human reproduction open

ORIGINAL ARTICLE

The impact of selected embryo culture conditions on ART treatment cycle outcomes: a UK national study

Catherine M. Castillo [1], **Joyce Harper** [2], **Stephen A. Roberts**[3,5], **Helen C. O'Neill**[2], **Edward D. Johnstone**[1,3], and **Daniel R. Brison** [1,3,4,*]

[1]Maternal & Fetal Health Research Centre, Division of Developmental Biology and Medicine, School of Medical Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester Academic Health Sciences Centre, Oxford Rd., Manchester M13 9WL, UK [2]Institute for Women's Health, University College London, London WC1E 6HX, UK [3]Maternal & Fetal Health Research Centre, St. Mary's Hospital, Manchester University National Health Service (NHS) Foundation Trust, Manchester Academic Health Sciences Centre, Oxford Rd., Manchester M13 9WL, UK [4]Department of Reproductive Medicine, Old St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester Academic Health Sciences Centre, Oxford Rd., Manchester M13 9WL, UK [5]Centre for Biostatistics, Division of Population Health, Health Services Research & Primary Care, School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Oxford Rd., Manchester M13 9PL, UK

*Correspondence address. Department of Reproductive Medicine, Old St. Mary's Hospital, Manchester University NHS Foundation Trust, Manchester Academic Health Sciences Centre, Oxford Rd., Manchester M13 9WL, UK. E-mail: Daniel.Brison@manchester.ac.uk
https://orcid.org/0000-0002-4307-1293

Submitted on March 30, 2019; resubmitted on September 13, 2019; editorial decision on September 27, 2019

STUDY QUESTION: Are selected embryo culture conditions namely media, oxygen level, and incubator type, associated with IVF live birth rate (LBR) and the health of singleton offspring at birth?

SUMMARY ANSWER: There were statistically significant differences in LBR between the eight culture media systems analysed; however, none of the embryo culture factors showed statistically significant associations with birth weight (BW) in multivariable regression analyses.

WHAT IS KNOWN ALREADY: In clinical ART culture media is the initial environment provided for the growth of human embryos. Pre-implantation development is a critical period of developmental plasticity, which could have long-lasting effects on offspring growth and health. Although some studies have shown an impact of culture medium type on BW, the interaction between culture medium type and associated culture conditions on both treatment success rates (LBR) and offspring BW is largely unexplored. This study aimed to examine these factors in a large multicentre national survey capturing the range of clinical practice.

STUDY DESIGN, SIZE, DURATION: In this cross-sectional study, data from a survey circulated to all UK IVF clinics requesting information regarding culture medium type, incubator type, and oxygen level used in ART between January 2011 and December 2013 were merged with routinely recorded treatment and outcome data held in the Human Fertilisation and Embryology Authority Register up to the end of 2014.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Forty-six (62%) UK clinics responded to the survey. A total of 75 287 fresh IVF/ICSI cycles were captured, including 18 693 singleton live births. IVF success (live birth, singleton or multiple; LB), singleton gestation and singleton gestation-adjusted BW were analysed using logistic and linear regression models adjusting for patient/treatment characteristics and clinic-specific effects.

MAIN RESULTS AND THE ROLE OF CHANCE: Culture medium type was shown to have some impact on LBR (multivariable logistic regression, (MRL); post-regression Wald test, $P < 0.001$), but not on BW (MLR; post-regression Wald test, $P = 0.215$). However, blastocyst culture had the largest observed effect on odds of LBR (odds ratio (OR) = 1.35, CI: 1.29–1.42), increased the risk of pre-term birth even when controlling for oxygen tension (MLR; OR = 1.42, CI: 1.23–1.63), and gestation-adjusted BW (MLR, $\beta = 38.97$ g, CI: 19.42–58.53 g) when compared to cleavage-stage embryo culture. We noted a very strong effect of clinic site on both LBR and BW, thus confounding between treatment practices and clinic site may have masked the effect of culture conditions.

LIMITATIONS, REASONS FOR CAUTION: Larger datasets with more inter-centre variation are also needed, with key embryo culture variables comprehensively recorded in national treatment registries.

WIDER IMPLICATIONS OF THE FINDINGS: This study is the largest investigation of laboratory environmental effects in IVF on both LBR and singleton BW. Our findings largely agree with the literature, which has failed to show a consistent advantage of one culture media type over another. However, we noted some association of LBR with medium type, and the duration of embryo exposure to laboratory conditions (blastocyst culture) was associated with both LBR and singleton health at birth. Because of the strong effect of clinic site noted,

further randomized controlled trials are needed in order to reliably determine the effect of embryo culture on IVF success rates and the growth and health of subsequent offspring.

WHAT DOES THIS MEAN FOR PATIENTS?

The first few days of conception (before embryos attach to their mother's womb) are a critical period where the embryo adapts to its surroundings, which could have a long-lasting impact on the growth and health of babies, long after their birth. In the IVF laboratory, human embryos are grown in a growth-supporting solution (liquid) inside incubators containing specific concentrations of oxygen and other gases before being transferred to the mother. This study aimed to determine whether key aspects of the IVF laboratory environment affected the chances of having a healthy single baby (measured as LBR and BW) using a large national survey that collected information about the range of clinical and laboratory practices in IVF clinics across the UK.

Our results show that the type of solution that embryos are grown in has a small effect on whether a baby is born alive, but not on weight at birth. However, we also saw that growing embryos in the laboratory for a longer time (5–6 days, compared to the standard 2–3 days) increased LBR, but also increased the chance of babies being born prematurely. We also noted very strong differences between individual clinics in both LBR and the BW of babies born in that clinic, which could not be explained by our survey results. We therefore think that these differences could be related to other clinical or laboratory practices that we did not record. The results of this large UK study add to previous studies, which have generally not shown an advantage of one culture solution type over another.

Introduction

IVF-conceived children accounted for almost 3% of UK babies born in 2016 (Office for National Statistics, 2017; Human Fertilisation and Embryology Authority, 2018), and approximately 8 million have been born worldwide over the past four decades (Mandal, 2018). Although the great majority of IVF children are born healthy, it has been established that IVF singletons have an increased risk of poor neonatal outcomes, including low birth weight (LBW), preterm birth (PTB) and small for gestational age (GA), compared to naturally conceived singletons (Kamphuis *et al.*, 2014; Fleming *et al.*, 2018). Some of these adverse outcomes are associated with specific treatment procedures (e.g. duration of embryo culture (to blastocyst) (Ishihara *et al.*, 2014), embryo freezing (Maheshwari *et al.*, 2017), fertilization method (Marino *et al.*, 2014), etc.) and known patient characteristics such as maternal age (Carolan, 2013), maternal parity (Shah, 2010), ovarian response (Sunkara *et al.*, 2015) and subfertility diagnosis (Pérez-López *et al.*, 2018). According to the Developmental Origins of Health and Disease's (DOHaD's) hypothesis, IVF occurs during a critical period of developmental plasticity, which could have long-lasting effects on offspring health (Fleming *et al.*, 2018; Roseboom, 2018). Environmental influences around the time of conception have the potential to translate into longer-term differences in health outcomes throughout human development. For example, LBW is associated with increased risk of adult disease such as insulin resistance (Norris *et al.*, 2012), type II diabetes (Harder *et al.*, 2007) and cardiovascular diseases (Mu *et al.*, 2012).

