human reproduction

ORIGINAL ARTICLE *Early pregnancy*

Controlled ovarian hyperstimulation leads to high progesterone and estradiol levels during early pregnancy

Ilkka Y. Järvelä[1,*], Sari Pelkonen[1], Outi Uimari[1], Kaarin Mäkikallio[1], Katri Puukka[2], Aimo Ruokonen[2], Aydin Tekay[1], and Hannu Martikainen[1]

[1]Department of Obstetrics and Gynaecology, Oulu University Hospital, PO Box 5000, 90014 Oulu, Finland [2]Department of Clinical Chemistry, Oulu University Hospital, PO Box 5000, 90014 Oulu, Finland

*Correspondence address. Tel: +358-8-3152011; Fax: +358-8-3154310; E-mail: ilkka.jarvela@oulu.fi

STUDY QUESTION: Are there differences in estrogen and progesterone secretion in singleton pregnancies, up to Week 11, between spontaneous pregnancies, after controlled ovarian hyperstimulation and fresh embryo transfer (COH + ET) and after frozen embryo transfer in a spontaneous cycle (FET)?

SUMMARY ANSWER: Serum progesterone and estradiol (E_2) concentrations after COH + ET were higher in early pregnancy, lasting up to Week 7–8, than FET and spontaneous pregnancies, while hormone levels after FET did not differ from spontaneous pregnancies.

WHAT IS ALREADY KNOWN: The risk of adverse perinatal outcomes after COH + ET seems to be increased when compared with spontaneous pregnancies. One of the reasons suggested for this is related to ovarian hyperstimulation.

STUDY DESIGN, SIZE, DURATION: This was a prospective cohort study consisting of three different groups of pregnant women which were followed-up weekly until Week 11 of their pregnancies. The spontaneous pregnancy group consisted of 41 women, the COH + ET group consisted of 39 and the FET group consisted of 30 women.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women in the control group with spontaneous conception were recruited from local prenatal clinics. Women in the COH + ET and FET groups were recruited from the Reproductive Unit of Oulu University Hospital. At each visit, a three-dimensional ultrasonography was performed to examine the ovarian volumes and vascularization. A blood sample was drawn to analyse progesterone and E_2 levels. The pregnancy outcome was included in the analysis.

MAIN RESULTS AND THE ROLE OF CHANCE: At pregnancy Week 5, the serum progesterone levels were higher after the COH + ET (median 312, inter-quartile range 183–480 nmol/l), when compared with the spontaneous (63, 52–80 nmol/l; $P < 0.001$) and FET (74, 48–96 nmol/l; $P < 0.001$) pregnancies. At Week 11, the P (189, 124–260 nmol/l) was still higher in the COH + ET group (FET 101, 78–120 nmol/l, $P < 0.001$; spontaneous 115, 80–139 nmol/l, $P < 0.01$) than the other two groups. The E_2 levels at Week 5 were also significantly higher after COH + ET (4.1, 2.2–6.6 nmol/l) than in the spontaneous pregnancies (1.1, 0.7–1.6 nmol/l, $P < 0.001$) or after FET (0.7, 0.6–0.9 nmol/l, $P < 0.001$). The volume of the ovaries and the intraovarian vasculature in the COH + ET group were significantly higher when compared with the other two groups ($P < 0.001$). The birthweight was negatively correlated with the serum P (R −0.340, $P < 0.01$) and E_2 (R = −0.275, $P < 0.05$) in pregnancy Weeks 5–8. In the multivariate analysis evaluating the factors affecting birthweight of the newborn, the significant factors were the length of gestation, maternal height and progesterone or E_2 secretion during Weeks 5–8.

LIMITATIONS, REASONS FOR CAUTION: Because of the low number of patients in this study, larger cohort studies are required to confirm the findings.

WIDER IMPLICATIONS OF THE FINDINGS: The findings here indicate that COH-induced increased luteal activity should be evaluated by measuring steroid levels or the ovarian size or vascularity, rather than number of oocytes retrieved. If unphysiologically high steroid activity during pregnancy after COH contributes to the risk of adverse perinatal outcomes after fresh embryo transfer, milder stimulation protocols or even freezing of all of the embryos should be considered.

Introduction

When comparing the outcomes between spontaneous and IVF singleton pregnancies, the latter seem to carry an increased risk for several perinatal complications, such as preterm delivery (<37 weeks; PTD) and fetal growth restriction (FGR) (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; Schieve *et al.*, 2004; Pandey *et al.*, 2012; Pinborg *et al.*, 2013). This increased risk is especially related to IVF pregnancies following controlled ovarian hyperstimulation and fresh embryo transfer (COH + ET) (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; Schieve *et al.*, 2004; Pandey *et al.*, 2012; Pinborg *et al.*, 2013). Pregnancies with the embryo transfer after freezing and thawing (FET) without COH show lower perinatal risks than COH + ET pregnancies (Wennerholm *et al.*, 2009; Maheshwari *et al.*, 2012). Furthermore, several studies comparing FET pregnancies with spontaneous pregnancies have revealed no increased risk for PTD and FGR in FET pregnancies (Pinborg *et al.*, 2010; Sazonova *et al.*, 2012) and PTD (Pelkonen *et al.*, 2010; Pinborg *et al.*, 2010; Sazonova *et al.*, 2012), although not in all cases (Wennerholm *et al.*, 2013).

