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No effect of embryo culture media on birthweight and length of newborns

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STUDY QUESTION: Does the type of media used to culture embryos for IVF influence the birthweight and length of neonates?

SUMMARY ANSWER: No significant differences were observed in birthweight and length among the three embryo culture media used for *in vitro* embryo culture.

WHAT IS KNOWN ALREADY: Since the establishment of IVF as an assisted reproductive technology (ART), many different culture systems have been used for the development of human embryos. Some studies have shown that the types of culture media influence the newborn birthweight; however, other studies have shown no effect. To further explore this contradictory issue, we compared the birthweight and length of neonates born after the transfer of embryos cultured in one of three commercially available media.

STUDY DESIGN, SIZE AND DURATION: This retrospective analysis of birthweight and length of newborns included 1201 women who delivered singletons and 445 women who delivered twins. The following three commercially available culture media were used: G5^{TM}, Global and Quinn's advantage media. Women who underwent IVF-ET cycles between 2008 and 2010 were analyzed.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: Patients younger than 40 years of age with a body mass index (BMI) <30 kg/m^2 were analyzed. Only data from singletons and twins born alive after the 20th week of gestation were included in the data analysis. Patients who received preimplantation genetic diagnosis (PGD) and donor oocytes were excluded.

MAIN RESULTS AND THE ROLE OF CHANCE: The analysis of 1201 singletons and 445 sets of twins showed no significant association between mean birthweight or mean birth length and the type of embryo culture medium. Inter-twin mean birthweight and length disparities were analyzed, but were not shown to be significantly different. Multiple linear regression analysis showed that maternal weight, maternal height, gestational age and infant gender were significantly related to birthweight, and paternal height, gestational age and newborn complications were significantly associated with birth length.

LIMITATIONS AND REASONS FOR CAUTION: The current study showed that birthweight and length of newborns were not associated with the embryo culture medium. More research needs to be performed to analyze the effects of other culture medium formulations and to evaluate the long-term effects of embryo culture medium on the health of children conceived through ART.

WIDER IMPLICATIONS OF THESE FINDINGS: Our retrospective study suggests that embryo culture medium does not influence neonatal birthweight and length; however, the effects of culture medium on epigenetic variation of embryos need to be studied further.

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Key words: IVF / embryo culture medium / birthweight / birth length

Introduction

Since the establishment of IVF as an assisted reproductive technology (ART), many different culture systems have been used for the development of human embryos (Quinn, 2012; Rieger, 2012). Recently, an increasing number of IVF centers have utilized commercially available and quality-controlled media, such as G1.3, G5, Cook, HTF and Quinn's advantage. However, some studies have shown that different medium systems change the expression of imprinted genes, including Igf 2, H19 and DMR2 (Khosla *et al.*, 2001; Rivera *et al.*, 2008; Market-Velker *et al.*, 2010). In particular, loss of DMR2 methylation induces the Beckwith–Wiedemann syndrome (Catchpoole *et al.*, 2000; Halliday *et al.*, 2004; Chang *et al.*, 2005). Dumoulin *et al.* (2010) reported that the use of Cook medium, compared with Vitrolife medium, resulted in singletons with a lower mean birthweight and led to higher inter-twin mean birthweight disparities and birthweight discordancy. A trend towards lower birthweight was also noted with the Cook medium compared with the Vitrolife medium after frozen embryo transfer (Nelissen *et al.*, 2012). However, Eaton *et al.* (2012) reported that G1.3, Global and G1.5 culture media did not influence neonatal birthweight following IVF. Another study demonstrated no significant relationships between HTF and Sage culture media and birthweight (Vergouw *et al.*, 2012). Whether or not, the measured effects in these studies be generalized to other culture media is not known. In an attempt to investigate this issue, we compared the birthweights and lengths of neonates conceived from the transfer of embryos cultured in one of three commercially available media.