The type of culture medium embryos are grown in has been associated with birth weight (BW) in prospective cohort studies (Zandstra *et al.*, 2015) as well as one prospective randomized controlled trial (RCT) (Kleijkers *et al.*, 2016; Sunde *et al.*, 2016). In this first prospective RCT of two commercially available culture media, Kleijkers *et al.* (2016) reported a 158 g difference in BW favouring a medium that was also comparatively associated with lower clinical pregnancy and implantation rates—two measures of IVF treatment efficacy, which are heavily focussed on in public reporting by clinics and the Human Fertilisation and Embryology Authority (HFEA). However, the effects of culture medium and the interaction with closely associated factors in the embryo culture environment, such as oxygen level and incubator type, are largely unknown. Two main types of commercially available culture media are used in routine practice. Sequential (or two-step) media are separated into complementary products: one specifically formulated to support development up to the cleavage stage on Day 3 and the other to support development to blastocyst on Day 5/6. This type of product (first manufactured by Vitrolife) requires the physical movement of embryos from the cleavage step medium to the blastocyst step medium in the context of extended embryo culture. Single-step media (such as LifeGlobal) are formulated to contain all of the components an embryo would require for development from the two pronuclei to blastocyst stage. Embryo culture incubators fall into a number of categories and there is a current lack of well-controlled studies comparing the relative environmental stability of particular incubator types as well as related associations with embryo development, treatment success rates and perinatal outcomes. The so-called 'big box' tissue culture incubator is considered to be the standard or conventional type of incubator. The more modern top-opening multi-chamber bench-top flatbed incubator contains multiple separate culture dish chambers designed to maintain individual stability across all mini-culture environments, temperature, pH, gas and humidity following disturbance for embryo inspection (Swain, 2014). Finally, time-lapse (TL) monitoring technology facilitates extended undisturbed embryo culture, allowing for constant monitoring of developing embryos without having to remove them from the incubator's protected environment. Considering the mechanical differences between older versus modern incubator models, including repeated incubator door openings etc., differences in environmental

control and recovery are likely to be real; however, the impact of these differences on IVF outcomes is largely unknown (Swain, 2014) and the most recent Cochrane review comparing TL systems with standard embryo incubation produced inconclusive results (Armstrong *et al.*, 2019). Some incubator types are also more amenable to use with low (5–6%) O_2. Although a protective effect of low versus atmospheric oxygen concentration for embryo culture in terms of improved embryo development and IVF treatment outcomes has been suggested in the literature (Mantikou *et al.*, 2013a; Bolton *et al.*, 2014), there is currently a lack of strong evidence due to the presence of numerous confounding factors including incubator type (Gardner, 2016; Nastri *et al.*, 2016).

An understanding of such complex multifactorial associations requires large sample sizes and a diversity of treatments within and between centres, which are not available in most existing cohort studies. It has also been difficult to establish any association between culture media type and treatment success rate (Mantikou *et al.*, 2013b; Chronopoulou and Harper, 2015). The proliferation of commercially available media and lack of detailed information on composition make conventional RCTs difficult and there are few adequately powered randomized trials prior to the introduction of new or revised media compositions (Morbeck *et al.*, 2017).

In order to reliably investigate the potential effects of IVF laboratory and clinical treatment factors, large multi-centre studies are needed. Such an effort could aid in identifying IVF treatment-related factors that could be altered to both increase success rate and reduce risk and potentially lead to modifications of embryo culture systems in order to optimize and reduce risks arising from IVF treatment in the future.

This study explored both treatment success (occurrence of a live birth per treatment cycle; LBR) as well as the neonatal outcomes (gestation and BW) of IVF-conceived singletons, in association with selected embryo culture system factors (medium type, oxygen level, and incubator type), in a multi-centre UK population, while accounting for known prognostic patient, IVF treatment, and pregnancy-associated factors.

Materials and Methods

This study was sponsored by the University of Manchester (UoM) and approved by the South East Coast (Surrey) Research Ethics Committee on 7 October 2015 (REC reference number 15/LO/1213).

A National Culture Media Questionnaire (NatCMQ) was developed to capture information describing embryo culture systems within fertility clinics across the UK (Supplementary Data). The main variable of interest was culture medium, with information about incubator type and oxygen exposure level also gathered. These data were then merged with fully anonymized IVF treatment procedure, patient prognostic, and pregnancy outcome data from the HFEA Register to examine the following IVF treatment outcomes: live birth (regardless of multiplicity) (LB), unadjusted BW in singletons, GA and PTB in singletons, and gestation-adjusted BW in singletons.

National survey data

All 74 HFEA-licenced fertility clinics in the UK that provided IVF treatment as of 1 January 2011 were invited to participate in the study via the UK Association of Clinical Embryologists (ACE) membership network. The NatCMQ was distributed to all ACE members on behalf of both UoM and University College London (UCL). The responses were received, and the centres were anonymized by J.H. and H.O. at UCL.

A total of 47 clinics submitted a completed NatCMQ. Of these, one clinic was excluded because its responses did not correspond to the study time period, giving an overall response rate of 62%.
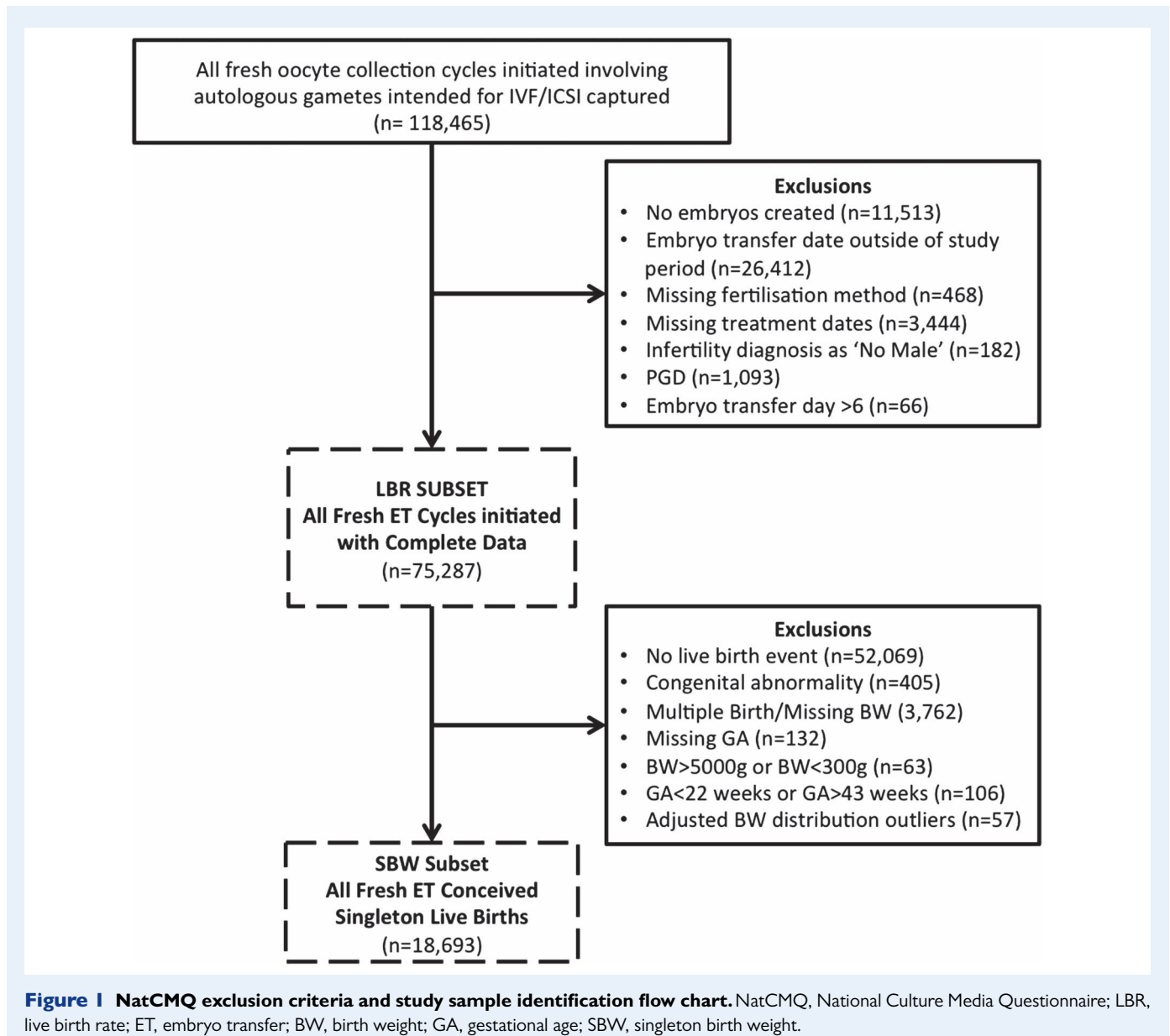
Embryo culture system information consisted of culture medium (eight categories: Cook Sequential, Irvine Single-Step, Life Global Single-Step, Medicult/Origio Sequential, Sage Sequential, Vitrolife Sequential, Vitrolife GTL, and more than one medium in parallel), incubator type category, and oxygen exposure level (where atmospheric oxygen was considered as 'high', 5–6% as 'low', and both used concurrently was categorized as 'both in parallel'). Incubators were categorized according to simultaneous use. These consisted of one category indicating the exclusive use of conventional front-opening standard 'box' incubators and eight other categories indicating the exclusive or simultaneous use of three other bench-top types of incubators. The incubator category coding system is described in Supplementary Table SI.

