700 nmol/l	57.42	72.11	~
16-αOHA + A 700 nmol/l	61.25	76.26	~

Appendix table 6.8.1. The values for E3 levels (fmol/100,000 viable cells/24 hours) produced by isolated cytotrophoblast cells incubated with various concentrations of A.

	Placenta 3	Placenta 4
16-αOHA alone	10.54	4.1
16-αOHA alone	13.56	2.61
16-αOHA alone	15.87	2.73
16-αOHA alone	12.24	2.96
16-αOHA alone	10.59	2.40
16-αOHA alone	15.30	2.52
16-αOHA + A 70 nmol/l	10.54	4.10
16-αOHA + A 70 nmol/l	13.56	2.61
16-αOHA + A 70 nmol/l	15.87	2.73
16-αOHA + A 70 nmol/l	12.24	2.96
16-αOHA + A 70 nmol/l	10.59	2.40
16-αOHA + A 70 nmol/l	15.30	2.52
16-αOHA + A 700 nmol/l	13.87	2.06
16-αOHA + A 700 nmol/l	10.98	1.90
16-αOHA + A 700 nmol/l	13.03	2.04
16-αOHA + A 700 nmol/l	14.25	2.35
16-αOHA + A 700 nmol/l	10.56	2.58
16-αOHA + A 700 nmol/l	8.72	3.69

Appendix table 6.8.2. The values for E3 levels (pmol/mg protein) produced by placental tissue minces incubated with 16-α OHA with/without A.

	E1	E2	TE	E3	P
Control	23.79	12.28	36.07	1.37	42.46
Control	27.36	13.02	40.38	1.56	37.22
Control	22.62	10.51	33.13	1.79	36.12
Control	16.84	10.09	26.93	1.46	55.01
Control	14.92	10.33	25.25	1.45	47.85
Control	16.28	14.55	30.83	1.55	47.98
CRF	21.67	13.94	35.61	2.44	42.46
CRF	21.12	12.24	33.36	2.59	71.65
CRF	27.67	14.18	41.85	2.46	63.02
CRF	21.37	10.35	31.72	2.38	44.77
CRF	18.99	14.46	33.45	2.50	61.82
CRF	16.41	13.88	30.29	2.65	74.26
ACTH	24.46	12.63	37.09	1.44	42.64
ACTH	13.56	13.78	27.34	1.60	61.25
ACTH	24.16	13.92	38.08	1.50	47.20
ACTH	22.55	13.79	36.34	1.43	47.84
ACTH	21.68	10.82	32.50	1.70	50.74
ACTH	19.12	15.64	34.76	3.23	55.71
IGF-I 20 ng/ml	21.65	18.93	40.58	1.19	54.27
IGF-I 20 ng/ml	21.41	17.44	38.86	1.21	39.72
IGF-I 20 ng/ml	18.49	19.60	38.09	1.24	61.85
IGF-I 20 ng/ml	16.48	16.22	32.70	1.28	55.73
IGF-I 20 ng/ml	17.40	18.31	35.71	1.29	51.32
IGF-I 20 ng/ml	16.93	18.61	35.54	1.56	30.74
IGF-1 400 ng/ml	22.03	9.87	31.90	1.37	39.43
IGF-1 400 ng/ml	27.10	9.70	36.80	1.39	42.51
IGF-1 400 ng/ml	27.68	11.36	39.04	1.45	40.81
IGF-1 400 ng/ml	27.38	10.83	38.21	1.55	48.19
IGF-1 400 ng/ml	25.74	11.43	37.17	1.44	33.82
IGF-1 400 ng/ml	25.26	11.71	36.97	1.55	44.92
IGF-II 20 ng/ml	22.67	12.80	35.47	1.25	47.65
IGF-II 20 ng/ml	28.04	12.39	40.43	1.50	55.52
IGF-II 20 ng/ml	22.42	14.46	36.88	1.48	62.26
IGF-II 20 ng/ml	17.05	11.79	28.84	1.43	32.21
IGF-II 20 ng/ml	23.23	12.06	35.29	1.66	33.65
IGF-II 20 ng/ml	16.55	13.71	30.26	1.93	62.81
IGF-II 400 ng/ml	36.39	10.51	46.90	1.15	34.88
IGF-II 400 ng/ml	37.29	10.84	48.13	1.51	34.15
IGF-II 400 ng/ml	41.64	11.37	53.01	1.61	45.64
IGF-II 400 ng/ml	21.34	9.12	30.46	1.63	36.46
IGF-II 400 ng/ml	25.85	10.34	36.19	1.65	33.20
IGF-II 400 ng/ml	23.80	9.28	33.08	1.75	55.18

Appendix table 6.9.1. The values for E1, E2, TE, E3 and P levels (all pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placenta B1.