The reason for the differences in the obstetric outcomes in spontaneous pregnancies and pregnancies with either fresh or frozen embryo transfers is unclear. Several factors related to the reproductive laboratory technology itself (De Geyter *et al.*, 2006; Ombelet *et al.*, 2006; Shih *et al.*, 2008; Pelinck *et al.*, 2010a,b; Nelissen *et al.*, 2012; Makinen *et al.*, 2013) or the patient characteristics (Thomson *et al.*, 2005; Romundstad *et al.*, 2008) may be involved. One of the factors possibly affecting pregnancy outcomes is COH, which causes a supraphysiologic endocrine uterine environment and suboptimal endometrial development (Hansen *et al.*, 2002; Chung *et al.*, 2006; Kalra *et al.*, 2011; Kansal Kalra *et al.*, 2011), which may finally result in adverse obstetric outcomes (Hansen *et al.*, 2002; Chung *et al.*, 2006; De Geyter *et al.*, 2006; Ombelet *et al.*, 2006; Wennerholm *et al.*, 2009; Pelinck *et al.*, 2010a,b).

Trophoblastic invasion and formation of the primary, secondary and tertiary villi take place during the first trimester of pregnancy (Jauniaux *et al.*, 2006), and abnormal placentation with a decreased trophoblastic invasion might lead to impaired fetal growth (Khong *et al.*, 1986; Labarrere and Althabe, 1987; Pijnenborg *et al.*, 1991), emphasizing the significance of this period of pregnancy. The main objective of the present study was to compare the hormonal profiles during the first trimester of pregnancy between three different types of pregnancies: spontaneous pregnancies, pregnancies following COH and the transfer of a fresh embryo (COH + ET) and pregnancies following the transfer of frozen–thawed embryos during a natural menstrual cycle (FET). The hormonal profile in each group was related to activity of the corpus luteum. In addition, the hormonal levels were related to the obstetric outcome.

Materials and Methods

Patients

This study was approved by the Ethics Committee of Oulu University Hospital in Oulu, Finland and was conducted according to the Declaration of Helsinki for Medical Research involving Human Subjects. Written informed consent was obtained from all of the voluntary participants. Women with a history of gynaecological pathology (e.g. endometriosis, fibroids and pelvic surgery) or currently smoking, as well as mothers with vanishing twin pregnancies were excluded. All recruited women were Caucasian.

The control group with spontaneous conception ($n = 41$) was recruited from local prenatal clinics, and the estimated date of delivery (EDD) was assessed according to the beginning of the last menstrual period, i.e. the EDD was 280 days later. No correction was performed according to the length of previous menstrual cycles, but the EDD was confirmed by an ultrasonographic measurement of crown-rump length at the 11th gestational week, and differences greater than ± 3 days were corrected.

The patients for the fresh (COH + ET, $n = 39$) and frozen embryo transfer (FET, $n = 30$) groups were recruited from the Reproductive Unit in the Department of Obstetrics and Gynaecology, Oulu University Hospital in Oulu, Finland. The COH was performed using a long GnRH agonist protocol (Martikainen *et al.*, 2001). The FSH stimulation was followed by oocyte retrieval 34–36 h after the hCG injection (5000–10 000 IU). In all COH + ET cases, one to two embryos were transferred into the uterine cavity 2 days after the oocyte retrieval. If a top-quality embryo was available, only one single embryo was transferred, and no blastocyst transfers were performed. Supernumerary good-quality embryos were frozen on the day of embryo transfer using a slow freezing protocol with 1,2-propanediol as the cryoprotectant (Martikainen *et al.*, 2001; Hyden-Granskog *et al.*, 2005). In the COH + ET pregnancies, the oocyte collection day was the day of fertilization and the EDD was set up 266 days (38 weeks) later. The frozen and thawed embryos (FET) were transferred during natural cycles with no ovarian stimulation, 2–5 days after a positive urinary LH-test. The EDD was calculated according to the age of the embryo on the transfer day. If the embryo was transferred on the same day as thawing (embryo age 2 days), the EDD was 264 days later. If the embryos were cultured overnight (embryo age 3 days), the EDD was 263 days later. Luteal support with vaginal micronized progesterone was started on the day of embryo transfer and used for 2 weeks in both the COH + ET and FET groups.

After recruitment at 4–8 weeks of gestation, the patients were followed-up weekly until Week 11. The follow-up visits in early pregnancy included blood sampling for hormonal assays and ultrasound examinations to determine ovarian volume and vasculature. At birth, the gestational age, birthweight and gender were registered. The birthweight Z-scores according to the gestational age at delivery and the gender were calculated by using the national birthweight data provided by the National Institutes for Health and Welfare (http://www.thl.fi/en_US/web/en/statistics/topics/reproductive_health/deliveries).

Biochemical assays

After blood sampling, the serum was collected and samples were stored at $-80°C$ for subsequent analyses. The maternal serum concentrations of progesterone were analysed using an automated chemiluminescence system (Advia Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). A radioimmunoassay was used for the estradiol (E_2) (Orion Diagnostica, Oulunsalo, Finland), according to the manufacturers' instructions. The respective intra-assay and inter-assay coefficients of variation were 4.7 and 8.4% for progesterone and 4.5 and 4.0% for E_2. External quality control of the hormone assays was organized by national (Labquality Ltd, Helsinki,

Finland) and international (Bio-Rad Laboratories EQAS, Irvine, CA, USA) companies.

Ovarian ultrasound examination

The mothers were examined weekly by using transvaginal three-dimensional (3-D) ultrasonography (Voluson Expert 730; GE Healthcare, Zipf, Austria), and the 3-D data collected were later analysed using specific computer software (Virtual Organ Computer-aided AnaLysis; VOCAL, GE Healthcare, Zipf, Austria), as described previously (Järvelä *et al.*, 2003, 2008). Vasculature supplying the corpus luteal tissue in the ovaries was examined by power Doppler with pre-installed settings (frequency, mid; dynamic set, 2; balance, $G > 170$; smooth, 5/5; ensemble, 16; line density, 7; and power Doppler map, 5). The setting conditions for the subpower Doppler mode were as follows: gain, -5.6; quality, normal; wall motion filter, low 1; and velocity range, 0.9 kHz.