Clinical data

Data from all IVF/ICSI treatment cycles involving the collection of fresh autologous oocytes performed at a participating fertility clinic between 1 January 2011 and 31 December 2013 were extracted from the HFEA register. All centres held current HFEA clinical treatment licences, which require that rigorous quality management systems be in place in line with the EU Tissues and Cells Directive and set out in detail in the HFEA Code of Practice that clinics must abide by. Thus, all laboratories contributing to the study had systems in place to ensure regular service and audit of key equipment function including routine monitoring of temperature, gases, routine analysis of key performance indicators, etc. Cycles where no embryo was created, no embryo transfer (ET) occurred, or PGD was used were excluded. Treatment cycles with missing treatment dates were excluded as these could not be matched to embryo culture system information extracted from the NatCMQ; further exclusions were cycles with implausible treatment dates (e.g. duration of culture, <0 or >6 days), missing fertilization method, and where the patient's recorded reason for seeking treatment was 'no male' (i.e. where treatment was undertaken in otherwise fertile women who lacked a male partner). Singletons with BW less than 300 g and greater than 5000 g or GA shorter than 22 and longer than 43 weeks were also excluded as probable data entry errors; these amounted to less than 0.1% of births.

For the analyses of singleton birth outcomes, only those cycles resulting in an LB were considered and all multiple births and births with a reported congenital anomaly or with missing values for key variables (child gender, BW, and GA) were excluded.

Patient characteristics included were patient and partner age, duration of infertility, number of previous fresh cycle IVF attempts, maternal parity, treatment funding source, and infertility diagnosis. IVF treatment procedure factors included fertilization method (IVF or ICSI), number of embryos transferred, and duration of embryo culture (in days), total number of oocytes collected, and viable embryos created, use of ovarian stimulation and the number of gestational sacs detected within 2 months of ET. GA was calculated as the difference in weeks between the ET and delivery dates, plus the number of days in culture, and an additional 2 weeks (average length of time since last menstrual period) consistent with other studies (Spong, 2013; Dupree *et al.*, 2016).

Figure 1 NatCMQ exclusion criteria and study sample identification flow chart. NatCMQ, National Culture Media Questionnaire; LBR, live birth rate; ET, embryo transfer; BW, birth weight; GA, gestational age; SBW, singleton birth weight.

Study size

This was a national study including all UK clinics providing IVF treatments. The study sample size was determined by the number of clinics that returned a completed NatCMQ and the number of cycles performed at each participating clinic during the study period.

Primary study outcomes

An LB was defined as an ET cycle resulting in a single or multiple LB. Patient-reported BW (unadjusted BW) was recorded in grams (g). PTB was defined as <37 weeks gestation. GA-adjusted BW was derived from unadjusted BW, GA, child gender, and maternal parity using the Gestation-Related Optimal Weight (GROW) formula (http://www. gestation.net/) based on standardized foetal growth data, and was centred to a nominal 40-week GA, male gender, and primiparity. Maternal parity was not available for cycles that did not result in an LB.

Statistical analyses

All treatment cycles conducted during the study period were assigned a culture medium, incubator category, and oxygen exposure level based on the date of oocyte collection and merged by clinic. The result was a fully anonymized collated dataset containing clinic-level information regarding embryo culture systems used and individual-level IVF treatment procedures performed, patient characteristics, and treatment outcomes. Two analysis datasets were defined: the LBR dataset, in which all LBs are included (expressed per ET cycle), and the analysis of BW in singleton LBs only (the SBW dataset). The dataset selection procedure is illustrated in Fig. 1.

Two multivariable logistic regression models were used to estimate the odds of an LB and PTB as binary outcome variables using the LBR and SBW datasets, respectively. Similar multivariable linear regression models predicting unadjusted BW, GA, and GA-adjusted BW as continuous outcome variables were fitted to the SBW dataset.

Table I Patient and treatment characteristics in a study of embryo culture conditions and ART outcomes.

Parameter	LBR dataset			SBW dataset
	ET cycles[1] n = 75 287	Total LBs[1,2] n = 23 167	Multiple LBs[1,2] n = 3737	Singletons[1,2] n = 18 693
Patient Characteristics				
Funding Source (NHS Funded)	39 187 (52)	12 682 (55)	1700 (45)	10 613 (57)
Infertility Duration (years)	5 (2.7)	4 (2.6)	5 (2.7)	4 (2.5)
Partner Age (years)	37 (5.8)	37 (5.6)	37 (5.3)	36 (5.6)
Maternal Age (years)	35 (4.5)	34 (4.2)	34 (3.9)	34 (4.2)
Previous IVF Attempts (n)				
0	39 714 (53)	13 056 (56)	1669 (45)	10 984 (58)
1	16 456 (22)	4680 (20)	843 (23)	3698 (20)
2	9236 (12)	2694 (12)	561 (15)	2046 (11)
3+	9881 (13)	2737 (12)	664 (18)	1965 (11)
Infertility Diagnosis[3]				
Uterine Factor/Endometriosis	6486 (9)	1855 (8)	292 (8)	1505 (8)
Male Factor	30 621 (41)	9833 (42)	1574 (42)	8013 (43)
Ovarian/Ovulatory Disorder	11 759 (16)	3884 (17)	628 (17)	3149 (17)
Tubal Disease	11 538 (15)	3452 (15)	594 (16)	2752 (15)
Treatment Parameters				
Culture Medium				
Cook Sequential	11 277 (15)	3527 (15)	599 (16)	2845 (15)
Irvine Single Step	896 (1)	272 (1)	32 (1)	228 (1)
Life Global Single Step	4826 (6)	1622 (7)	267 (7)	1284 (7)
Medicult/Origio Sequential[4]	6701 (9)	1916 (8)	300 (8)	1523 (8)
Sage Sequential	32 694 (43)	10 171 (44)	1762 (47)	8040 (43)
Vitrolife Sequential	12 507 (17)	3444 (15)	473 (13)	2886 (15)
Vitrolife G-TL	433 (1)	133 (<1)	9 (<1)	124 (1)
Tow or more in Parallel	5575 (7)	1975 (9)	275 (7)	1677 (9)
Missing	368 (1)	107 (<1)	20 (<1)	86 (1)
Incubator				
Standard Box Only	24 656 (33)	7557 (33)	1284 (34)	6109 (33)
Standard box and BTFI	5982 (8)	1716 (7)	288 (8)	1388 (8)
Standard box and BTFI/MINC™	3934 (5)	1181 (5)	144 (4)	968 (5)
Standard box, BTFI, and ES	4493 (6)	1595 (7)	253 (7)	1308 (7)
Standard box and MINC™	9791 (13)	2769 (12)	416 (11)	2237 (12)
Standard box and ES	5960 (8)	1808 (8)	211 (6)	1546 (8)
BTFI Only	12 636 (17)	4170 (18)	820 (22)	3159 (17)
BTFI and MINC™	279 (<1)	93 (<1)	8 (<1)	81 (<1)
MINC™ Only	6377 (8)	1945 (9)	261 (7)	1626 (9)
Missing	1179 (2)	333 (1)	52 (<1)	271 (1)
Oxygen Exposure Level				
Atmospheric (high)	14 367 (19)	4408 (19)	735 (20)	3595 (19)
5–6% Oxygen (low)	46 886 (62)	14 859 (64)	2444 (65)	11 957 (64)
Both in parallel (concurrently)	13 706 (18)	3822 (17)	536 (14)	3087 (17)
Missing	328 (1)	78 (<1)	22 (1)	54 (<1)