	E1 baseline	E1 24 hrs	E2 24hrs	TE 24 hrs	E3 24 hrs	P 24 hrs
Control	57.87	2.78	0.58	3.36	4.05	36.00
Control	~	3.90	0.68	4.58	4.58	40.64
Control	63.27	2.68	0.50	3.18	3.76	44.39
Control	24.37	2.31	0.39	2.70	3.22	81.79
Control	~	2.68	0.41	3.09	3.99	51.26
Control	47.96	4.42	0.59	5.01	8.36	71.36
CRF	206.35	2.79	0.51	3.30	3.45	80.45
CRF	77.66	3.57	0.57	4.14	3.35	49.42
CRF	77.84	2.08	0.37	2.46	6.63	53.77
CRF	~	2.11	0.38	2.49	3.21	56.54
CRF	109.87	2.01	0.38	2.39	3.47	62.01
CRF	54.84	3.70	0.56	4.26	4.69	84.30
ACTH	86.79	3.96	0.75	4.72	3.21	26.58
ACTH	~	3.74	0.64	4.38	4.23	42.90
ACTH	~	3.24	0.66	3.90	3.72	85.13
ACTH	~	4.35	0.66	5.02	2.54	80.07
ACTH	~	3.56	0.54	4.10	2.97	51.42
ACTH	58.13	4.77	0.74	5.51	2.12	48.43
IGF-I 20 ng/ml	68.41	4.00	0.74	4.74	6.52	31.35
IGF-I 20 ng/ml	64.20	2.85	0.51	3.36	5.16	85.07
IGF-I 20 ng/ml	215.15	3.46	0.60	4.06	3.36	40.55
IGF-I 20 ng/ml	133.31	3.12	0.50	3.62	3.28	52.57
IGF-I 20 ng/ml	~	7.28	1.95	9.23	3.15	79.63
IGF-I 20 ng/ml	~	3.84	1.32	5.16	3.43	54.60
IGF-1 400 ng/ml	~	3.83	0.76	4.59	4.07	80.90
IGF-1 400 ng/ml	688.01	3.27	0.49	3.76	3.21	40.67
IGF-1 400 ng/ml	143.67	5.71	1.25	6.96	2.88	43.88
IGF-1 400 ng/ml	35.17	2.11	0.30	2.41	4.19	79.34
IGF-1 400 ng/ml	1230.92	3.49	0.71	4.20	5.88	51.61
IGF-1 400 ng/ml	65.90	2.70	0.40	3.10	4.08	76.07
IGF-II 20 ng/ml	106.58	2.53	0.47	3.00	3.95	97.21
IGF-II 20 ng/ml	143.33	3.32	0.55	3.87	5.32	66.59
IGF-II 20 ng/ml	8.17	11.29	2.74	14.03	5.93	54.54
IGF-II 20 ng/ml	96.74	3.58	0.58	4.16	4.87	95.08
IGF-II 20 ng/ml	570.05	3.70	0.85	4.55	5.20	130.73
IGF-II 20 ng/ml	105.91	3.27	0.48	3.75	4.28	71.68
IGF-II 400 ng/ml	69.93	3.24	0.70	3.94	4.12	78.83
IGF-II 400 ng/ml	~	4.37	1.32	5.69	4.72	47.51
IGF-II 400 ng/ml	139.23	4.83	0.95	5.78	3.77	90.34
IGF-II 400 ng/ml	133.83	2.42	0.48	2.90	2.59	106.69
IGF-II 400 ng/ml	174.92	4.23	0.77	5.00	5.26	95.18
IGF-II 400 ng/ml	406.45	4.09	0.63	4.72	3.73	58.70
No precursor	104.73	3.03	0.73	3.76	4.16	42.48
No precursor	193.48	3.54	1.16	4.70	3.66	82.62
No precursor	49.85	2.84	0.56	3.40	3.38	57.34
No precursor	884.52	3.31	0.85	4.16	4.17	111.17
No precursor	74.81	2.81	0.55	3.36	4.16	65.03
No precursor	~	3.19	0.61	3.80	4.61	75.62

Appendix table 6.9.2. The values for E1, E2, TE, E3 and P levels produced by tissue explants obtained from placenta B1. All explants were incubated with appropriate precursors except when stated in the table. The values at 24 hours are expressed as pmol/mg protein. Basline levels of E1 are expressed as fmol/mg protein. All other basline values were too low to measure.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P baseline	P 24hrs
Control	2.05	2.71	4.76	0.19	1.42	480.7
Control	2.55	3.10	5.65	0.21	1.91	480.7
Control	2.37	3.34	5.71	0.17	1.93	293.3
Control	2.19	2.69	4.88	0.16		278.2
Control	2.38	3.22	5.60	0.20		293.5
Control	2.23	3.10	5.33	0.14		284.8
CRF	1.87	4.00	5.86	0.29	0.75	239.1
CRF	1.99	3.62	5.61	0.28	1.29	230.9
CRF	1.90	3.96	5.87	0.34	1.69	299.4
CRF	1.73	3.74	5.47	0.29		262.6
CRF	1.79	4.00	5.79	0.30		240.7
CRF	1.72	3.65	5.37	0.33		252.8
ACTH	2.31	2.97	5.28	0.24	1.83	1058.4
ACTH	2.06	3.90	5.96	0.21	2.93	1266.4
ACTH	2.01	3.97	5.98	0.20	2.32	898.4
ACTH	2.05	3.74	5.79	0.23		779.5
ACTH	1.80	4.24	6.04	0.20		664.8
ACTH	2.00	3.32	5.32	0.24		806.6
IGF-I 20 ng/ml	1.80	3.43	5.23	0.35	1.88	324.3
IGF-I 20 ng/ml	1.69	3.87	5.56	0.28	1.61	297.5
IGF-I 20 ng/ml	1.53	4.04	5.57	0.36	2.32	360.1
IGF-I 20 ng/ml	1.52	4.48	6.00	0.34		220.0
IGF-I 20 ng/ml	1.86	4.50	6.36	0.34		246.1
IGF-I 20 ng/ml	1.87	4.26	6.13	0.21	2.48	199.9
IGF-1 400 ng/ml	1.84	3.10	4.94	0.17	1.75	1142.5
IGF-1 400 ng/ml	1.88	1.98	3.85	0.19	.620	441.4
IGF-1 400 ng/ml	1.77	3.51	5.28	0.20	0.78	459.5
IGF-1 400 ng/ml	1.44	2.66	4.10	0.20		240.9
IGF-1 400 ng/ml	1.21	2.34	3.54	0.20		364.1
IGF-1 400 ng/ml	1.21	2.56	3.77	0.19		229.1
IGF-II 20 ng/ml	1.15	4.79	5.94	0.32	1.49	188.2
IGF-II 20 ng/ml	1.63	5.08	6.71	0.36	1.49	236.7
IGF-H 20 ng/ml	2.09	4.73	6.82	0.37		588.4
IGF-II 20 ng/ml	1.58	4.55	6.13	0.38		340.6
IGF-II 20 ng/ml	1.50	4.16	5.66	0.47		582.1
IGF-II 20 ng/ml	1.73	3.88	5.61	0.44		435.9
IGF-II 400 ng/ml	1.70	3.95	5.65	0.28	0.74	608.4
IGF-II 400 ng/ml	1.99	4.05	6.04	0.35	0.90	170.5
IGF-II 400 ng/ml	1.82	4.15	5.97	0.34	3.31	526.5
IGF-II 400 ng/ml	1.34	3.68	5.02	0.36		253.8
IGF-II 400 ng/ml	1.62	4.44	6.06	0.31		317.3
IGF-II 400 ng/ml	1.90	4.29	6.19	0.41		316.9