The data analysis of the ovaries using VOCAL has been described in our earlier studies (Järvelä *et al.*, 2003, 2008). Briefly, the manual mode of the VOCAL Contour Editor was used to cover the entire 3-D volume of the ovary, with $30°$ rotation steps (Järvelä *et al.*, 2007). Hence, six contour planes were analysed for each ovary to cover $180°$. After obtaining the total ovarian volume, the programme calculated the ratio of coloured voxels to all voxels; this fraction of the total volume (%) was expressed as the vascularization index (VI). The vascularized volume (ml) in the ovary was calculated by multiplying the total ovarian volume by the VI.

All of the ultrasound examinations and VOCAL analyses were performed by a single observer (I.Y.J.). Our earlier studies have revealed good intraobserver intraclass correlation coefficients for the reproducibility of these measurements (Järvelä *et al.*, 2003, 2008).

Statistics

The primary outcome was the steroid levels during the follow-up. Power analysis was based on the assumption that serum levels of progesterone are increased by 50% (estimated from the study by Johnson *et al.*, 1994) at pregnancy Weeks 5–6 in COH + ET group in comparison with spontaneous pregnancies (mean 70 nmol/l; with SD 50 nmol/l). The power analysis revealed that 22 subjects would be needed in each group for the study to have 80% power, when an α error was set at a significant level of 0.05. A total of 28 subjects needed to be recruited in each group after adjusting for a possible 20% dropout rate.

The Statistical Package for the Social Sciences (SPSS) 20.0.0.2 for Mac (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. The statistical analyses were performed from Week 5 onwards, since only a few spontaneous pregnancies were recruited at Week 4. The general linear model for repeated measures was employed to detect the differences within and between the groups. The areas under the curve (AUCs) for the hormone levels were calculated for two periods in early pregnancy, Weeks 5–8 and 8–11, because before 8 weeks of spontaneous pregnancy the corpus luteum is the main origin for progesterone production and after that the responsible organ is the placenta (Csapo *et al.*, 1973a,b; Csapo and Pulkkinen, 1978). In the comparisons of the serum hormone levels and ovarian volumes between the groups, the Kruskall–Wallis anlaysis of variance test was used.

To explore the independent factors affecting the birthweight, a linear regression model, including the gestational age, gender, maternal age and height, previous delivery (yes/no), and progesterone and E_2 secretion in pregnancy Weeks 5–8 (AUC), was evaluated. No data on maternal weight gain or paternal age were available. The continuous factors were gestational age, maternal age and height, and the fixed factors included gender and previous delivery (yes/no). A *P*-value of <0.05 was considered to be statistically significant. All steroid and ovarian volume values in the results are given as the median and inter-quartile range (IQR).

Results

The perinatal characteristics are shown in Table I. The spontaneous pregnancy group was younger with more previous pregnancies when compared with the other groups. In the COH + ET group, the birthweight and gestational age at delivery were lower than those in the spontaneous pregnancy group.

The progesterone and E_2 levels are presented in Fig. 1. The analysis of the repeated measurements during pregnancy Weeks 5–11 revealed changes both within and between the groups, as described below.

Progesterone in early pregnancy

The progesterone secretion (median, IQR) at Week 5 was the highest in the COH + ET group, compared with the FET and spontaneous pregnancy groups (312, 183–480 nmol/l versus 74, 48–96 nmol/l versus 63, 52–80 nmol/l; $P < 0.001$), and it remained higher than in the FET and spontaneous pregnancy groups, even at Week 11 (189, 124–260 nmol/l versus 101, 78–120 nmol/l versus 115, 80–139 nmol/l; $P < 0.001$). In the COH + ET group, the daily progesterone secretion decreased 3.0 (1.3–5.0) nmol/l per day between Weeks 5 and 11, whereas it increased in the FET group 0.6 (0.2–1.4) nmol/l and in spontaneous pregnancy group 0.9 (0.2–1.7) nmol/l per day ($P < 0.001$). The total progesterone secretion in the COH + ET group between Weeks 5 and 8 (AUC_{5-8}: 5380, 3310–9310 nmol/l) was 4-fold higher when compared with that in the FET and spontaneous pregnancy groups (1400, 1060–1930 nmol/l and 1426, 1190–1630 nmol/l, $P < 0.001$), and it remained higher between Weeks 8 and 11 in the COH + ET group (AUC_{8-11}: 4300, 2870–5950 nmol/l), compared with the FET and spontaneous pregnancy groups (1690, 1340–2302 nmol/l and 2058, 1690–2430 nmol/l, $P < 0.001$). In the spontaneous pregnancy and FET groups, the progesterone secretion was higher ($P < 0.001$) between Weeks 8 and 11 than at Weeks 5–8, with no difference between these groups, but in the COH + ET group, the progesterone secretion was greater during the earlier period at 5–8 weeks ($P < 0.001$).

In the COH + ET group, the progesterone AUC_{5-8} correlated with the ovarian volume (Spearman correlation coefficient R 0.671, $P < 0.001$) and ovarian vascularized volume ($R = 0.666$, $P < 0.001$), but not with the number of oocytes collected ($R = 0.089$, NS). The number of collected oocytes did not correlate with the ovarian volume or ovarian vasculature.

In the analysis including all mothers, the progesterone AUC_{5-8} correlated negatively with the birthweight and birthweight *Z*-score (Table II), but not with the gestational age. In addition, a positive correlation was found between the progesterone AUC_{5-8} and both the ovarian volume and vasculature. The progesterone AUC_{8-11} correlated with the ovarian volume and vasculature, but not with the perinatal parameters.