(Continued)

Table I Continued

Parameter	LBR dataset			SBW dataset
	ET cycles[1] n = 75 287	Total LBs[1,2] n = 23 167	Multiple LBs [1,2] n = 3737	Singletons[1,2] n = 18 693
Total Oocytes Collected (n)	10.2 (5.7)	11.4 (5.7)	11.7 (5.6)	11 (5.6)
Embryos Created (n)	6 (3.9)	7 (4.0)	7 (4)	7 (3.9)
Total Embryos Transferred (n)				
1	31 135 (41)	9935 (43)	146 (4)	9466 (51)
2	41 254 (55)	12 730 (55)	3511 (94)	8834 (47)
3	2898 (4)	502 (2)	80 (2)	393 (2)
eSET (versus no eSET)	19 426 (26)	7367 (32)	108 (3)	7033 (38)
Fertilisation Method				
IVF	42 913 (57)	13 176 (57)	2212 (59)	8167 (44)
ICSI	32 374 (43)	9991 (43)	1525 (41)	10 526 (56)
Ovarian Stimulation (Yes)	74 266 (99)	23 043 (99)	3727 (99)	18 584 (99)
Duration of Culture (Days)				
Up to Cleavage Stage (0–3)	43 762 (58)	10 553 (46)	1895 (51)	8319 (45)
Up to Blastocyst Stage (4–6)	31 525 (42)	12 614 (54)	1842 (49)	10 374 (55)
Pregnancy Outcomes				
Previous Live Births (n)				
0				17 118 (92)
1				1529 (8)
2–3				46 (<1)
Unadjusted BW (g)				3282 (584)
Gestational Age (weeks)				39 (2)
Pre-term Birth (Yes)				1840 (10)
Adjusted BW (g)				3411 (486)
Gender				
Male				9419 (50)
Female				9274 (50)
Number of Sacs Detected				
1				17 906 (96)
2				722 (4)
3				(<1)

ET, embryo transfer; LB, live birth; NHS, National Health Service; BTF, bench-top flatbed; ES, EmbryoScope[TM]; eSET, elective single-embryo transfer.

[1]Values are number (column percentage) for categorical variables and mean (SD) for continuous variables unless otherwise stated.

[2]Total Multiple LBs and Singletons do not sum to total Successful LBs because of exclusions made in deriving the SBW subset.

[3]Infertility diagnosis categories are not mutually exclusive, therefore categories do not add up to 100%.

[4]For cleavage stage ET cycles involving embryos cultured in Medicult/Origio Sequential, a small number of embryos cultured using Embryo Assist medium were pooled with a majority that were cultured in ISM1.

'Univariable' models predicting each outcome included just the variable of interest (i.e. each available embryo culture, IVF treatment, and prognostic patient characteristic individually) as one covariate and a categorical treatment centre ID variable as a second covariate. The multivariable linear regression models predicting these outcomes included as covariates all embryo culture factors, IVF treatment factors (total oocytes collected, embryos created, days in culture, elective single embryo transfer (eSET), fertilization method, and ovarian stimulation (LBR only)), and patient prognostic characteristic variables (treatment funding source, previous years of infertility, maternal and paternal ages, previous IVF attempts, and infertility diagno-

sis) as well as clinic site as a categorical variable. Analogous logistic regression models were used to analyse LB and PTB; number of sacs detected was not available for unsuccessful cycles and therefore excluded from the LBR analysis. Ovarian stimulation was excluded from the GA, BW, and PTB analyses due to numbers that were too low to be included in multivariable regression analyses. Non-linear terms (quadratic and cubic) were considered for continuous predictors and included where these reached statistical significance. Global Wald tests for the effects of culture medium, oxygen exposure level, and incubator group were performed to provide an overall test of the effect of each culture variable. A value of $P < 0.05$ was

Table II **The total number of ETs performed and the number of clinic sites for all embryo culture system parameter conditions.**

	Cleavage ETs[1,2]	Blastocyst ETs[1,2]	Centres[3]
Culture Medium			
Cook Sequential	6172 (55)	5105 (45)	10
Irvine Single Step	287 (32)	609 (68)	2
Life Global Single Step	3083 (64)	1743 (36)	5
Medicult/Origio Sequential	4199 (63)	2502 (37)	4
Sage Sequential	19 045 (58)	13 649 (42)	26
Vitrolife Sequential	8190 (65)	4317 (35)	14
Vitrolife G-TL	105 (24)	338 (76)	1
Two or more in Parallel	2369 (42)	3206 (58)	10
Undetermined	312 (85)	56 (15)	1
Incubator Group			
Standard Box Only	16 179 (66)	8477 (34)	26
BTF1 Only	6322 (50)	6314 (50)	8
MINC Only	3166 (50)	3211 (50)	7
Standard box and BTF1	3995 (67)	1987 (33)	5
Standard box and MINC	6227 (64)	3564 (36)	8
Standard box and ES	2639 (44)	3321 (56)	3
BTF1 and MINC	209 (75)	70 (25)	1
Standard box, BTF1, and MINC	2608 (66)	1326 (34)	2
Standard box, BTF1, and ES	1524 (34)	2969 (66)	2
Two or more in parallel	893 (76)	286 (24)	2
Oxygen Concentration			
High	9665 (67)	4702 (33)	11
Low	24 851 (53)	22 035 (47)	31
Both in Parallel	9041 (66)	4665 (34)	10
Undetermined	205 (63)	123 (37)	1

[1] Values are count (row percentage) for all categories. Values are not mutually exclusive as some centres switched from one category to another within the study period. Therefore, centre totals do not add up to 46.

[2] Values represent all non-missing observations and therefore column totals do not add up to 75 287 (the total number of cycles captured in the LB dataset).

considered statistically significant. All statistical analyses were performed in Stata (version 13.1; Stata Corporation, College Station, TX, USA).

Results

Study population

Table I describes characteristics of the patients and treatment parameters for all ET cycles, for LBs (singleton and multiples), as captured in the LBR dataset, and singleton pregnancy outcomes captured in the SBW dataset. Table II shows the total number of centres that reported using each embryo culture system parameter, and the number of cleavage- and blastocyst-stage ETs performed in those centres.

The most widely used culture medium was Sage Sequential, used in more than half of clinics and comprising 443 of all included ET cycles, with Cook sequential (15%) and Vitrolife media (18%) also with substantial representation, such that these three media comprised 75% of the sample. The most commonly used incubator type was the standard box CO_2 incubator, used in 33% of all

ET cycles, with the majority of clinics using these alone or in combination with bench-top incubators. The oxygen exposure level is to a large degree linked to the type of incubator that is in use, as both bench-top flatbed and EmbryoScope[TM] incubators were designed to minimize environmental disturbances and facilitate a stable and controlled low oxygen concentration environment. A majority of ET cycles captured in this study (62%) involved embryos that were cultured in low oxygen, with 19% of cycles in high oxygen, and 11 centres with only high-oxygen incubators available.