Appendix table 6.10.1. The values for E1, E2, TE, E3 and P levels (pmol/100,000 viable cells) produced by cytotrophoblast cells isolated from placenta B2.

	E1 baseline	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 baseline	E3 24 hrs	P baseline	P 24 hrs
Control	48.59	37.41	4.35	41.76	0.33	9.27	1.59	118.7
Control	~	18.60	2.38	20.98	0.56	11.71	1.37	109.1
Control	57.59	25.28	2.47	27.75	3.28	9.15	~	105.7
Control	26.24	22.54	4.30	26.84	0.88	7.75	1.37	107.1
Control	~	20.14	3.86	24.01	~	8.58	1.46	178.6
Control	29.93	29.22	3.43	32.66	0.51	6.89	0.32	181.1
CRF	67.21	11.46	1.58	13.05	7.19	14.79	1.62	119.0
CRF	65.83	37.75	4.99	42.74	3.80	10.71	~	119.1
CRF	93.53	21.51	3.03	24.54	8.89	17.18	6.61	82.0
CRF	~	9.82	1.73	11.54	2.13	8.81	1.40	219.2
CRF	119.67	20.75	3.61	24.36	4.66	6.23	5.88	123.2
CRF	18.16	11.15	1.38	12.53	3.66	8.58	0.67	129.8
ACTH	63.69	18.73	2.37	21.10	0.16	7.62	2.35	103.5
ACTH	~	15.50	2.42	17.92	1.44	8.30	2.16	147.8
ACTH	~	15.27	2.09	17.36	3.54	11.27	1.08	175.0
ACTH	~	22.79	3.47	26.26	1.38	5.76	5.85	278.1
ACTH	~	13.22	2.18	15.40	~	6.20	0.86	221.2
ACTH	24.98	14.23	2.67	16.89	7.50	7.96	3.12	195.9
IGF-I 20 ng/ml	38.18	29.96	2.75	32.70	~	9.02	4.52	282.3
IGF-I 20 ng/ml	87.47	31.66	3.36	35.02	~	10.89	1.49	135.4
IGF-I 20 ng/ml	118.20	25.64	5.11	30.75	2.21	10.35	0.83	121.8
IGF-I 20 ng/ml	90.84	33.30	4.71	38.02	0.36	7.68	~	165.9
IGF-I 20 ng/ml	<	12.75	1.76	14.51	2.86	5.52	3.15	185.4
IGF-I 20 ng/ml	~	14.62	2.02	16.63	0.89	7.43	<	124.9
IGF-1 400 ng/ml	~	17.49	2.57	20.06	0.63	10.85	~	222.2
IGF-1 400 ng/ml	135.17	17.75	2.23	19.98	~	10.89	1.27	150.9
IGF-1 400 ng/ml	54.95	20.87	3.17	24.03	1.76	9.70	1.49	124.4
IGF-1 400 ng/ml	38.90	20.03	3.97	24.00	1.44	7.97	4.13	62.0
IGF-1 400 ng/ml	52.45	30.74	3.49	34.23	~	7.75	4.77	270.6
IGF-1 400 ng/ml	41.73	7.51	2.30	9.81	~	17.19	17.68	194.4
IGF-II 20 ng/ml	196.66	38.57	4.92	43.49	0.94	11.76	1.18	220.5
IGF-II 20 ng/ml	203.65	53.09	6.26	59.35	0.92	9.60	9.51	109.7
IGF-II 20 ng/ml	106.20	22.32	1.29	23.610	2.53	13.21	4.96	137.6
IGF-II 20 ng/ml	35.97	13.95	2.08	16.03	3.12	5.93	0.73	138.0
IGF-II 20 ng/ml	177.61	10.71	1.43	12.14	2.75	9.91	2.00	299.3
IGF-II 20 ng/ml	45.76	11.96	1.56	13.52	1.58	7.23	1.40	114.3
IGF-II 400 ng/ml	~	16.19	2.00	18.19	3.32	6.26	14.53	127.6
IGF-II 400 ng/ml	~	13.50	1.97	15.47	~	9.48	15.30	195.8
IGF-II 400 ng/ml	43.22	20.87	2.32	23.19	3.38	12.93	0.48	164.6
IGF-II 400 ng/ml	82.71	23.96	3.04	27.00	1.30	8.37	~	286.9
IGF-II 400 ng/ml	92.86	15.10	3.37	18.46	1.08	6.32	2.86	127.5
IGF-II 400 ng/ml	226.98	20.71	2.75	23.46	0.35	7.16	16.47	104.5
No precursor	39.29	6.04	1.02	7.06	2.21	7.66	2.89	132.9
No precursor	92.11	12.80	1.41	14.21	1.95	8.28	~	139.4
No precursor	29.92	6.50	1.06	7.55	2.11	7.58	2.93	140.5
No precursor	27.01	6.45	0.83	7.29	2.02	5.91	1.40	116.0
No precursor	~	7.11	0.98	8.08	0.29	6.24	1.53	128.8
No precursor	~	5.60	0.78	6.38	~	4.78	6.65	98.1