Materials and methods

Patients

This retrospective study was approved by the Ethics Committee of Peking University Third Hospital. Women who underwent IVF-ET cycles between 2008 and 2010 in the Reproductive Medical Centre of Peking University Third Hospital were analyzed. Women underwent controlled ovarian hyperstimulation with a GnRH agonist or GnRH antagonist protocol as described previously (Liu *et al.*, 2012). Ovarian follicle development was monitored based on serum estradiol (E2) levels and transvaginal ultrasonographic measurements. When at least one follicle reached a mean diameter of 18 mm and the E2 concentration exceeded 500 pg/ml, 10 000 units of urinary hCG (Serono, Aubonne, Switzerland) were administered before ultrasonography-guided oocyte retrieval. Luteal support was initiated on the day after oocyte retrieval using 60 mg of progesterone (Xianju Pharmacy, Zhejiang, China; Xiaoying *et al.*, 2012).

Laboratory protocols

The following three commercially available culture media were used: G5^{TM} (Vitrolife, Gottenburg, Sweden); Global (IVF Online, Toronto, Canada) and Quinn's advantage medium (SAGE, CA, USA). The corresponding sera supplemented the media: HSA solution^{TM} (Vitrolife); HSA solution (IVF Online) and Quinn's advantage SPS (SAGE). Mineral oil was obtained from Sigma (St. Louis, MO, USA) and used after washing and sterile filtration. The clinical pregnancy rates were 42.9, 40.8 and 39.3% for G5^{TM}, Global and Quinn's advantage media, respectively; there were no significant differences in clinical pregnancy rates among the three culture media. The ratio of cycles using each culture medium was ~3:3:1 (G5^{TM}: Global:Quinn's advantage). IVF and ICSI were performed according to the laboratory's routine insemination procedures on the day of oocyte retrieval (D0). The presence of two pronuclei was observed 16–18 h after insemination or injection, and the zygotes were then cultured in 25 μl of pre-equilibrated cleavage medium droplets (G1, Global HTF and Quinn's advantage cleavage medium). The embryos were cultured in incubators at 37°C under 5 or 6% CO_2 as described previously (Ming *et al.*, 2012). The morphology of embryos was evaluated 68–72 h after insemination with respect to cell number, fragmentation and symmetry. The number of embryos transferred was determined based on the patient age, number of IVF cycles and embryo quality.

Data collection

In the present study, only patients younger than 40 years of age with a body mass index (BMI) $<30\ kg/m^2$ were analyzed. Patients were excluded if they received preimplantation genetic diagnosis or donor oocytes. Furthermore, only data from singletons and twins born alive after the 20th week of gestation were included in the data analysis. The criteria for data collection were the same as used in previous reports; specifically, gestational age, low birthweight (LBW) and high birthweight (HBW) were defined as described previously (Nelissen *et al.*, 2012).

Statistics analysis

All statistical analyses were performed with SPSS software. The basic characteristics of the patients were compared using Student's *t*-tests (continuous variables) and categorical variables were evaluated with χ^2 tests. Multiple linear regression analyses were used to evaluate the association between culture media and birthweight or length, while controlling for the effects of possible confounding factors, including fertilization methods (IVF or ICSI), maternal age, paternal age, maternal height, paternal height, maternal weight, paternal weight, subfertility types, duration of subfertility, cause of subfertility, gestational age, infant gender, number of transferred embryos, number of cycles and newborn complications (neonatal brain injury, congenital heart disease, Down syndrome, hypertrophic pyloric stenosis, icterus hepatitis or congenital cartilage disease).

Results

Data from 1646 patients were analyzed. Among the 1201 women who delivered singletons, 596 had embryos cultured in the G5^{TM} medium, 460 had embryos cultured in the Global medium and 145 had embryos cultured in the Quinn's advantage medium. Among the 445 women who delivered twins, 196 had embryos cultured in the G5^{TM} medium, 159 had embryos cultured in the Global medium and 90 had embryos cultured in the Quinn's advantage medium. The parental and cycle characteristics are shown in Table I. Smoking was not included in our analysis because few women smoke in China.