E_2 in early pregnancy

The serum E_2 (Fig. 1) was initially significantly higher at pregnancy Week 5 in the COH + ET group (median, IQR: 4.1, 2.2–6.6 nmol/l) compared with the FET (0.7, 0.6–0.9 nmol/l; $P < 0.001$) or spontaneous pregnancy groups (1.1, 0.7–1.6 nmol/l; $P < 0.001$). The secretion at pregnancy Week 11 was higher in the spontaneous pregnancy group (8.6, 6.9–11.1 nmol/l) than in the COH + ET group (5.3, 3.7–7.7 nmol/l; $P < 0.001$) and the FET group (6.6, 4.1–8.1 nmol/l; $P < 0.002$).

Table I Perinatal characteristics of spontaneous control pregnancies and pregnancies with controlled ovarian hyperstimulation and fresh embryo transfer (COH + ET) and frozen embryo transfer (FET).

	Spontaneous pregnancy (n = 41)	COH + ET (n = 39)	FET (n = 30)	P		
				Control versus COH + ET	Control versus FET	COH + ET versus FET
Mother						
Age, years	25.7 (0.7)	31.2 (0.7)	32.0 (0.8)	<0.001	<0.001	0.388
Weight, kg	61.8 (1.5)	63.1 (2.1)	64.3 (1.8)	0.330	0.190	0.755
Height, cm	164.6 (0.9)	164.7 (1.0)	165.8 (1.2)	0.810	0.520	0.575
BMI (kg/m^2)	22.8 (0.5)	23.2 (0.7)	23.4 (0.7)	0.638	0.351	0.708
Gravidity	2.0 (1–6)	1.1 (0–4)	1.5 (1–3)	0.016	0.285	0.147
Parity	0.6 (0–3)	0.2 (0–1)	0.4 (0–2)	0.008	0.379	0.086
IVF	0	69.2% (27)				
ICSI	0	30.8% (12)				
Eggs after COH	–	11 (4–25)	–			
Newborn						
Male	46.3%	51.7%	46.7%	0.207	1.000	0.341
GA	281 (267–298)	275 (241–295)	279 (266–292)	0.018	0.140	0.243
Birthweight in grams (range)	3691 (2255–4950)	3366 (1890–4385)	3550 (2646–4385)	0.010	0.190	0.180
Birthweight Z-score	0.23 (0.18)	−0.13 (0.16)	0.02 (0.21)	0.083	0.482	0.403

GA, gestational age.

The values given are %(*n*), mean (SE) and median (range). Kraskal–Wallis test was used for statistical analysis.

In the COH + ET group, the daily E_2 secretion between Weeks 5 and 11 remained rather stable (change of 0.04, −0.01 to 0.08 nmol/l per day), whereas it increased in the spontaneous pregnancy group (0.18, 0.14–0.23 nmol/l per day; $P < 0.001$) and FET group (0.12, 0.07–0.17 nmol/l per day; $P < 0.001$); the increase was higher in the spontaneous pregnancy group than in the FET group ($P < 0.03$).

The E_2 secretion between pregnancy Weeks 5 and 8 (AUC$_{5-8}$) was higher in the COH + ET group (100, 54–132 nmol/l) than in the spontaneous pregnancy group (54, 48–77 nmol/l, $P < 0.001$); in the FET group the secretion was the lowest (33, 25–47 nmol/l, $P < 0.001$). The E_2 secretion between Weeks 8 and 11 (AUC$_{8-11}$) was highest in the spontaneous pregnancy group (142, 114–177 nmol/l) and significantly lower in both the COH + ET (99, 76–144 nmol/l, $P < 0.001$) and FET groups (101, 59–136 nmol/l, $P < 0.001$). The E_2 secretion between 8 and 11 weeks was higher in all groups than between 5 and 8 weeks ($P < 0.05$).

The E_2 AUC$_{5-8}$ was negatively correlated with the birthweight ($P = 0.006$) and birthweight Z-score ($P = 0.009$; Table II), but not with the gestational age (all groups together). The E_2 AUC$_{8-11}$ did not correlate with any of those mentioned above, or with any of the ovarian parameters.

Ovarian volumes and vasculature

In the spontaneous pregnancy group and in the FTE group, the dominant ovary was significantly smaller at all times than either of the ovaries in the COH + ET group (Fig. 2, $P < 0.001$). However, the volumes of the ovaries in each group decreased significantly during the follow-up ($P < 0.001$). The median AUC (IQR) of the ovarian volume (both ovaries together in the COH + ET group, only dominant ovary with the corpus luteum in the spontaneous and FET groups) during pregnancy Weeks 5–8 was 1309 ml (861–3280) in the COH + ET group, 246 ml (180–306) in the spontaneous pregnancy group ($P < 0.001$ versus COH + ET) and 250 ml (218–319) in the FET group ($P < 0.001$ versus COH + ET). Between Weeks 8 and 11, the AUC of the dominant ovarian volume was 834 ml (527–1687) in the COH + ET group, 214 ml (177–277) ($P < 0.001$ versus COH + ET) in the spontaneous pregnancy group and 242 ml (198–296) in the FET group ($P < 0.001$ versus COH + ET).

The ovarian volumes remained significantly higher in the COH + ET group than the dominant ovarian volume in the spontaneous pregnancy group or in the FTE group at pregnancy Week 11 (Fig. 2).

As found for ovarian volume, the volume of the blood flow supplying the corpus luteal tissue was higher at all times in the COH + ET group (Fig. 2, $P < 0.001$), whereas no difference existed between the FET and spontaneous pregnancy group. The AUC of the vascularized volume between pregnancy Weeks 5 and 8 was 195 ml (119–395) in the COH + ET group, 36 ml (22–53) in the spontaneous pregnancy group ($P < 0.001$ versus COH + ET) and 37 ml (27–56) in the FET group ($P < 0.001$ versus COH + ET). Between pregnancy Weeks 8 and 11, the AUC was 133 ml (105–182) in the COH + ET group, 21 ml (12–46) in the spontaneous pregnancy group ($P < 0.001$ versus COH + ET) and 38 ml (26–44) in the FET group ($P < 0.001$ versus COH + ET).