Appendix table 6.10.2. The values for E1, E2, TE, E3 and P levels produced by placental tissue explants obtained from placenta B2. All explants were incubated with appropriate precursors except when stated in the table. The values for baseline levels of E1 are expressed as fmol/mg protein whilst all other values are expressed as pmol/mg protein.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P baseline	P 24 hrs
Control	3.73	4.01	7.74	0.69	19.62	436.3
Control	3.61	4.04	7.65	0.64	34.60	471.1
Control	4.06	3.68	7.74	0.67	35.68	306.8
Control	3.59	3.39	6.98	0.72		181.5
Control	3.96	3.97	7.93	0.73		378.0
Control	3.82	3.75	7.57	0.68		233.9
CRF	2.75	5.25	8.00	0.88	20.55	235.8
CRF	2.43	5.01	7.44	0.98	59.22	167.6
CRF	2.23	5.72	7.95	0.92	39.34	276.2
CRF	2.84	5.15	7.99	0.89		288.7
CRF	2.75	6.34	9.09	0.90		838.3
CRF	3.21	5.55	8.75	0.92		387.3
ACTH	2.98	5.48	8.46	1.17	31.96	862.7
ACTH	2.74	4.56	7.30	0.89	27.76	776.8
ACTH	2.90	5.38	8.28	1.32	22.61	787.3
ACTH	2.74	4.80	7.54	0.87		493.6
ACTH	2.80	4.81	7.61	0.89		368.9
ACTH	3.32	4.82	8.14	0.89	19.72	736.9
IGF-I 20 ng/ml	3.82	4.75	8.57	1.14	18.09	407.8
IGF-I 20 ng/ml	3.35	4.88	8.24	0.94	25.76	186.9
IGF-I 20 ng/ml	3.68	5.49	9.17	1.09		251.9
IGF-I 20 ng/ml	3.36	5.06	8.41	1.02		169.5
IGF-I 20 ng/ml	3.92	4.71	8.63	1.08		115.9
IGF-I 20 ng/ml	3.78	4.90	8.68	0.72		176.7
IGF-1 400 ng/ml	2.75	3.54	6.29	0.88	33.20	253.9
IGF-1 400 ng/ml	3.23	4.56	7.79	0.83	69.42	218.5
IGF-1 400 ng/ml	2.68	4.69	7.37	0.66	45.79	278.6
IGF-1 400 ng/ml	3.67	4.70	8.37	0.74		103.4
IGF-1 400 ng/ml	2.84	4.02	6.86	0.74		114.2
IGF-1 400 ng/ml	2.81	4.02	6.83	0.73		71.8
IGF-II 20 ng/ml	4.10	4.23	8.33	1.53	~	598.1
IGF-II 20 ng/ml	3.50	5.03	8.53	1.57	~	216.4
IGF-II 20 ng/ml	3.49	5.39	8.88	1.47	~	418.6
IGF-II 20 ng/ml	3.71	4.91	8.62	1.80		306.5
IGF-II 20 ng/ml	3.82	5.22	9.04	1.62		293.3
IGF-II 20 ng/ml	4.32	4.33	8.65	1.70		257.7
IGF-II 400 ng/ml	2.77	5.90	8.67	1.19	41.02	235.2
IGF-II 400 ng/ml	2.49	5.58	8.07	1.27	32.60	373.0
IGF-II 400 ng/ml	2.73	5.77	8.50	1.22	40.67	286.6
IGF-II 400 ng/ml	3.02	5.90	8.92	1.01		125.1
IGF-II 400 ng/ml	2.92	5.17	8.09	1.04		147.5
IGF-II 400 ng/ml	2.65	4.87	7.52	1.25		104.1