Live birth rate

Fig. 2 shows the covariate-adjusted LBR (LBs per ET cycle) for the three embryo culture system parameters, with the full regression parameters shown in Supplementary Table SII. Univariable analyses suggested statistically significant differences in LBR with respect to all three embryo culture system parameters: incubator type, oxygen exposure level, and culture medium, although the LBRs varied only moderately for any parameter, ranging from 25 to
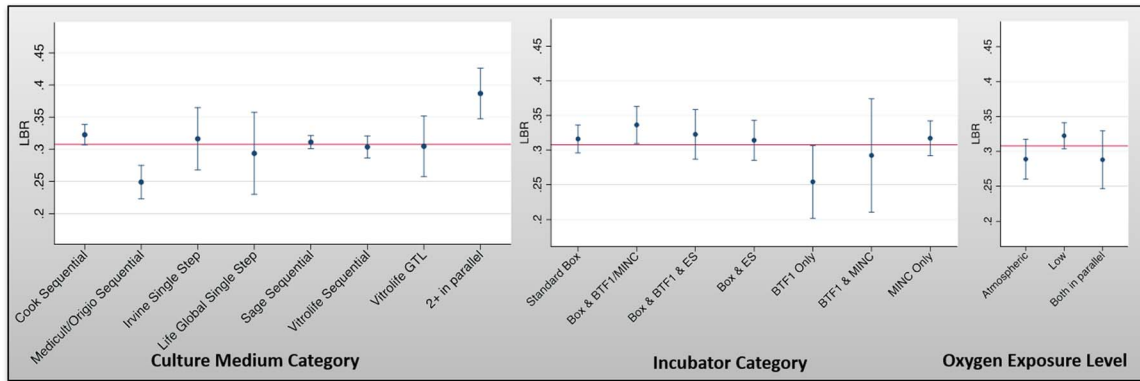
Figure 2 Estimated mean LBR by laboratory parameter category. The LBR per ET for each category of the three primary laboratory parameters; adjusted for patient and treatment characteristics (i.e. derived from the fully adjusted multivariable logistic regression model). Points represent the estimated mean LBR for each subcategory; error bars represent the 95% CI. Red lines indicate the mean LBR for the full sample (0.31).
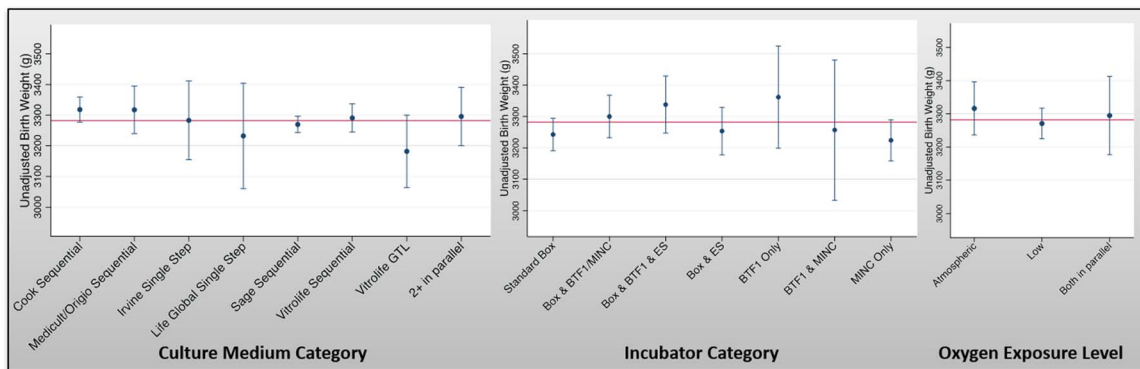

Figure 3 Estimated mean unadjusted BW by laboratory parameter category. Unadjusted BW by laboratory parameters; adjusted for patient and treatment characteristics (i.e. derived from the fully adjusted multivariable linear regression model). Points represent the estimated mean unadjusted BW value for each subcategory; error bars represent the 95% CI. Red lines indicate the mean unadjusted BW value for the full sample (3282 g).

39%. Neither incubator type nor oxygen exposure level remained significantly associated with LBR in the multivariable regression model, indicating that this association may arise from confounding with other treatment variables. However, in the multivariable model, culture media categories significantly differed from one another in terms of LBR ($P < 0.001$) (Fig. 2). Taking the most widely used medium Sage sequential as the reference, MediCult/Origio Sequential had a significantly lower LBR. Of the cycle and patient factors included in the full model, length of time of embryo culture had the largest effect size, with blastocyst-stage ET associated with higher LBR compared to cleavage-stage ET (odds ratio (OR) = 1.35, 95% CI: 1.29–1.42).

Singleton BW

Unadjusted BW

Fig. 3 shows the estimated mean unadjusted BW value for each embryo culture system parameter in the multivariable regression model. Full model parameters for both the univariable and multivariable models

are given in Supplementary Table SIII. None of the embryo culture factors (i.e. culture medium, incubator type, and oxygen exposure level) were statistically associated with unadjusted BW.

Gestational age

Fig. 4 shows the estimated mean GA value for each embryo culture system parameter in the multivariable linear regression model. Full model parameters for both the univariable and multivariable models are given in Supplementary Table SIV. Neither culture medium type nor oxygen exposure level was significantly associated with GA ($P = 0.087$ and $P = 0.338$, respectively). However, mean GA did marginally differ between incubator categories ($P = 0.045$). Of the cycle and patient factors included in the full model, blastocyst-stage ET was associated with lower GA ($\beta = -0.15$ weeks, 95% CI: -0.23 to -0.06) as was infertility diagnosis (e.g. $\beta = -0.22$ weeks, 95% CI: -0.30 to -0.13 for ovarian disorders) and spontaneous foetal reduction from three or two sacs ($\beta = -0.98$ weeks, 95% CI: -1.76 to -0.20 and $\beta = -0.75$ weeks, 95% CI: 0.91 to -0.59, respectively).
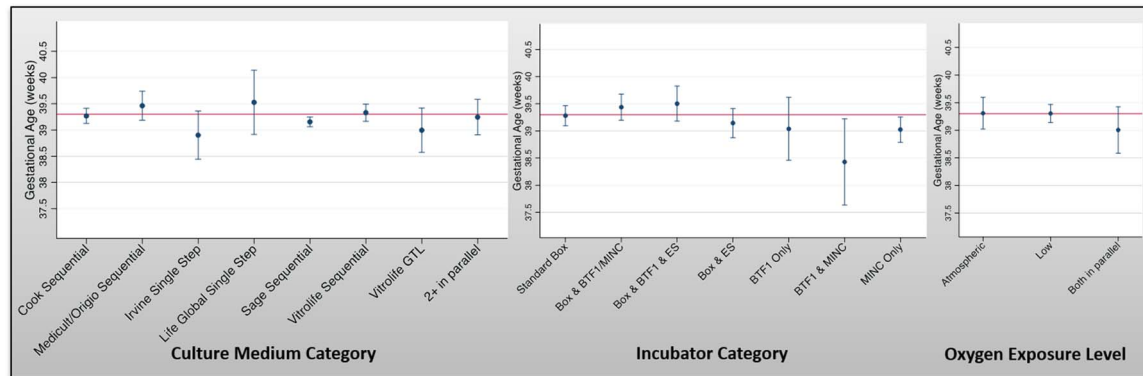
Figure 4 Estimated mean GA by laboratory parameter category. GA by time-dependent laboratory parameters; adjusted for patient and treatment characteristics (i.e. derived from the fully adjusted multivariable linear regression model). Points represent the estimated mean GA value for each subcategory; error bars represent the 95% CI. Red lines indicate the average GA value for the full sample (mean = 39.3 weeks).
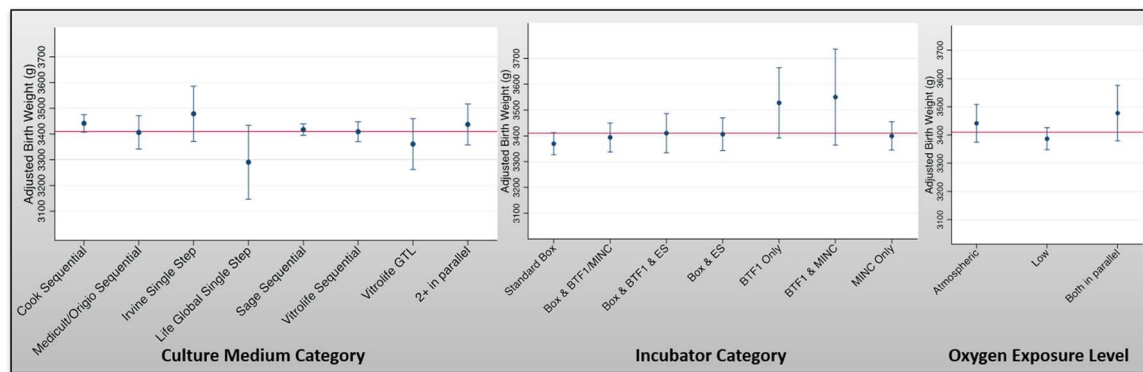