Pregnancy outcome

To evaluate the independent factors affecting the fetal birthweight, we performed multiple linear regression analyses. Since both the

progesterone AUC_{5-8} and E_2 AUC_{5-8} correlated negatively with the birthweight, they were included in the analysis. When combined together in the analysis, neither of them was a significant factor. When only E_2 or progesterone AUC was included in the model, both were significant factors with the length of gestation and maternal height (Tables III and IV).
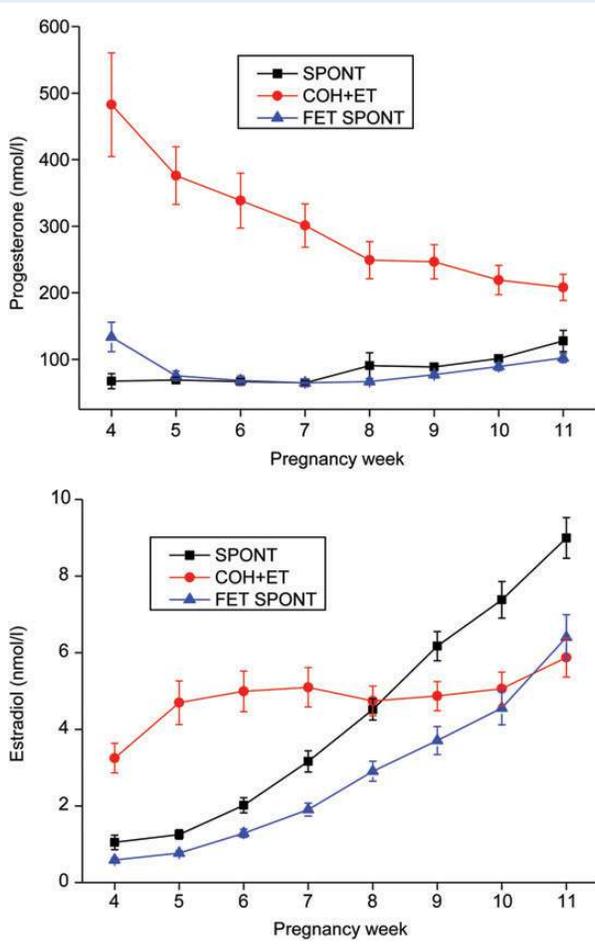

Figure 1 Mean serum progesterone and estradiol levels (error bars representing SE) from pregnancy Weeks 4–11 in three study groups. For statistical differences, see results. SPONT, spontaneous pregnancies ($n = 41$); COH + ET, controlled ovarian hyperstimulation and fresh embryo transfer ($n = 39$); FET SPONT, frozen embryo transfer in spontaneous menstrual cycle ($n = 30$).

Discussion

Our study revealed that in pregnancies after COH + ET, the luteal activity was significantly increased, which lasted up to pregnancy Week 11, whereas the FET pregnancies resembled spontaneous pregnancies.

During spontaneous pregnancy the corpus luteum is rescued by hCG from the trophoblastic tissue of the blastocyst. Corpus luteum is able to secrete sufficient steroid to produce a physiological environment for the trophoblastic invasion and placental maturation to take place. At 7–8 weeks of pregnancy the placental trophoblastic tissue is mature enough to produce the steroids required for maintenance of the pregnancy and the corpus luteum is no longer crucial. In fact, corpus luteal function is declining even before this, in spite of an increasing stimulus from the rising levels of hCG secreted from the growing placenta (Tulchinsky et al., 1972; Tulchinsky and Hobel, 1973; Järvelä et al., 2008).

No other luteal steroid is considered as essential as progesterone in maintaining pregnancy (Csapo et al., 1973a,b). Progesterone promotes endometrial decidualization, inhibits smooth muscle contractility, decreases prostaglandin formation and suppresses T-lymphocyte cell-mediated responses involved in tissue rejection (Moriyama and Sugawa, 1972; Siiteri et al., 1977). If the corpus luteum is removed during the first trimester, a pregnancy is only maintained with progesterone supplementation (Csapo et al., 1973a,b; Csapo and Pulkkinen, 1978). In spontaneous pregnancies, maternal serum progesterone levels remain quite stable up to the luteoplacental shift, and start to rise thereafter (Tulchinsky et al., 1972; Tulchinsky and Hobel, 1973; Järvelä et al., 2008).

Corpus luteum also produces other steroids, such as 17-hydroxyprogesterone, androstenedione and E_2, none of which are as essential as progesterone (Tulchinsky et al., 1972; Tulchinsky and Hobel, 1973). Clinically, the most potent of them is E_2. During the first trimester of pregnancy there is a steady rise in E_2 secretion (Tulchinsky et al., 1972; Tulchinsky and Hobel, 1973; Järvelä et al., 2008). At first, the corpus luteum is responsible for the secretion but by the end of first trimester the main origin is the placenta (Tulchinsky et al., 1972; Tulchinsky and Hobel, 1973; Järvelä et al., 2008).

Table II Spearman correlations between progesterone (P) and estradiol (E_2) secretion (AUC_{5-8} corresponding to Weeks 5–8 and AUC_{8-11} to Weeks 8–11), GA at birth, birthweight, birthweight Z-score, placental weight, and ovarian volume and vasculature at 5–8 (AUC_{5-8}) and 8–11 weeks (AUC_{8-11}) in mothers from all three groups.

