TITLE PAGE

Factors influencing the outcome of intrauterine insemination (IUI): age, clinical variables and significant thresholds.

Running title: Outcome of intrauterine insemination

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SUMMARY

The aim was to investigate the influence of various biological factors upon the outcome of intrauterine insemination (IUI). The total IUI history (856 cycles) of 352 couples was studied. Livebirth showed a strong negative correlation with female age but no correlation with male age. Anti-Mullerian hormone (AMH) and antral follicle count (AFC) correlated negatively with female age, and follicle stimulating hormone (FSH) correlated positively. Significant thresholds were found for all three variables, and also for total motile count (TMC) in the prepared sperm. Calculating pregnancy losses per positive pregnancy showed a strong correlation with increasing female age. This was highly significant for biochemical losses but not for foetal heart miscarriages. Male age had no effect on rate of pregnancy loss. In conclusion female age, FSH, AMH and TMC are good predictive factors for livebirth and therefore relate to essential in vivo steps in the reproductive process.

INTRODUCTION

Intrauterine insemination is widely acknowledged to be a useful treatment for infertile couples, especially in the initial stages of treatment (Merviel *et al*. 2010). It can appeal to patients by being less expensive and invasive than in vitro insemination (IVF), as well as being repeatable at shorter time intervals. However a major drawback is that success rates per cycle have been considerably lower than rates cited for IVF (Steures *et al*. 2004, Custers *et al*. 2007, Malizia *et al*. 2009). The present article seeks to investigate the effect of a range of female and male clinical variables upon the outcome of IUI with the additional aim of confirming which factors are the most significant in influencing the success rate.

MATERIALS AND METHODS

Patients

As this was a no-intervention retrospective study no ethical permission was required. Information was gathered on all IUI cycles carried out at the Centre for Reproductive and Genetic Health, London, UK (CRGH) over the calendar year 2009. In order to obtain a complete picture of each patient's total experience of IUI this data was combined with further data on all the IUI cycles that the same patients had experienced over the years 2003 to 2011. Cycles using frozen or donor sperm were excluded, so that 352 couples having 856 IUI cycles remained in the study. It was found that couples who had consecutive failed IUI cycles often chose to have many repeated cycles, and if results were expressed per cycle this would give undue prominence to these relatively unsuccessful patients. To avoid this, and to ensure that equal weight was given to each couple in the study, the results (apart from those shown in Tables 3 and 4) are expressed per treated couple. Livebirth and miscarriage rates are calculated as the average for each couple. In this paper a livebirth means either a single or a multiple birth.

Preparation of semen samples for IUI. The sample was subjected to centrifugation at 300 x g for 20 min in a 45% and 90% PureSperm-100 density gradient (Nidacon International, Gothenburg, Sweden). The pellet was washed twice in Quinn's Sperm Washing Medium (Cat. ART-1005, Cooper Surgical, Trumbull, CT, USA), suspended in 0.5 ml Sperm Washing Medium and kept at room temperature before being used for insemination.

IUI procedure

The study used ovarian hyperstimulation with clomiphene citrate or gonadotrophin since there is strong evidence that this increases the livebirth rate of IUI cycles (Veltman-Verhulst *et al* 2011, Arici *et al* 1994). Stimulation with hMG is a more aggressive and expensive treatment than stimulation with clomiphene citrate (Dickey *et al* 2002), and in this study the

choice of stimulant was geared to each couple individually in an effort to achieve an optimum result. In addition, patients with severe male factor infertility were advised to proceed to IVF/ICSI rather than to IUI and did not enter the study. Cohlen *et al* (1998) reported that a significant relationship between use of ovarian stimulation and pregnancy rate did not hold at low sperm concentrations. The dose of ovarian stimulant used was decided by considering the patient's age, previous response, hormonal profile and presence or absence of polycystic ovaries. The insemination process was the same for both types of stimulation. With the patient in the lithotomy position a speculum was introduced into the vagina. Using an IUI catheter the prepared sperm sample was injected into the uterus. Patients were asked to do a urine pregnancy test 16 days after the procedure. In the case of a positive result a blood level of β–hCG was determined. This was followed by pregnancy scans at around 7 and 10 weeks of gestation. None of these patients was lost to follow-up.

Measurement of FSH and AMH. Day 3 blood was sent to The Doctors Laboratory (TDL), London, UK for immunoassay. AMH is expressed in pmol/l. To convert these values to ng/ml one needs to divide by 7.2.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows was used. The statistical methods were non-parametric for the initial part of the paper and parametric for Tables 3 and 4.

RESULTS

In an initial study of the results it was found that the livebirth and miscarriage rates showed no significant difference between the patient groups having the two different drug regimes for ovarian stimulation (results not shown). Therefore the patients were considered as one cohort regardless of the drug used for ovarian stimulation. Table 1 gives details on the 352 couples in the study. Their main reasons for infertility were diagnosed as male factor (24%), uterine problems and endometriosis (8%), ovarian cysts (7%) and unexplained (59%). The data in Table 1 show no significant differences between the absence and presence of male factor.