Figure 5 Estimated mean adjusted BW by laboratory parameter category. Adjusted BW by embryo culture system parameters; adjusted for patient and treatment characteristics (i.e. derived from the fully adjusted multivariable linear regression model). Points represent estimated mean adjusted BW for each variable category; error bars represent the 95% CI. Red lines indicate the global average adjusted BW value for the full sample (mean = 3410 g).

PTB

The logistic regression analyses predicting PTB for primary embryo culture and IVF treatment factors are shown in Supplementary Table SV. Oxygen level, but not embryo culture media or incubator type, was associated with PTB in the univariable model; however, none of the embryo culture system factors were associated with PTB in the multivariable regression model. PTB was associated with extended embryo culture in both the univariable and multivariable regression model and with eSET in the multivariable model. Singletons conceived from blastocyst-stage ETs were more likely to be born before 37 weeks gestation (OR = 1.42, 95% CI: 1.23–1.63) as compared to those conceived from cleavage-stage ETs, as were those born following diagnosis of all three female-factor diagnoses (uterine factor OR = 1.21, 95% CI: 102–1.45; ovarian disorder OR = 1.18, 95% CI: 1.04–1.35; and tubal disease OR = 1.27, 95% CI: 1.11–1.46), and spontaneous foetal reduction from three or two sacs (OR = 2.37, 95% CI: 0.93–6.01 and OR = 2.2, 95% CI: 1.80–2.72, respectively). In contrast, singletons born from eSET were less likely to be born preterm (OR = 0.86, 95% CI: 0.76–0.98) than those conceived from multiple ET.

Gestation-adjusted BW

Fig. 5 shows an effect plot illustrating the estimated mean adjusted BW value for each embryo culture system parameter in the multivariable linear regression model, with both regression analyses shown with full model parameters in Supplementary Table SVI. None of the embryo culture system factors (culture medium, incubator category, or oxygen exposure) were significantly associated with adjusted BW (Fig. 5). However, blastocyst culture was associated with higher adjusted BW (β = 38.9 g, 95% CI: 19.4–58.5 g) compared to cleavage-stage embryo culture (Supplementary Table SVI). Vanishing twin syndrome had the largest effect size on adjusted BW, with spontaneous twin and triplet reductions associated with lower average adjusted BW (β = −66.4 g, 95% CI: −103.9 to −28.9 g and β = −321.7 g, 95% CI: −504.4 to −139.0, respectively). Tubal disease, National Health Service (NHS) (versus patient) funding and duration of infertility were also both negatively associated with adjusted BW (β = 34.2 g, 95% CI: 13.3–55.2 g; β = 55.3 g, 95% CI: 37.4–73.2 g; and β = −10.6 g, 95% CI −18.4 to −2.9, respectively).

Discussion

The aim of this study was to capture the range of clinical practice in the UK with respect to the use of embryo culture media, oxygen level, and incubator type in order to examine their impact on neonatal health, measured as gestation length and BW, and success rate, measured as LBR per cycle. Since changes in ART technology tend to be driven by impact on success rates (Harper *et al.*, 2011), the hypothesis was that if embryo culture system factors altered success rate by altering embryo quality, they might also have an impact on neonatal health.

We observed some association between the type of culture medium system used and LBR. MediCult/Origio Sequential medium was associated with a lower average probability of an LB in a fully adjusted regression model. On average, MediCult/Origio sequential medium yielded the lowest LBR compared to at least three other sequential media included in this study (i.e. Cook sequential, Sage sequential, and Vitrolife sequential). Interestingly, the category with the highest observed LBR was that of two or more in parallel, suggesting that the simultaneous use of more than one medium in side-by-side comparisons may be beneficial in terms of success rates. Thus, our findings add to the literature, which has previously failed to show a consistent advantage of one media type over another (Mantikou *et al.*, 2013b; Chronopoulou and Harper, 2015; Sfontouris *et al.*, 2016; Dieamant *et al.*, 2017). It is also of note that despite careful quality control of embryo culture conditions as mandated in the UK by the HFEA Code of Practice and comprehensive adjustment for confounders and embryo culture conditions as far as possible (including testing non-linear terms), there were still significant unexplained differences between clinic centres in the fully adjusted regression models. The analysis showed statistically significant differences between clinics with reference to all of the outcomes studied (LBR, and mean unadjusted and adjusted BW) except PTB. This indicates that despite including all available patients, IVF treatment, and pregnancy outcome covariates, there remained unobserved clinic-specific factors associated with these outcomes that we could not account for in our statistical models.

Previous studies have observed a beneficial effect of low oxygen tension (5% O_2) on odds of a live birth (Meintjes *et al.*, 2009; Kasterstein *et al.*, 2013). In our study, although low oxygen exposure was associated with increased LBR in the univariable analysis, this effect was not statistically significant in the multivariable regression model. This may be because oxygen concentration is linked to, and therefore confounded with, incubator type; therefore, adjusting for incubator category may indirectly control for this effect.

None of the embryo culture parameters were statistically associated with unadjusted BW, GA, or adjusted BW in the corresponding multivariable regression models. However, the power to detect differences was limited as, despite the large number of cycles and births, there were relatively few informative changes of culture conditions within centres and, after adjusting for centre effects, many of the comparisons of interest were based on relatively small numbers. Therefore, we cannot draw definitive conclusions about any relationship between the embryo culture factors included here and PTB and/or singleton BW as small, but clinically important effects would not have been detected. We further note that the particular media comparisons, which have been shown to have an impact on BW in previous RCTs (Zandstra *et al.*, 2015; Kleijkers *et al.*, 2016), were not present in our data.

In terms of adjusted BW, the magnitude of clinic effect sizes ranged from −199 g to 186 g. This represents a significant effect of the clinic site on BW and therefore long-term offspring health and suggests that further research is required on datasets containing more detailed covariates and more changes within centres over a longer timeframe.

Although none of the three embryo culture factors analysed was associated with LBR, GA, or BW, the length of time which embryos were kept in those culture conditions was significant. Blastocyst culture had the largest observed effect on LBR compared to cleavage-stage embryo culture, consistent with extensive previous literature (Blake et al., 2007; Glujovsky et al., 2016). We were not able to analyse frozen embryo transfer (FET) cycles or cumulative pregnancy rate due to the limited duration of our study. However, previous studies have suggested that although extended embryo culture yields higher success rates per fresh ET, it results in lower cumulative success rates when all embryos (fresh and thawed) are transferred. This is consistent with extended embryo culture being a useful but crude selection method, which results in loss of viable embryos (Glujovsky and Farquhar, 2016). Blastocyst-stage ET was also associated with higher risk of PTB in our study, as observed in other previous studies (Maheshwari *et al.*, 2013; Dar *et al.*, 2014; Chambers *et al.*, 2015). Notably, the effect of blastocyst culture on PTB was evident even when controlling for oxygen tension used, in contrast to previous suggestions (Gardner, 2016). Blastocyst ET was also associated with higher adjusted BW. Other studies have also observed an association between blastocyst transfer and BW (Zhu *et al.*, 2014b; Kaartinen *et al.*, 2015), while others have not been able to show a difference (Vos *et al.*, 2014; Chambers *et al.*, 2015; Martins *et al.*, 2017). Although the magnitude of this increase in BW observed was relatively small, it is similar to that seen from FET cycles (Maheshwari *et al.*, 2017). High-BW babies are reminiscent of Large Offspring Syndrome seen in animals exposed to blastocyst culture (Young *et al.*, 1998; McEvoy *et al.*, 2000) and high BW (e.g. macrosomia and LGA) status carries its own health risks, so this effect requires further consideration and monitoring (Maheshwari *et al.*, 2017).