The birth outcomes of singletons were compared as shown in Table II. The mean birthweight was 3246.10 ± 22.06 g in the G5^{TM} group, 3293.88 ± 26.26 g in the Global group and 3291.24 ± 43.45 g in the Quinn's group. The mean birth length of singletons was 50.19 ± 0.13 cm in the G5^{TM} group, 50.32 ± 0.15 cm in the Global group and 50.45 ± 0.25 cm in the Quinn's group. There was no significant association between the mean birthweight or length and type of culture medium. Furthermore, we investigated the potential interactions between gender, gestational age, LBW, HBW and culture medium. Again, no significant differences were observed. The effects of the stimulation protocols on birthweight and length were also compared, as shown in Supplementary data, Table SI.

Table I Patients and cycle characteristics.

Characteristic	G5™ (*n* = 792)	Global (*n* = 619)	Quinn's advantage (*n* = 235)	*P*-value
Primary indication for IVF treatment				
Male factor	152 (19.19)	136 (21.97)	34 (14.47)	0.056
Tubal factor	270 (34.09)	205 (33.12)	88 (37.45)	0.056
Male and tubal factor	332 (41.92)	262 (42.33)	99 (42.13)	0.056
Unexplained	38 (4.80)	16 (2.58)	14 (5.96)	0.056
Duration of subfertility (years)	4.57 ± 3.22	4.72 ± 3.18	4.72 ± 3.31	0.679
Primary subfertility	485 (61.24)	345 (55.74)	134 (57.02)	0.100
Cycles with ICSI	281 (35.48)	255 (41.20)	87 (37.02)	0.086
Maternal characteristics				
Age (years)	31.21 ± 4.05	31.17 ± 3.84	31.54 ± 3.96	0.451
Age ≥38 years	52 (6.57)	26 (4.20)	9 (3.83)	0.080
Height (cm)	161.61 ± 4.48	161.89 ± 4.91	161.34 ± 4.53	0.268
Weight (kg)	57.10 ± 7.32	57.52 ± 7.79	56.45 ± 6.79	0.164
Body mass index	21.87 ± 2.72	21.94 ± 2.73	21.69 ± 2.51	0.492
Paternal characteristics				
Age (years)	32.65 ± 4.06	32.78 ± 4.15	32.78 ± 3.93	0.815
Height (cm)	173.34 ± 5.28	173.49 ± 5.40	173.17 ± 5.20	0.722
Weight (kg)	73.010 ± 9.69	73.56 ± 10.30	74.25 ± 10.20	0.278
Body mass index	24.30 ± 2.79	24.40 ± 2.91	24.71 ± 2.80	0.150

Data are presented as numbers (%) or mean ± SD.

The birth outcomes of twins were compared, as shown in Table III. The mean birthweight was 2500.63 ± 30.74 g in the G5™ group, 2554.78 ± 35.58 g in the Global group and 2483.42 ± 53.68 g in the Quinn's group. The mean birth length of twins was 48.58 ± 0.28 cm in the G5™ group, 48.03 ± 0.27 cm in the Global group and 47.88 ± 0.42 cm in the Quinn's group. There was no significant association between the mean birthweight or length and the type of embryo culture medium. Furthermore, the inter-twin mean birthweight and length disparities were analyzed. No significant differences were observed in the birthweight and length disparities among the three embryo culture media.

Finally, multiple linear regression was used to determine the relationship between embryo culture medium and birthweight, birth length and gestational age in neonates with fertilization methods, maternal age, paternal age, maternal height, paternal height, maternal weight, paternal weight, subfertility types, duration of subfertility, cause of subfertility, gestational age, gender, number of transferred embryos, number of cycles and newborn complications. As shown in Table IV, birthweight after embryo culture was associated with maternal height, maternal weight, gestational age at birth and gender. There was also a potential interaction between birth length and paternal height, gestational age at birth and newborn complications (neonatal brain injury, congenital heart disease, Down syndrome, hypertrophic pyloric stenosis, icterus hepatitis and congenital cartilage disease). The data from the multiple linear regression analysis of gestational age at birth also indicated that there were no significant differences among the three culture media.