	Birthweight	Birthweight Z-score	GA at birth	Ovarian volume, AUC_{5-8}	Ovarian volume, AUC_{8-11}	Ovarian vasculature, AUC_{5-8}	Ovarian vasculature, AUC_{8-11}
P AUC_{5-8}, R (P)	−0.340 (0.002)	−0.313 (0.005)	−0.163 (0.150)	0.769 (<0.001)	0.741 (<0.001)	0.806 (<0.001)	0.758 (<0.001)
P AUC_{8-11}, R (P)	−0.198 (0.072)	−0.170 (0.124)	−0.113 (0.308)	0.659 (<0.001)	0.651 (<0.001)	0.784 (<0.001)	0.698 (<0.001)
E_2 AUC_{5-8}, R (P)	−0.275 (0.014)	−0.252 (0.025)	−0.109 (0.338)	0.580 (<0.001)	0.614 (<0.001)	0.592 (<0.001)	0.607 (<0.001)
E_2 AUC_{8-11}, R (P)	−0.018 (0.867)	−0.063 (0.564)	0.129 (0.240)	0.056 (0.651)	0.036 (0.755)	0.079 (0.552)	0.001 (0.993)
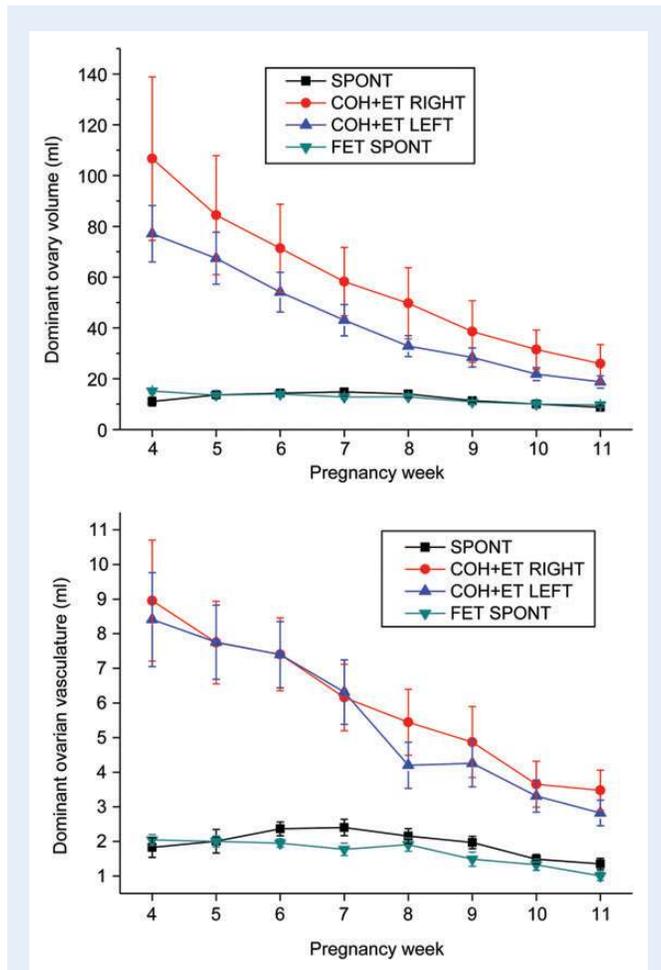
Figure 2 Mean ovarian volume and vasculature (error bars representing SE) from pregnancy Weeks 4–11 in three study groups. For statistical differences, see results. In COH + ET, the stimulated ovaries are presented separately; in SPONT and FET SPONT, only the dominant ovary is presented.

Progesterone

In the current study, pregnancies after FET were similar to the spontaneous pregnancies, and the progesterone levels did not differ from those in the spontaneous pregnancies, as expected, since in both of them there was only one single corpus luteum. On the contrary, in the COH + ET pregnancy group, the progesterone levels were five to six times higher at pregnancy Week 5. This was expected, since the COH resulted in several corpora lutea, which were activated by hCG. After the first measurements, there was a constant decline in progesterone levels, but at the 11th week the levels were still higher than in the spontaneous or FET pregnancy groups. No specific progesterone rise, reflecting a luteoplacental shift, could be detected in the COH + ET pregnancies during the first trimester of pregnancy.

As far as we know, this is the first study to evaluate progesterone secretion in FET pregnancies. Only two previous studies (Johnson *et al.*, 1994; Costea *et al.*, 2000) have reported the progesterone secretion following COH + ET. Johnson *et al.* (1994) compared spontaneous singleton pregnancies with COH + ET pregnancies stimulated with

Table III Model 1: Multivariate analysis of factors affecting birthweight.

Factors	B	P	95% Confidence interval for B	
			Lower bound	Upper bound
GA at delivery	26.307	<0.001	16.634	35.980
Maternal height	25.017	0.006	7.626	42.408
Maternal BMI	15.825	0.284	−13.467	45.117
Maternal age	−0.041	0.154	−33.311	6.975
P AUC$_{5-8}$	−0.33	0.015	−0.060	−0.007
Gender				
Female				
Male	262.013	0.164	−110.190	634.216
Previous delivery				
Yes	154.723	0.332	−161.755	471.202
No				

Progesterone secretion at 5–8 weeks of pregnancy (P AUC$_{5-8}$) is considered to reflect luteal activity.

Table IV Model 2: Multivariate analysis of factors affecting birthweight.