We also noted a significant effect of the number of embryos transferred and implanting on the neonatal health of a surviving singleton. Reduction in foetal sacs from three or two (vanishing twin syndrome) resulted in decreases in BW and GA, and an increase in PTB, whereas transfer of a single embryo (eSET) reduced PTB. This finding further supports the benefits of SET instead of multiple ET, in line with existing literature where the risks of vanishing twin syndrome have also been observed (Lawlor and Nelson, 2012; Hart and Norman, 2013; Magnus *et al.*, 2017). Specific infertility diagnoses, especially ovarian disorders, were associated with small effects on GA, PTB, and BW, and NHS-funded patients had slightly heavier babies.

Limitations

The moderate response rate at 60% of all UK clinics limits the generalizability of results. Since detailed data regarding the embryo culture environment were not recorded at the individual cycle or embryo level, only clinic-level information was available. Therefore, it was not always possible to determine exactly which embryos were cultured in a certain media that went into a particular incubator with a certain oxygen exposure level when different types of each of these factors were in use simultaneously in a given clinic. This situation resulted in

the inclusion of 'parallel' categories of culture medium, incubator, and oxygen exposure so as not to have to exclude these observations.

Given the observed statistically significant clinic site effects, more information regarding the use of embryo culture systems (such as medium equilibration, humid versus dry incubation, culture platform, medium to oil supplement ratio, etc.) could potentially account for some clinic-level differences. For example, one study observed an association between BW and protein source supplementation when comparing neonates resulting from embryos cultured in media from the same manufacturer (Zhu et al., 2014a).

Likewise, we were unable to collect patient-level clinical treatment information regarding controlled ovarian stimulation, such as the ovulation induction protocol, drug selection and dosage, trigger criteria, and measures of ovarian response. For example, elevated peak oestradiol levels, which are known to alter endometrial gene expression and narrow the window of implantation (Boomsma et al., 2010), are associated with increased miscarriage rates and low birth rates (Shapiro et al., 2011); this suggests that the oestrogen-dominated endometrium may be less receptive to embryos, reducing the success rate of fresh ET. Moreover, the resulting sub-optimal peri-implantation maternal environment may also lead to effects on the embryo and placenta (Choux et al., 2015), leading to altered foetal growth, and BW (Pereira et al., 2017).

Furthermore, additional characteristics of the patients and a longer study period capturing more changes in embryo culture systems would improve future studies. It is also possible that additional and potentially influential confounders exist, which are not in the routinely collected data: ethnicity, maternal and paternal height/weight, and pre-existing health conditions would certainly be among the candidates.

Therefore, given the large impact of clinic site on BW that we report here, we believe that it is now imperative that national registries contain more detailed information on culture environment variables, in line with previous (Sunde et al., 2016) and recent (Macklon et al., 2019) suggestions. However, definitive RCTs are also needed in order to reliably determine the effect of embryo culture on IVF success rates and the growth and health of subsequent offspring.

Conclusion

This study is the largest UK-based investigation of the effect of culture medium and associated factors on IVF treatment outcome and singleton BW. Some impact of culture media type on IVF success is shown, but the possible effects of laboratory environmental influences on singleton BW appear to be confounded with treating clinic centre. Future RCTs are needed together with routine collection of more detailed data in order to unravel these potential effects on the health of IVF conceived offspring.

Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

Acknowledgements

We would like to thank the HFEA, particularly Suzanne Hodgson and Howard Ryan, for supporting this study and allowing us access to their national dataset; the UK ACE for the support with questionnaire data collection and for allowing us mass access to our national community of embryology practitioners and researchers.

Authors' roles

Funding

Conflict of interest

The authors have no competing interests.

References

Armstrong S, Bhide P, Jordan V, Pacey A, Marjoribanks J, Farquhar C. Time-lapse systems for embryo incubation and assessment in assisted reproduction. *Cochrane Database Syst Rev* 2019 Art. No.: CD011320; doi: 10.1002/14651858.CD011320.pub4.

Blake D, Farquhar C, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev* 2007; Art. No.: CD002118. doi: 10.1002/14651858.CD002118.pub3.

Bolton VN, Cutting R, Clarke H, Brison DR. ACE consensus meeting report: culture systems. *Hum Fertil* 2014;**17**:239–251.

Boomsma CM, Kavelaars A, Eijkemans MJC, Fauser BCJM, Heijnen CJ, Macklon NS. Ovarian stimulation for in vitro fertilization alters the intrauterine cytokine, chemokine, and growth factor milieu encountered by the embryo. *Fertil Steril* 2010;**94**:1764–1768.

Carolan M. Maternal age ≥45 years and maternal and perinatal outcomes: a review of the evidence. *Midwifery* 2013;**29**:479–489.

Chambers GM, Chughtai AA, Farquhar CM, Wang YA. Risk of preterm birth after blastocyst embryo transfer: a large population study using contemporary registry data from Australia and New Zealand. *Fertil Steril* 2015;**104**:997–1003.

Choux C, Carmignac V, Bruno C, Sagot P, Vaiman D, Fauque P. The placenta: phenotypic and epigenetic modifications induced by assisted reproductive technologies throughout pregnancy. *Clin Epigenetics* 2015;7:87.

Chronopoulou E, Harper JC. IVF culture media: past, present and future. *Hum Reprod Update* 2015;**21**:39–55.

Dar S, Lazer T, Shah PS, Librach CL. Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: a systematic review and meta-analysis. *Hum Reprod Update* 2014;**20**:439–448.

Dieamant F, Petersen CG, Mauri AL, Comar V, Mattila M, Vagnini LD, Renzi A, Petersen B, Ricci J, Oliveira JBA *et al.* Single versus sequential culture medium: which is better at improving ongoing pregnancy rates? A systematic review and meta-analysis. *JBRA Assist Reprod* 2017;**21**:240–246.

Dupree JM, Dickey RM, Lipshultz LI. Inequity between male and female coverage in state infertility laws. *Fertil Steril* 2016;**105**:1519–1522.

Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, Barker M, Saffery R, Yajnik CS, Eckert JJ *et al.* Origins of lifetime health around the time of conception: causes and consequences. *Lancet* 2018;**391**:1842–1852.

Gardner DK. The impact of physiological oxygen during culture, and vitrification for cryopreservation, on the outcome of extended culture in human IVF. *Reprod Biomed Online* 2016;**32**:137–141.

Glujovsky D, Farquhar C. Cleavage-stage or blastocyst transfer: what are the benefits and harms? *Fertil Steril* 2016;**106**:244–250.

Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev* 2016; Art. No.: CD002118. DOI: 10.1002/14651858.CD002118.pub5.

Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. *Am J Epidemiol* 2007;**165**:849–857.

Harper J, Magli MC, Lundin K, Barratt CLR, Brison D. When and how should new technology be introduced into the IVF laboratory? *Hum Reprod* 2011;**27**:303–313.

Hart R, Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment: part I-general health outcomes. *Hum Reprod Update* 2013;**19**:232–243.

Human Fertilisation & Embryology Authority. *Fertility Treatment 2014–2016: Trends and Figures*. 2018. https://www.hfea.gov.uk/media/2563/hfea-fertility-trends-and-figures-2017-v2.pdf (Accessed March 1st 2018).

Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril* 2014; **101**:128–133.

Kaartinen NM, Kananen KM, Rodriguez-Wallberg KA, Tomás CM, Huhtala HS, Tinkanen HI. Male gender explains increased birthweight in children born after transfer of blastocysts. *Hum Reprod* 2015;**30**:2312–2320.

Kamphuis EI, Bhattacharya S, van der Veen F, Mol BW, Templeton A, Evidence Based IVF Group. Are we overusing IVF? *BMJ* 2014;**348**:g252.

Kasterstein E, Strassburger D, Komarovsky D, Bern O, Komsky A, Raziel A, Friedler S, Ron-El R. The effect of two distinct levels of oxygen concentration on embryo development in a sibling oocyte study. *J Assist Reprod Genet* 2013;**30**: 1073–1079.

Kleijkers SHM, Mantikou E, Slappendel E, Consten D, Echten-Arends J, Wetzels AM, Wely M, Smits LJM, Montfoort APA, Repping S *et al.* Influence of embryo culture medium (G5 and HTF) on pregnancy

and perinatal outcome after IVF: a multicenter RCT. *Hum Reprod* 2016;**31**:2219–2230.

Lawlor DA, Nelson SM. Effect of age on decisions about the numbers of embryos to transfer in assisted conception: a prospective study. *Lancet* 2012;**379**:521–527.

Macklon N, Ahuja K, Fauser B. Building an evidence base for IVF 'add-ons'. *Reprod Biomed Online* 2019;**38**:853–856.

Magnus MC, Ghaderi S, Morken N-H, Magnus P, Bente Romundstad L, Skjærven R, Wilcox AJ, Eldevik HS. Vanishing twin syndrome among ART singletons and pregnancy outcomes. *Hum Reprod* 2017;**32**:2298–2304.

Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2013;**100**:1615–1621 e10.

Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* 2017;**24**:35–58.

Mandal A. *8 Million Babies Born Through IVF Says Study*. News Medical Life Sciences. https://www.news-medical.net/news/20180704/8-million-babies-born-through-IVF-says-study.aspx (Accessed July 5th 2018).

Mantikou E, Bontekoe S, Wely M, Seshadri S, Repping S, Mastenbroek S. Low oxygen concentrations for embryo culture in assisted reproductive technologies. *Hum Reprod Update* 2013a;**19**: 209–209.

Mantikou E, Youssef MAFM, Wely M, Veen F, Al-Inany HG, Repping S, Mastenbroek S. Embryo culture media and IVF/ICSI success rates: a systematic review. *Hum Reprod Update* 2013b;**19**: 210–220.

Marino JL, Moore VM, Willson KJ, Rumbold A, Whitrow MJ, Giles LC, Davies MJ. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. *PLoS One* 2014;**9**:e80398.

Martins WP, Nastri CO, Rienzi L, Poel SZ, Gracia C, Racowsky C. Blastocyst *vs* cleavage-stage embryo transfer: systematic review and meta-analysis of reproductive outcomes. *Ultrasound Obstet Gynecol* 2017;**49**:583–591.

McEvoy TG, Sinclair KD, Young LE, Wilmut I, Robinson JJ. Large offspring syndrome and other consequences of ruminant embryo culture *in vitro*: relevance to blastocyst culture in human ART. *Hum Fertil* 2000;**3**:238–246.

Meintjes M, Chantilis SJ, Douglas JD, Rodriguez AJ, Guerami AR, Bookout DM, Barnett BD, Madden JD. A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. *Hum Reprod* 2009;**24**:300–307.

Morbeck DE, Baumann NA, Oglesbee D. Composition of single-step media used for human embryo culture. *Fertil Steril* 2017;**107**: 1055–1060 e1.

Mu M, Wang S-F, Sheng J, Zhao Y, Li H-Z, Hu C-L, Tao F-B. Birth weight and subsequent blood pressure: a meta-analysis. *Arch Cardiovasc Dis* 2012;**105**:99–113.

Nastri CO, Nóbrega BN, Teixeira DM, Amorim J, Diniz LMM, Barbosa MWP, Giorgi VSI, Pileggi VN, Martins WP. Low versus

atmospheric oxygen tension for embryo culture in assisted reproduction: a systematic review and meta-analysis. *Fertil Steril* 2016;**106**: 95–104 e17.

Norris SA, Osmond C, Gigante D, Kuzawa CW, Ramakrishnan L, Lee NR, Ramirez-Zea M, Richter LM, Stein AD, Tandon N *et al*. Size at birth, weight gain in infancy and childhood, and adult diabetes risk in five low- or middle-income country birth cohorts. *Diabetes Care* 2012;**35**:72–79.

Office for National Statistics. *Birth Characteristics in England and Wales: 2016*. 2017. https://www.ons.gov.uk/peoplepopulationand community/birthsdeathsandmarriages/livebirths/bulletins/birth characteristicsinenglandandwales/2016 (Accessed March 1st 2018).

Pereira N, Elias RT, Christos PJ, Petrini AC, Hancock K, Lekovich JP, Rosenwaks Z. Supraphysiologic estradiol is an independent predictor of low birth weight in full-term singletons born after fresh embryo transfer. *Hum Reprod* 2017;**32**:1410–1417.

Pérez-López FR, Villagrasa-Boli P, Muñoz-Olarte M, Morera-Grau Á, Cruz-Andrés P, Hernandez AV. Association between endometriosis and preterm birth in women with spontaneous conception or using assisted reproductive technology: a systematic review and meta-analysis of cohort studies. *Reprod Sci* 2018;**25**: 311–319.

Roseboom TJ. Developmental plasticity and its relevance to assisted human reproduction. *Hum Reprod* 2018;**33**:546–552.

Sfontouris IA, Martins WP, Nastri CO, Viana IGR, Navarro PA, Raine-Fenning N, Poel S, Rienzi L, Racowsky C. Blastocyst culture using single versus sequential media in clinical IVF: a systematic review and meta-analysis of randomized controlled trials. *J Assist Reprod Genet* 2016;**33**:1261–1272.

Shah PS. Parity and low birth weight and preterm birth: a systematic review and meta-analyses. *Acta Obstet Gynecol Scand* 2010;**89**: 862–875.

Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders. *Fertil Steril* 2011;**96**:344–348.

Spong CY. Defining "term" pregnancy: recommendations from the defining "term" pregnancy workgroup. *JAMA* 2013;**309**:2445–2446.

Sunde A, Brison D, Dumoulin J, Harper J, Lundin K, Magli MC, Abbeel EV, Veiga A. Time to take human embryo culture seriously. *Hum Reprod* 2016;**31**:2174–2182.

Sunkara SK, La Marca A, Seed PT, Khalaf Y. Increased risk of preterm birth and low birthweight with very high number of oocytes following IVF: an analysis of 65 868 singleton live birth outcomes. *Hum Reprod* 2015;**30**:1473–1480.

Swain JE. Decisions for the IVF laboratory: comparative analysis of embryo culture incubators. *Reprod Biomed Online* 2014;**28**: 535–547.

Vos AD, Janssens R, Velde HV, Haentjens P, Bonduelle M, Tournaye H, Verheyen G. The type of culture medium and the duration of in vitro culture do not influence birthweight of ART singletons. *Hum Reprod* 2014;**30**:20–27.

Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;**3**:155–163.

Zandstra H, Van Montfoort APA, Dumoulin JCM. Does the type of culture medium used influence birthweight of children born after IVF? *Hum Reprod* 2015;**30**:530–542.

Zhu J, Li M, Chen L, Liu P, Qiao J. The protein source in embryo culture media influences birthweight: a comparative study between G1 v5 and G1-PLUS v5. *Hum Reprod* 2014a;**29**:1387–1392.

Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, Qiao J. Effect of in vitro culture period on birthweight of singleton newborns. *Hum Reprod* 2014b;**29**:448–454.