Discussion

Our retrospective study demonstrates that the type of embryo culture medium does not influence the mean birthweight or mean birth length.

It is well known that birthweight is a commonly used measure for the assessment of perinatal outcome, which is related to morbidity and mortality (Land, 2006). Therefore, many IVF centers focus on the effect of *in vitro* culture of embryos on neonatal birthweight. The birthweights of neonates were not found to differ significantly when embryos were cultured in the Global or Vitrolife medium (Eaton *et al.*, 2012), and another study demonstrated that different culture media do not affect the mean birthweight of newborns (Vergouw *et al.*, 2012). In contrast, Dumoulin *et al.* (2010) reported that embryos cultured in the Cook medium had significantly lower mean birthweights than embryos cultured in the Vitrolife medium. However this result may be due to the lower number of blastomeres in the transferred embryos in the Cook medium group. Additionally, the mothers in the Cook group were shorter and weighed less, which can influence neonatal birthweight, and the number of samples was small (Vitrolife group, $n = 110$; Cook group, $n = 78$). In our retrospective study, there were 792 neonates in the Vitrolife G5™ group, 619 neonates in the Global group and 235 neonates in the Quinn's group. These numbers are large enough to detect the effects of different media on neonatal birthweight. Cheung *et al.* (2002) found that birth length is strongly associated with both neonatal and post-neonatal mortality. Therefore, the birth lengths of singletons

Table II Neonatal characteristics of live born singletons.

	G5™ (*n* = 596)	Global (*n* = 460)	Quinn's advantage (*n* = 145)	RR (95% CI) G5™–Global	RR (95% CI) G5™–Quinn's advantage	RR (95% CI) Global–Quinn's advantage	*P*-value
Boys	312 (52.35)	254 (55.22)	71 (48.97)	0.948 (0.847–1.061)	1.069 (0.890–1.284)	1.128 (0.937–1.357)	0.376
Gestational age at birth (weeks)	38.45 ± 0.08	38.25 ± 0.11	38.26 ± 0.17	—	—	—	0.270
Preterm birth (<37weeks)	54 (9.06)	52 (11.30)	18 (12.41)	0.801 (0.559–1.150)	0.730 (0.442–1.205)	0.911 (0.551–1.505)	0.335
Birthweight (g)	3246.10 ± 22.06	3293.88 ± 26.26	3291.24 ± 43.45	—	—	—	0.327
Birth length (cm)	50.19 ± 0.13	50.32 ± 0.15	50.45 ± 0.25	—	—	—	0.594
Low birthweight (<2500 g)	34 (5.70)	25 (5.43)	6 (4.14)	1.050 (0.635–1.734)	1.379 (0.590–3.221)	1.313 (0.550–3.139)	0.756
High birthweight (>4500 g)	3 (0.50)	6 (1.30)	3 (2.07)	0.386 (0.097–1.535)	0.243 (0.050–1.193)	0.630 (0.160, 2.489)	0.166

Data are presented as numbers (%) or mean ± SD. RR, rate ratio; 95% CI, confidence interval.

Table III Neonatal outcomes of live born twins.