Factors	B	P	95% Confidence interval for B	
			Lower bound	Upper bound
GA at delivery	25.761	<0.001	16.164	35.359
Maternal height	26.358	0.005	8.468	44.248
Maternal BMI	16.042	0.272	−12.925	45.008
Maternal age	−0.055	0.053	−0.111	0.001
E$_2$ AUC$_{5-8}$	−2.584	0.039	−5.035	−0.133
Gender				
Female				
Male	211.399	0.263	−162.796	585.594
Previous delivery				
Yes	150.173	0.347	−166.590	466.936
No				

Estradiol secretion at 5–8 weeks of pregnancy (E$_2$ AUC$_{5-8}$) is considered to reflect luteal activity.

either clomiphene citrate–hMG or buserelin–hMG combinations. They also observed that the progesterone concentrations were higher in the COH + ET pregnancies up to pregnancy Weeks 11–12, and thereafter the difference disappeared (Johnson *et al.*, 1994). Costea *et al.* (2000) observed that the progesterone levels were higher up to eight pregnancy weeks after COH + ET (Costea *et al.*, 2000).

Earlier studies have attempted to evaluate the effect of COH on pregnancy outcome, and the number of oocytes retrieved has been considered to be a sign of luteal activity (Griesinger *et al.*, 2008; Shih *et al.*, 2008). However, no connection between the number of oocytes and the incidence of low-birthweight or preterm birth has been observed

(Griesinger et al., 2008; Shih et al., 2008). Since the main progesterone source during early pregnancy is the corpus luteal tissue, progesterone may be considered to be a sign of luteal activity following COH. Here, we wanted to evaluate which parameters correlated with the early pregnancy progesterone levels. No correlation was observed between the number of oocytes retrieved and the progesterone levels, suggesting that the number of oocytes retrieved cannot be considered to be an indicator of ovarian hyperstimulation or luteal activity during the first trimester of pregnancy. We observed a strong correlation between the progesterone levels and ovarian size, and the amount of intraovarian vasculature, especially during pregnancy Weeks 5–8, when the luteoplacental shift has not yet taken place and the ovarian luteal tissue is responsible for maintaining the pregnancy by secreting progesterone (Csapo et al., 1973a,b; Csapo and Pulkkinen, 1978). Therefore, it appears that if the connection between COH-induced increased luteal activity and the obstetric outcome must be evaluated as a sign of luteal activity, one should evaluate the steroid levels or the ovarian size or vascularity, rather than the number of oocytes retrieved.

According to an Internet-based survey (Vaisbuch et al., 2012), most IVF-doctors continue the luteal phase support up to gestational Week 8 or longer. The practice is difficult to justify since we have shown here that the progesterone levels following COH–ET are significantly higher, at least up to pregnancy Week 11, than in spontaneous pregnancies. A recent review of luteal phase support duration suggested also that supplementation beyond the first positive pregnancy test might be unnecessary (Liu et al., 2012).

Estradiol

The role of E_2 during human pregnancy is still poorly understood. High E_2 levels on the day of hCG administration during COH are associated with an increased risk for small for gestational age and preeclampsia, which can be overcome by freezing all of the embryos and transferring them after thawing in an unstimulated cycle (Imudia et al., 2012, 2013).

It has been proposed that estrogens may augment uterine blood flow during pregnancy (Resnik et al., 1974), which has been confirmed in baboons (Albrecht et al., 2006; Aberdeen et al., 2012; Bonagura et al., 2012). During the first trimester of a baboon pregnancy, low levels of estrogen are required to permit the normal progression of trophoblastic vascular invasion (Albrecht et al., 2006). During the second trimester, there is a surge in estrogen synthesis, which is believed to play a physiological role in suppressing further trophoblastic invasion (Albrecht et al., 2006). When the E_2 levels during the first trimester of a baboon pregnancy are artificially elevated, the trophoblastic invasion and remodelling of the uterine spiral arteries are considerably diminished (Bonagura et al., 2012), leading to disruption in the uteroplacental blood flow (Aberdeen et al., 2012). Therefore, abnormally high early pregnancy E_2 levels could prevent the normal trophoblastic invasion of the decidual and myometrial spiral arteries and cause abnormal placentation, which is believed to restrict fetal growth during the last period of pregnancy (Khong et al., 1986; Labarrere and Althabe, 1987; Pijnenborg et al., 1991).

In the present study, COH + ET resulted in considerably higher E_2 levels during early pregnancy when compared with the spontaneous or FET pregnancies. A similar finding was published by Johnson et al. (1994), who discovered high E_2 levels after FSH stimulation combined with either clomiphene citrate or a GnRH agonist (Johnson et al., 1994). It is very likely that the rescued luteal tissue in the stimulated ovaries was responsible for the increased E_2 production between pregnancy Weeks 5 and 8 (Johnson et al., 1994).

Between pregnancy Weeks 8 and 11, serum E_2 was lower in both the COH and FET groups in comparison with spontaneous pregnancies. At this phase of pregnancy, the luteoplacental shift has already taken place, suggesting that placental E_2 synthesis might be impaired. Earlier studies measuring estriol for second trimester. Down syndrome screening have also observed lower levels in pregnancies achieved by IVF (Barkai et al., 1996; Frishman et al., 1997; Wald et al., 1999).

Because the E_2 levels were lower at all times in the FET than in the spontaneous pregnancies, COH cannot be the cause. The FET pregnancies differed from the spontaneous pregnancies in that the embryos were cultured in the laboratory environment before freezing, and also after thawing. Some of the earlier studies have shown that the type of culture media (Dumoulin et al., 2010; Nelissen et al., 2012) as well as the duration of the culture period (Makinen et al., 2013) may affect the birthweight and perinatal outcome of the newborn, reflecting changes in the fetoplacental function and growth. In addition, the FET patients received luteal support for 2 weeks following the embryo transfer, which may also have had an impact.

The clinical significance of low E_2 levels after FET remains unclear. As mentioned earlier, a low first trimester E_2 level is a prerequisite to permit normal progression of the trophoblastic vascular invasion in baboons (Albrecht et al., 2006; Aberdeen et al., 2012; Bonagura et al., 2012). If low levels of E_2 are required for normal trophoblastic invasion in human pregnancies as well, then the low levels of E_2 observed after FET may not be clinically so harmful as the higher levels observed after COH + ET.