Appendix table 6.11.1. The values for E1, E2, TE, E3 and P levels (pmol/100,000 viable cells) produced by cytotrophoblast cells isolated from placenta B3.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P basline	P 24 hrs
Control	7.20	1.12	8.32	5.46	0.60	109.39
Control	5.29	0.80	6.09	4.47	3.18	88.79
Control	4.28	0.67	4.95	6.07	~	97.34
Control	3.15	0.63	3.78	6.94	9.92	67.29
Control	2.63	0.74	3.37	7.64	0.41	99.66
Control	4.18	0.71	4.89	5.76	24.96	89.07
CRF	2.77	0.57	3.34	5.70	27.35	441.67
CRF	7.44	1.18	8.62	4.85	16.15	100.23
CRF	4.00	0.76	4.76	3.80	1.43	104.18
CRF	8.91	2.59	11.50	5.78	2.64	137.31
CRF	4.38	0.84	5.22	6.84	~	304.71
CRF	8.22	1.48	9.70	6.11	4.77	95.02
ACTH	4.35	0.72	5.07	9.55	17.33	230.61
ACTH	5.40	1.00	6.40	11.16	8.27	166.60
ACTH	16.03	3.14	19.17	6.88	2.70	158.90
ACTH	5.19	0.96	6.15	7.60	10.75	153.94
ACTH	4.23	0.99	5.22	6.27	8.08	231.31
ACTH	5.47	1.12	6.59	5.58	2.42	142.56
IGF-I 20 ng/ml	4.53	0.94	5.47	4.44	3.37	61.18
IGF-I 20 ng/ml	3.87	0.75	4.62	4.95	22.67	58.64
IGF-I 20 ng/ml	4.08	0.79	4.87	7.19	1.46	100.93
IGF-I 20 ng/ml	2.99	0.41	3.40	4.45	9.73	-
IGF-I 20 ng/ml	5.04	1.49	6.53	4.39	0.22	71.42
IGF-I 20 ng/ml	6.98	1.66	8.63	4.91	~	175.82
IGF-1 400 ng/ml	3.34	0.75	4.09	8.07	~	74.32
IGF-1 400 ng/ml	9.91	1.15	11.06	2.47	~	72.22
IGF-1 400 ng/ml	6.20	1.15	7.35	9.34	7.41	110.76
IGF-1 400 ng/ml	7.35	1.33	8.68	5.04	1.88	114.16
IGF-1 400 ng/ml	3.81	0.87	4.68	4.31	1.08	95.34
IGF-1 400 ng/ml	4.31	0.72	5.03	4.75	~	102.62
IGF-II 20 ng/ml	4.34	0.88	5.22	3.30	~	85.70
IGF-II 20 ng/ml	4.28	0.75	5.03	7.57	3.56	73.39
IGF-II 20 ng/ml	6.18	1.20	7.38	7.35	1.24	134.74
IGF-II 20 ng/ml	3.07	0.39	3.46	8.49	9.60	147.39
IGF-II 20 ng/ml	7.46	1.39	8.85	4.92	5.18	159.54
IGF-II 20 ng/ml	5.88	1.18	7.06	4.95	~	93.46
IGF-II 400 ng/ml	6.69	1.03	7.72	3.36	~	86.78
IGF-II 400 ng/ml	8.08	1.22	9.30	5.71	~	120.20
IGF-II 400 ng/ml	5.55	0.91	6.46	7.36	~	82.20
IGF-II 400 ng/ml	3.84	1.10	4.94	5.26	12.05	143.26
IGF-II 400 ng/ml	4.72	0.54	5.26	4.72	4.32	103.48
IGF-II 400 ng/ml	3.49	0.85	4.34	4.58	~	90.38
No precursor	2.43	0.50	2.92	3.36	~	61.56
No precursor	3.65	0.74	4.39	9.49	~	116.52
No precursor	3.45	0.79	4.24	6.53	~	74.63
No precursor	3.19	0.75	3.94	6.30	~	91.43
No precursor	3.03	0.62	3.64	6.14	1.91	85.29
No precursor	2.26	1.13	3.38	4.84	4.74	67.26