	G5™ (*n* = 196)	Global (*n* = 159)	Quinn's advantage (*n* = 90)	RR (95% CI) G5™–Global	RR (95% CI) G5™–Quinn's advantage	RR (95% CI) Global–Quinn's advantage	*P*-value
Monozygotic twins (*n*)	12 (6.12)	7 (4.40)	5 (5.56)	1.416 (0.544–3.686)	1.109 (0.379–53.274)	0.783 (0.241–2.543)	0.773
Gestational age (weeks)	36.20 ± 0.16	36.32 ± 0.16	36.21 ± 0.21	—	—	—	0.862
Preterm birth (<37 weeks)	84 (42.86)	75 (47.17)	47 (52.22)	0.840 (0.552–1.279)	0.686 (0.416–1.133)	0.817 (0.487–1.371)	0.324
Low birthweight (<2500 g)	135 (34.44)	115 (36.16)	76 (42.22)	0.927 (0.680–1.263)	0.719 (0.501–1.032)	0.775 (0.533–1.127)	0.195
Very low birthweight (<1500 g)	8 (2.04)	5 (1.57)	8 (4.44)	1.304 (0.422–4.026)	0.448 (0.165–1.213)	0.343 (0.111–1.066)	0.109
Mean birthweight (g)	2500.63 ± 30.74	2554.78 ± 35.58	2483.42 ± 53.68	—	—	—	0.397
Mean birthweight disparity (g)	325.54 ± 20.80	334.65 ± 26.83	299.72 ± 29.54	—	—	—	0.684
Mean birthweight disparity (%)	15.19 ± 0.01	15.40 ± 0.01	15.00 ± 0.02	—	—	—	0.740
Mean birth length (cm)	48.58 ± 0.28	48.03 ± 0.27	47.88 ± 0.42	—	—	—	0.245
Mean birth length disparity (cm)	1.05 ± 0.195	1.08 ± 0.148	1.33 ± 0.23	—	—	—	0.655
Mean birth length disparity (%)	2.30 ± 0.00	2.34 ± 0.00	2.43 ± 0.01	—	—	—	0.670

Data are presented as numbers (%) or mean ± SD. RR, rate ratio; 95% CI, confidence interval.

Table IV Results of multiple regression analysis among live born singletons.

	Birthweight (g)			Birth length (cm)			Gestational age at birth (weeks)		
	β	t	*P*-value	*β*	t	*P*-value	*β*	t	*P*-value
Media 1	0.050	1.777	0.076	0.032	0.884	0.377	−0.043	−1.436	0.151
Media 2	0.042	1.496	0.135	0.053	1.435	0.152	−0.019	−0.641	0.522
ICSI (versus IVF)	−0.005	−0.141	0.888	−0.027	−0.608	0.543	0.084	2.239	0.025*
Maternal age (per year)	−0.032	−0.708	0.479	−0.113	−1.869	0.062	−0.020	−0.411	0.681
Paternal age (per year)	−0.054	−1.316	0.188	0.036	0.666	0.506	0.001	0.018	0.985
Maternal height (per cm)	0.072	2.477	0.013*	0.013	0.332	0.740	−0.007	−0.215	0.830
Maternal weight (per kg)	0.148	5.130	0.000*	0.073	1.888	0.059	0.025	0.799	0.425
Paternal height (per cm)	0.045	1.410	0.159	0.084	1.985	0.048*	0.046	1.334	0.182
Paternal weight (per kg)	−0.029	−0.916	0.360	0.006	0.147	0.883	−0.066	−1.907	0.057
Types of subfertility	0.018	0.605	0.546	−0.022	−0.568	0.570	−0.018	−0.583	0.560
Duration of subfertility (per year)	0.006	0.184	0.854	0.010	0.250	0.803	−0.034	−1.007	0.314
Male versus other	0.041	0.874	0.382	0.032	0.513	0.608	0.153	3.071	0.002*
Unexplained versus other	0.036	0.850	0.396	0.030	0.525	0.600	0.114	2.473	0.014*
Tubal versus other	−0.034	−1.152	0.250	−0.015	−0.383	0.702	0.002	0.066	0.948
Gestational age at birth (per week)	0.345	12.796	0.000*	0.224	6.328	0.000*	—	—	—
Gender (male versus female)	0.083	3.103	0.002*	0.068	1.914	0.056	−0.085	−2.952	0.003*
No. of transferred embryos	0.026	0.856	0.392	0.006	0.136	0.892	−0.072	−2.160	0.031*
Cycle number (>1 versus 1)	−0.004	−0.150	0.881	0.031	0.838	0.402	−0.029	−0.962	0.336
Newborn complications (yes or no)	−0.034	−1.265	0.206	−0.086	−2.465	0.014*	−0.078	−2.700	0.007*

β is the regression coefficient.
*$P < 0.05$.