Relationships between partners' ages, livebirth and pregnancy loss.

Table 2 shows relationships between variables by Spearman correlation. In Table 2(a) a highly significant negative relationship between female age and livebirth is evident in couples as a whole and in couples without male factor. It is also clear that the ages of the male and female partners of a couple are closely related. Although couples in column B show a weak negative correlation between male age and livebirth, this correlation was abolished when the confounding factor of female age was eliminated. This is dealt with later in the paper.

Table 2(b) deals with the effect of age on pregnancy loss. Since IUI has a relatively high occurrence of negative cycles, which by definition exclude pregnancy loss, the rate of pregnancy loss for each couple was expressed as the percentage of pregnancy losses per positive pregnancy, i.e. the number of losses per sum of livebirths plus losses. The table gives strong evidence for an increase in pregnancy losses as female age increases. The results also indicate that increasing female age increases the rate of biochemical losses but has little or no effect upon miscarriages that show a foetal heart. No significant effect of male age upon pregnancy losses was seen.

Relationships involving other variables. The TMC of the prepared sperm sample used in the insemination was calculated as volume multiplied by concentration of motile spermatozoa. It did not correlate over its whole range with male age or with livebirth rate (n=348). FSH correlated positively with female age (n = 310, $P < 0.001$). AMH (n = 284) and AFC (n = 233) each correlated negatively with female age ($P < 0.001$).

Table 3 shows a significant threshold for each variable. The chance of a livebirth was significantly different according to whether the value of the variable lay below or above the threshold.

In Table 4 the statistical method of partial correlation was used to investigate the effect of male age upon livebirth (compare Table 2a). When the confounding factor of female age was eliminated male age had no significant effect on livebirth.

DISCUSSION

Spearman correlation over the entire female age range showed a highly significant negative correlation between female age and livebirth. This is supported by previous studies which have shown that the pregnancy rate in IUI is lower in the higher female age ranges (Dovey *et al*. 2008, Merviel *et al.* 2010).

FSH, AMH and TMC all showed highly significant thresholds (Table 3). FSH gave no sign of being inferior to AMH in detecting the adverse effect of diminished ovarian reserve upon livebirth. The results indicate that the most useful predictors of livebirth in the female are age, FSH and AMH. The results for TMC suggest a poor chance of pregnancy for those IUI cycles where the TMC is below 1.5×10^6.

With regard to threshold values found by previous workers, for FSH in IVF cycles the thresholds stated vary between 11.4 and 25.0 IU/l (Jain *et al*. 2004). Scott *et al*. (2008) have proposed that the use of statistical methods other than those based on clinical outcome, such as those using receiver operating characteristic (ROC) curves, leads to erroneous threshold values They obtained threshold values between 18 and 15 IU/l for five patient groups of increasing female age. These values are slightly above our threshold value for FSH which was also found by clinical outcome. Considering only IUI cycles for FSH, Magarelli *et al.* (1996) state a threshold of $\leq$ 23 IU/l for positive pregnancy. The difference between this value and our value of < 14.6 IU/l could be due to the fact that our threshold measured livebirth events rather than pregnancy events.

In the case of AMH, studies on IVF cycles which state single cut-off values give values between 0.35 ng/ml and 14 pmol/l (Nakuda *et al.* 2007, Hazout *et al.* 2004, Gleicher *et al.* 2010, Gnoth *et al.* 2008, Lekamge *et al.* 2007). Our finding of $\geq$ 2.9 pmol/l, which equals

≥ 0.40 ng/ml, is in the lower region of these values. Li *et al* (2010) in their study of IUI cycles state two cut-off values which were found by using ROC curves.

Because of the relatively high rate of negative cycles seen in IUI it was found necessary to calculate the pregnancy losses per positive pregnancy (i.e. per sum of livebirths and pregnancy losses) to clarify whether female age has an effect on pregnancy loss. When this was done female age showed a highly significant correlation with total pregnancy losses and also with biochemical losses but not with those miscarriages showing a foetal heart. Our observation that male age over the range studied had no significant effect on livebirth rate or rate of pregnancy losses is supported by Bellver *et al.* (2008).

The process leading to a biochemical pregnancy loss is known to start with implantation, since β–hCG is produced, but the pregnancy terminates before formation of the foetal heart. Although foetal heart miscarriages were equal in number to biochemical losses no significant correlation was seen between foetal heart miscarriages and female age. This implies that some foetal heart miscarriages have causes that are less affected by age than those causing biochemical losses. Further investigation of these aspects could help in understanding the mechanisms of foetal loss in assisted conception cycles.

To conclude, the most significant factors for predicting livebirth outcome in this group of patients were female age, FSH and AMH. On the male side the importance of sperm concentration and motility was shown by the highly significant relationship between TMC and livebirth.

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