Appendix table 6.11.2. The values for E1, E2, TE, E3 and P levels (pmol/mg protein) produced by tissue explants obtained from placenta B3. All explants were incubated with appropriate precursors except when stated in the table.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P 24 hrs
Control	2.03	5.93	7.96	0.78	311.3
Control	2.04	5.74	7.78	0.86	313.2
Control	1.86	6.16	8.01	1.16	290.3
Control	2.32	5.46	7.78	0.92	311.3
Control	2.06	5.46	7.52	0.63	340.7
Control	2.17	5.61	7.77	1.04	301.3
CRF	2.29	5.58	7.87	1.03	168.1
CRF	2.32	6.34	8.66	1.07	181.5
CRF	2.15	6.07	8.22	1.04	122.8
CRF	1.87	4.50	6.37	1.02	95.9
CRF	1.84	4.89	6.73	1.04	193.6
CRF	2.36	5.27	7.63	1.05	200.6
ACTH	1.53	5.82	7.35	1.05	183.9
ACTH	1.53	5.73	7.26	0.96	208.8
ACTH	1.47	5.89	7.36	0.90	235.3
ACTH	1.23	4.62	5.85	0.98	234.3
ACTH	0.95	5.37	6.32	1.10	207.1
ACTH	1.79	5.16	6.95	0.84	171.9
IGF-I 20 ng/ml	1.93	5.68	7.61	0.93	182.1
IGF-I 20 ng/ml	1.59	5.59	7.18	1.04	236.2
IGF-I 20 ng/ml	2.16	5.76	7.92	0.97	258.7
IGF-I 20 ng/ml	1.80	5.13	6.93	1.07	171.6
IGF-I 20 ng/ml	0.97	5.86	6.83	1.00	228.3
IGF-I 20 ng/ml	1.99	4.97	6.96	1.12	228.1
IGF-1 400 ng/ml	1.71	5.09	6.80	0.79	210.5
IGF-1 400 ng/ml	2.44	6.32	8.76	0.79	327.4
IGF-1 400 ng/ml	2.43	5.90	8.33	0.96	255.7
IGF-1 400 ng/ml	1.47	4.70	6.17	0.92	204.7
IGF-1 400 ng/ml	1.79	5.00	6.79	0.89	186.6
IGF-1 400 ng/ml	1.65	4.23	5.88	1.07	199.6
IGF-II 20 ng/ml	2.23	5.75	7.98	0.90	240.0
IGF-II 20 ng/ml	1.44	5.68	7.12	0.97	117.5
IGF-II 20 ng/ml	2.13	5.29	7.42	0.88	141.0
IGF-II 20 ng/ml	2.65	6.21	8.86	0.92	208.6
IGF-II 20 ng/ml	1.99	6.14	8.13	1.05	232.6
IGF-II 20 ng/ml	2.46	6.54	8.99	0.96	194.4
IGF-II 400 ng/ml	1.88	5.49	7.37	0.93	258.5
IGF-II 400 ng/ml	2.47	5.74	8.21	0.88	289.6
IGF-II 400 ng/ml	2.66	5.88	8.54	1.08	257.9
IGF-II 400 ng/ml	1.91	6.13	8.04	0.96	225.7
IGF-II 400 ng/ml	1.89	5.43	7.32	0.76	263.1
IGF-II 400 ng/ml	1.82	5.96	7.78	0.78	291.9
Cholesterol 20 μmol					18.0
Cholesterol 20 μmol					13.2
Cholesterol 20 μmol					17.0
Cholesterol 20 μmol					47.5
Cholesterol 20 μmol					35.5
Cholesterol 20 μmol					17.2

Appendix table 6.12. The values for E1, E2, TE, E3 and P levels (pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placenta B4.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P 24 hrs
Control	3.01	5.54	8.55	0.73	149.9
Control	2.92	5.32	8.24	0.71	97.0
Control	2.94	5.64	8.58	0.73	123.7
Control	1.97	5.35	7.32	0.70	204.1
Control	2.71	5.20	7.91	0.64	226.8
Control	2.26	4.47	6.73	0.67	162.4
CRF	2.26	3.50	5.76	0.86	131.5
CRF	3.50	5.33	8.83	1.01	141.6
CRF	2.74	4.74	7.48	0.69	123.0
CRF	2.52	4.68	7.20	0.63	94.2
CRF	2.80	5.27	8.07	0.71	104.2
CRF	3.25	5.16	8.41	0.88	143.4
ACTH	2.74	5.24	7.98	0.69	125.1
ACTH	1.81	4.60	6.41	0.73	114.0
ACTH	2.74	4.67	7.41	0.79	126.6
ACTH	3.58	5.90	9.48	0.77	149.9
ACTH	2.78	5.95	8.73	0.77	150.5
ACTH	2.71	4.86	7.57	0.70	139.5
IGF-I 20 ng/ml	2.10	4.53	6.63	0.88	120.8
IGF-I 20 ng/ml	2.10	5.00	7.10	0.83	115.8
IGF-I 20 ng/ml	1.75	4.80	6.55	0.93	101.5
IGF-I 20 ng/ml	2.56	7.08	9.64	0.81	103.8
IGF-I 20 ng/ml	2.86	5.64	8.50	0.74	110.3
IGF-I 20 ng/ml	2.64	4.71	7.35	0.70	119.3
IGF-1 400 ng/ml	2.89	5.17	8.06	0.85	148.2
IGF-1 400 ng/ml	2.67	4.75	7.42	0.83	136.4
IGF-1 400 ng/ml	3.44	5.74	9.18	0.76	131.9
IGF-1 400 ng/ml	2.99	4.70	7.69	0.81	81.8
IGF-1 400 ng/ml	2.74	4.47	7.21	0.76	131.7
IGF-1 400 ng/ml	2.92	4.32	7.24	0.75	123.9
IGF-II 20 ng/ml	2.91	5.38	8.29	0.69	46.3
IGF-II 20 ng/ml	2.13	3.07	5.20	0.58	123.1
IGF-II 20 ng/ml	2.19	4.98	7.17	0.68	159.6
IGF-II 20 ng/ml	2.55	5.41	7.96	0.77	157.2
IGF-II 20 ng/ml	2.47	5.36	7.83	0.73	155.8
IGF-II 20 ng/ml	2.41	4.64	7.05	0.76	116.0
IGF-II 400 ng/ml	2.75	5.35	8.10	0.75	185.3
IGF-II 400 ng/ml	1.41	5.41	6.82	0.91	135.8
IGF-II 400 ng/ml	2.38	6.00	8.38	0.77	87.7
IGF-II 400 ng/ml	2.35	4.89	7.24	0.67	130.4
IGF-II 400 ng/ml	1.89	6.12	8.01	0.58	171.4
IGF-II 400 ng/ml	3.07	5.35	8.42	0.86	146.5
Cholesterol 20 μmol					24.1
Cholesterol 20 μmol					10.1
Cholesterol 20 μmol					5.0
Cholesterol 20 μmol					8.8
Cholesterol 20 μmol					6.4
Cholesterol 20 μmol					1.3