and twins were also compared, which have not been analyzed in previous studies. Similar to the birthweights, there were no significant differences in the mean birth lengths of embryos cultured in different media. Furthermore, the inter-twin mean birthweight and length disparities were analyzed, but were not shown to be significantly different between media types. It seems possible that the embryo culture medium does not influence the neonatal birthweight or length. Fu and Yu (2011) constructed newborn weight-for-gestational age nomograms based on a computerized perinatal database. The weights of 28 052 singleton deliveries derived from spontaneous conceptions were analyzed. Fu and Yu (2011) reported that the neonatal mean weights were 3160, 3282 and 3388 g at 38, 39 and 40 weeks of gestation, respectively. The mean birth length of naturally conceived children born was ~50.5 cm (Jie *et al.*, 2011). Indeed, the birthweights and lengths of newborns derived from IVF in this study are similar to newborns derived from spontaneous conceptions. Although this study has also confirmed a lack of effect due to culture media, the present analysis is limited because only three commercially available culture media were evaluated. Additional research needs to be conducted to analyze the effects of other culture media formulations.

Multiple linear regression analysis was performed to determine the relationship between confounding factors and birthweight and length. Maternal weight, gestational age and neonatal gender were significantly related to birthweight in agreement with earlier studies (Oken *et al.*, 2003). Surprisingly, maternal height also correlated with the birthweight in the current study. It is possible that race influences newborn birthweight. The present study is the first study in which the relationship between birth length and paternal height, gestational age and newborn complications was investigated. When the analysis was adjusted for the gestational age at birth, significant differences in birthweight were not observed among the three commercial embryo culture media. However, we showed that the gestational age was related to the fertilization method, cause of infertility, gender, number of transferred embryos and newborn complications. Previous studies have demonstrated that there are significant relationships between the fertilization method, etiology of infertility, and fetal gender and length of gestation (Lao *et al.*, 2011; Nelson and Lawlor, 2011). Transfer of multiple embryos results in a high rate of multiple pregnancies and preterm delivery (Barri *et al.*, 2011). Craigo (2011) also reported that newborn complications increased the incidence of preterm infants. Therefore, it is possible that the fertilization method, infertility types, gender, number of transferred embryos and newborn complications influence gestational age.

Epigenetic programming of gametes and early post-fertilization embryos is essential for the development of a new organism, a process that requires DNA methylation, histone modification, chromatin remodeling and interfering RNA (Shi and Wu, 2009; Hales *et al.*, 2011). Increasing data suggest that ovarian stimulation and embryo culture medium affect genetic imprinting. Serum-containing medium decreases the expression of H19 and Igf2 and reduces birthweight compared with serum-free medium in mice (Khosla *et al.*, 2001). Although the media were supplemented by different sera,

effects of culture media on birthweight and length were not demonstrated in the present study. The birthweight and length of newborns were also not found to differ significantly when patients used different stimulation protocols. It is possible that the culture environment still influences the epigenetic variation of the embryo, which may not be represented by birthweight and length. Therefore, the effects of embryo culture medium on the health of ART-derived children need additional research.

In conclusion, the present study showed that no significant differences exist in birthweight and length after the use of the three embryo culture media for *in vitro* embryo culture. However, more research needs to be performed to evaluate the long-term effects of culture medium on embryo development.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

P.L. conceived and designed the study; S.L.L., M.L. and Y.L. coordinated data collection; L.X.C. analyzed the data; S.L.L. wrote the paper and all authors interpreted the data.

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Conflict of interest

None declared.

References

Barri PN, Coroleu B, Clua E, Tur R. Prevention of prematurity by single embryo transfer. *J Perinat Med* 2011;**39**:237–240.

Catchpoole D, Smallwood AV, Joyce JA, Murrell A, Lam W, Tang T, Munroe D, Reik W, Schofield PN, Maher ER. Mutation analysis of H19 and NAP1L4 (hNAP2) candidate genes and IGF2 DMR2 in Beckwith–Wiedemann syndrome. *J Med Genet* 2000;**37**:212–215.