Appendix table 6.13. The values for E1, E2, TE, E3 and P levels (pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placenta B5.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P 24 hrs
Control	3.02	10.26	13.28	2.65	286.0
Control	2.94	9.29	12.23	3.16	283.8
Control	3.86	12.54	16.41	3.17	225.6
Control	4.02	9.77	13.79	2.84	378.1
Control	3.65	4.68	8.33	3.17	213.1
Control	3.97	8.83	12.80	3.42	248.8
CRF	2.59	13.15	15.74	3.06	229.6
CRF	3.25	16.23	19.48	3.16	236.6
CRF	3.19	18.11	21.30	2.96	212.3
CRF	3.43	9.86	13.29	2.55	177.9
CRF	4.07	11.45	15.52	3.47	316.8
CRF	3.98	11.59	15.57	3.52	266.6
ACTH	3.19	15.28	18.47	2.83	259.8
ACTH	2.26	8.91	11.17	3.11	282.3
ACTH	3.29	12.14	15.43	3.11	202.9
ACTH	3.16	10.49	13.65	3.70	203.2
ACTH	3.84	10.44	14.28	4.46	327.5
ACTH	2.91	10.46	13.37	3.24	208.1
IGF-I 20 ng/ml	2.74	10.47	13.21	2.87	241.4
IGF-I 20 ng/ml	2.79	11.95	14.74	2.80	261.5
IGF-I 20 ng/ml	2.74	10.12	12.86	2.73	201.6
IGF-I 20 ng/ml	3.49	8.57	12.06	3.19	268.6
IGF-I 20 ng/ml	3.25	10.55	13.80	3.75	272.0
IGF-I 20 ng/ml	3.53	10.80	14.33	3.94	257.5
IGF-1 400 ng/ml	2.75	11.55	14.30	2.73	234.5
IGF-1 400 ng/ml	2.64	13.41	16.05	2.65	170.7
IGF-1 400 ng/ml	2.63	12.46	15.09	2.30	162.5
IGF-1 400 ng/ml	3.43	18.63	22.06	3.16	249.2
IGF-1 400 ng/ml	2.88	10.44	13.32	3.26	279.5
IGF-1 400 ng/ml	2.77	9.39	12.16	3.25	223.6
IGF-II 20 ng/ml	2.99	12.76	15.75	2.61	222.3
IGF-II 20 ng/ml	2.99	10.93	13.92	2.58	283.7
IGF-II 20 ng/ml	3.24	11.19	14.43	2.50	342.7
IGF-II 20 ng/ml	3.87	9.77	13.64	2.96	362.7
IGF-II 20 ng/ml	2.72	9.59	12.31	3.33	331.1
IGF-II 20 ng/ml	3.87	10.36	14.23	3.50	285.3
IGF-II 400 ng/ml	3.21	12.66	15.87	2.55	412.9
IGF-II 400 ng/ml	4.05	14.66	18.71	2.02	452.4
IGF-II 400 ng/ml	4.16	12.78	16.93	2.72	407.7
IGF-II 400 ng/ml	2.40	9.79	12.19	2.86	321.2
IGF-II 400 ng/ml	3.24	9.66	12.90	2.66	393.7
IGF-II 400 ng/ml	3.73	8.83	12.56	2.92	344.6
Cholesterol 20 μmol					2.8
Cholesterol 20 μmol					18.3
Cholesterol 20 μmol					13.9
Cholesterol 20 μmol					6.2
Cholesterol 20 μmol					9.9
Cholesterol 20 μmol					12.9

Appendix table 6.14. The values for E1, E2, TE, E3 and P levels (pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placenta B6.

	E1 24 hrs	E2 24 hrs	TE 24 hrs	E3 24 hrs	P 24 hrs
Control	2.92	7.65	10.57	0.78	1174.0
Control	3.05	7.73	10.78	1.07	1033.1
Control	3.07	0.81	3.88	1.10	907.2
Control	2.33	7.83	10.16	1.06	740.0
Control	2.71	8.18	10.89	1.19	836.7
Control	2.42	7.85	10.27	0.72	904.4
CRF	2.39	6.71	9.10	0.83	884.3
CRF	2.90	8.74	11.64	0.90	1007.2
CRF	2.82	6.79	9.61	1.19	877.3
CRF	2.33	6.66	8.99	0.86	874.6
CRF	2.72	7.07	9.79	1.00	676.6
CRF	2.47	8.06	10.53	0.99	789.5
ACTH	2.44	8.62	11.06	1.12	1034.0
ACTH	2.34	8.13	10.47	0.94	1076.2
ACTH	2.88	7.45	10.33	0.69	1098.2
ACTH	2.85	7.51	10.36	0.86	732.3
ACTH	2.46	8.31	10.77	1.14	638.2
ACTH	2.32	8.14	10.46	0.84	730.5
IGF-I 20 ng/ml	2.38	8.39	10.77	1.05	1047.6
IGF-I 20 ng/ml	2.53	7.77	10.31	1.11	913.7
IGF-I 20 ng/ml	2.74	8.17	10.91	0.91	1015.6
IGF-I 20 ng/ml	2.29	8.66	10.95	1.04	486.5
IGF-I 20 ng/ml	3.05	8.18	11.23	0.88	530.2
IGF-I 20 ng/ml	2.77	8.52	11.29	0.97	392.7
IGF-1 400 ng/ml	3.69	7.45	11.14	0.49	943.0
IGF-1 400 ng/ml	3.41	8.28	11.69	1.07	950.6
IGF-1 400 ng/ml	3.34	8.16	11.51	0.98	1105.3
IGF-1 400 ng/ml	2.33	10.18	12.51	0.90	461.0
IGF-1 400 ng/ml	2.73	8.02	10.75	1.15	493.9
IGF-1 400 ng/ml	2.48	7.29	9.77	1.08	455.5
IGF-II 20 ng/ml	2.16	7.73	9.89	0.94	1085.8
IGF-II 20 ng/ml	2.51	7.38	9.89	1.19	837.0
IGF-II 20 ng/ml	2.54	6.67	9.21	0.98	970.6
IGF-II 20 ng/ml	2.63	7.93	10.56	1.00	426.8
IGF-II 20 ng/ml	2.48	6.54	9.02	0.87	376.9
IGF-II 20 ng/ml	2.99	7.75	10.74	0.81	576.6
IGF-II 400 ng/ml	2.98	8.31	11.29	0.71	547.6
IGF-II 400 ng/ml	2.47	7.03	9.50	1.12	758.2
IGF-II 400 ng/ml	2.44	7.17	9.61	1.09	793.9
IGF-II 400 ng/ml	2.76	7.58	10.34	1.03	326.7
IGF-II 400 ng/ml	2.41	8.39	10.80	1.07	601.6
IGF-II 400 ng/ml	2.59	6.86	9.45	1.09	562.4

Appendix table 6.15. The values for E1, E2, TE, E3 and P levels (pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placenta B7.

	Pregnenolone 10 μmol	Pregnenolone 20 μmol
Control	241.9	175.2
Control	379.5	139.2
Control	311.1	153.3
Control	348.1	290.8
Control	318.7	273.7
Control	237.5	237.3
CRF	297.3	193.6
CRF	362.5	190.2
CRF	282.8	266.3
CRF	282.3	224.5
CRF	200.0	263.4
CRF	287.8	202.2
ACTH	238.8	311.8
ACTH	237.5	203.8
ACTH	294.7	265.8
ACTH	220.5	279.9
ACTH	241.6	228.6
ACTH	288.2	281.5
IGF-I 20 ng/ml	320.3	187.9
IGF-I 20 ng/ml	293.2	248.3
IGF-I 20 ng/ml	286.7	206.6
IGF-I 20 ng/ml	235.1	166.9
IGF-I 20 ng/ml	262.4	146.2
IGF-I 20 ng/ml	192.2	184.6
IGF-1 400 ng/ml	387.5	193.2
IGF-1 400 ng/ml	226.1	271.1
IGF-1 400 ng/ml	183.9	268.0
IGF-1 400 ng/ml	326.2	128.1
IGF-1 400 ng/ml	173.6	187.1
IGF-1 400 ng/ml	340.9	178.7
IGF-II 20 ng/ml	223.9	176.8
IGF-II 20 ng/ml	182.2	253.0
IGF-II 20 ng/ml	270.4	210.5
IGF-II 20 ng/ml	367.7	164.7
IGF-II 20 ng/ml	201.2	204.7
IGF-II 20 ng/ml	240.1	126.4
IGF-II 400 ng/ml	174.3	164.5
IGF-II 400 ng/ml	168.7	248.6
IGF-II 400 ng/ml	213.5	209.1
IGF-II 400 ng/ml	243.6	180.9
IGF-II 400 ng/ml	265.4	222.5
IGF-II 400 ng/ml	239.3	156.6

Appendix table 6.16. The values for P levels (pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placenta B8 and incubated for 24 hours with pregnenolone as the chosen precursor.

	Placenta B7	Placenta B8
Control- Cholesterol alone	35.04	78.48
Control- Cholesterol alone	38.64	65.83
Control- Cholesterol alone	31.48	94.89
Control- Cholesterol alone	27.06	97.94
Control- Cholesterol alone	53.55	95.18
Control- Cholesterol alone	39.43	91.93
CRF	20.99	116.26
CRF	29.10	78.48
CRF	18.67	92.95
CRF	23.18	86.18
CRF	23.88	97.50
CRF	21.88	85.54
ACTH	37.49	73.11
ACTH	25.98	72.73
ACTH	19.94	73.81
ACTH	20.64	68.27
ACTH	20.57	65.00
ACTH	13.36	72.06
IGF-I 20 ng/ml	19.08	72.06
IGF-I 20 ng/ml	16.00	57.18
IGF-I 20 ng/ml	3.18	59.88
IGF-I 20 ng/ml	14.09	68.43
IGF-I 20 ng/ml	15.84	62.26
IGF-I 20 ng/ml	11.10	64.55
IGF-II 20 ng/ml	5.66	77.27
IGF-II 20 ng/ml	16.95	66.14
IGF-II 20 ng/ml	16.63	55.01
IGF-II 20 ng/ml	13.52	49.19
IGF-II 20 ng/ml	13.39	60.48
IGF-II 20 ng/ml	19.78	61.85
No cholesterol precursor	42.48	101.28
No cholesterol precursor	32.02	50.31

Appendix table 6.17. The values for P levels (pmol/100,000 viable cells) produced by isolated cytotrophoblast cells obtained from placentae B7 and B8 and incubated for 24 hours with cholesterol (20 μmol/l) as the chosen precursor.