Chang AS, Moley KH, Wangler M, Feinberg AP, Debaun MR. Association between Beckwith–Wiedemann syndrome and assisted reproductive technology: a case series of 19 patients. *Fertil Steril* 2005;**83**:349–354.

Cheung YB, Yip PS, Karlberg JP. Size at birth and neonatal and postneonatal mortality. *Acta Paediatr* 2002;**91**:447–452.

Craigo SD. Indicated preterm birth for fetal anomalies. *Semin Perinatol* 2011;**35**:270–276.

Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD, Geraedts JP *et al*. Effect of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;**25**:605–612.

Eaton JL, Lieberman ES, Stearns C, Chinchilla M, Racowsky C. Embryo culture media and neonatal birthweight following IVF. *Hum Reprod* 2012;**27**:375–379.

Fu J, Yu M. A hospital-based birth weight analysis using computerized perinatal data base for a Chinese population. *J Matern Fetal Neonatal Med* 2011;**24**:614–618.

Hales BF, Grenier L, Lalancette C, Robaire B. Epigenetic programming: from gametes to blastocyst. *Birth Defects Res A Clin Mol Teratol* 2011; **91**:652–665.

Halliday J, Oke K, Breheny S, Algar E, Amor DJ. Beckwith–Wiedemann syndrome and IVF: a case–control study. *Am J Hum Genet* 2004; **75**:526–528.

Jie W, Liyun Z, Jianhua P, Jian Z, Xiaoguang Y. Nutrition and development status of neonate in 16 counties in 8 provinces in China. *J Hyg Res* 2011; **40**:238–239.

Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol Reprod* 2001;**64**:918–926.

Land JA. How should we report on perinatal outcome? *Hum Reprod* 2006; **21**:2638–2639.

Lao TT, Sahota DS, Suen SS, Law LW. The impact of fetal gender on preterm birth in a southern Chinese population. *J Matern Fetal Neonatal Med* 2011;**24**:1440–1443.

Liu N, Ma Y, Li R, Jin H, Li M, Huang X, Feng HL, Qiao J. Comparison of follicular fluid amphiregulin and EGF concentrations in patients undergoing IVF with different stimulation protocols. *Endocrine* 2012; **42**:708–716.

Market-Velker BA, Fernandes AD, Mann MR. Side-by-side comparison of five commercial media systems in a mouse model: suboptimal in vitro culture interferes with imprint maintenance. *Biol Reprod* 2010; **83**:938–950.

Ming L, Liu P, Qiao J, Lian Y, Zheng X, Ren X, Huang J, Wu Y. Synchronization between embryo development and endometrium is a contributing factor for rescue ICSI outcome. *Reprod Biomed Online* 2012;**24**:527–531.

Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, Land JA, Evers JL, Dumoulin JC. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. *Hum Reprod* 2012;**27**:1966–1976.

Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. *PLoS Med* 2011;**8**:e1000386.

Oken E, Kleinman KP, Rich-Edwards J, Gillman MW. A nearly continuous measure of birth weight for gestational age using a United States national reference. *BMC Pediatr* 2003;**3**:6–15.

Quinn P. Culture systems: sequential. *Methods Mol Biol* 2012; **912**:211–230.

Rieger D. Culture systems: physiological and environmental factors that can affect the outcome of human ART. *Methods Mol Biol* 2012; **912**:333–354.

Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM, Bartolomei MS. Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. *Hum Mol Genet* 2008;**17**:1–14.

Shi L, Wu J. Epigenetic regulation in mammalian preimplantation embryo development. *Reprod Biol Endocrinol* 2009;**7**:59–69.

Vergouw CG, Hanna Kostelijk E, Doejaaren E, Hompes PG, Lambalk CB, Schats R. The influence of the type of embryo culture medium on neonatal birthweight after single embryo transfer in IVF. *Hum Reprod* 2012;**27**:2619–2626.

Xiaoying Z, Lina W, Xiumei Z, Ying L, Ping L, Jie Q. Effect of hCG priming on embryonic development of immature oocytes collected from unstimulated women with polycystic ovarian syndrome. *Reprod Biol Endocrinol* 2012;**10**:40–46.