Fetal birthweight

Since both progesterone and E_2 concentrations during pregnancy Weeks 5–8 correlated negatively with the birthweight, we performed multivariate analyses of factors independently affecting the weight of the newborn. In the first model, the factors included the GA at birth, maternal height and progesterone concentration during pregnancy Weeks 5–8. In the second model, the independent factors were GA at birth, maternal height and E_2 concentration during pregnancy Weeks 5–8. It appears that increased luteal activity during early pregnancy may be related to fetoplacental growth during later pregnancy.

During the first 4 weeks after fertilization (pregnancy Weeks 2–6) embryonal blastogenesis takes place (Opitz, 1993; Halliday et al., 2010). Disorders in blastogenesis affect the formation of the midline and mesoderm, causing defects of fusion, lateralization, segmentation, morphogenic movement and asymmetry (Opitz, 1993). Recently, a study comparing birth defects after assisted reproduction technology observed that blastogenic birth defects were markedly increased after COH + ET, but not after FET (Halliday et al., 2010). According to the present findings, during this period the steroid secretion following COH was most prominently elevated when compared with the spontaneous and FET pregnancies. To compare the defects in blastogenesis between the groups would have required a considerably higher number of patients and, therefore, defects in blastogenesis were not selected as an outcome in this study.

The major limitation of this study was that the number of patients per group groups was small and, therefore, minor differences between them could not been detected. In order to confirm the findings, larger cohort studies are required.

Conclusion

COH resulted in increased luteal activity up to pregnancy Week 11, being most apparent up to Week 8. The maternal steroid profile during early pregnancy after COH may be considered to be unphysiologically high. Pregnancies following FET during a spontaneous menstrual cycle closely resembled the spontaneous pregnancies, and seemed to provide a more physiological environment for early fetal development than COH–ET. If fresh embryo transfer is to be used, we propose that milder ovarian stimulation protocols should be considered.

Authors' roles

This study has been designed and executed by I.Y.J. All of the authors have participated in drafting the manuscript and in critical discussions.

Funding

Conflict of interest

The authors declare no conflicts of interest.

References

Aberdeen GW, Bonagura TW, Harman CR, Pepe GJ, Albrecht ED. Suppression of trophoblast uterine spiral artery remodeling by estrogen during baboon pregnancy: impact on uterine and fetal blood flow dynamics. *Am J Physiol Heart Circ Physiol* 2012;**302**:H1936–H1944.

Albrecht ED, Bonagura TW, Burleigh DW, Enders AC, Aberdeen GW, Pepe GJ. Suppression of extravillous trophoblast invasion of uterine spiral arteries by estrogen during early baboon pregnancy. *Placenta* 2006;**27**:483–490.

Barkai G, Goldman B, Ries L, Chaki R, Dor J, Cuckle H. Down's syndrome screening marker levels following assisted reproduction. *Prenat Diagn* 1996;**16**:1111–1114.

Bonagura TW, Babischkin JS, Aberdeen GW, Pepe GJ, Albrecht ED. Prematurely elevating estradiol in early baboon pregnancy suppresses uterine artery remodeling and expression of extravillous placental vascular endothelial growth factor and alpha1beta1 and alpha5beta1 integrins. *Endocrinology* 2012;**153**:2897–2906.

Chung K, Coutifaris C, Chalian R, Lin K, Ratcliffe SJ, Castelbaum AJ, Freedman MF, Barnhart KT. Factors influencing adverse perinatal outcomes in pregnancies achieved through use of in vitro fertilization. *Fertil Steril* 2006;**86**:1634–1641.

Costea DM, Gunn LK, Hargreaves C, Howell RJ, Chard T. Delayed luteo-placental shift of progesterone production in IVF pregnancy. *Int J Gynaecol Obstet* 2000;**68**:123–129.

Csapo AI, Pulkkinen M. Indispensability of the human corpus luteum in the maintenance of early pregnancy. Luteectomy evidence. *Obstet Gynecol Surv* 1978;**33**:69–81.

Csapo AI, Pulkkinen MO, Kaihola HL. The effect of estradiol replacement therapy on early pregnant luteectomized patients. *Am J Obstet Gynecol* 1973a;**117**:987–990.

Csapo AI, Pulkkinen MO, Wiest WG. Effects of luteectomy and progesterone replacement therapy in early pregnant patients. *Am J Obstet Gynecol* 1973b;**115**:759–765.

De Geyter C, De Geyter M, Steimann S, Zhang H, Holzgreve W. Comparative birth weights of singletons born after assisted reproduction and natural conception in previously infertile women. *Hum Reprod* 2006;**21**:705–712.

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.

Frishman GN, Canick JA, Hogan JW, Hackett RJ, Kellner LH, Saller DN Jr. Serum triple-marker screening in in vitro fertilization and naturally conceived pregnancies. *Obstet Gynecol* 1997;**90**:98–101.

Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: a registry study. *Hum Reprod* 2008;**23**:2549–2554.

Halliday JL, Ukoumunne OC, Baker HW, Breheny S, Jaques AM, Garrett C, Healy D, Amor D. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. *Hum Reprod* 2010;**25**:59–65.

Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med* 2002;**346**:725–730.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *Br Med J* 2004;**328**:261.

Hyden-Granskog C, Unkila-Kallio L, Halttunen M, Tiitinen A. Single embryo transfer is an option in frozen embryo transfer. *Hum Reprod* 2005;**20**:2935–2938.

Imudia AN, Awonuga AO, Doyle JO, Kaimal AJ, Wright DL, Toth TL, Styer AK. Peak serum estradiol level during controlled ovarian hyperstimulation is associated with increased risk of small for gestational age and preeclampsia in singleton pregnancies after in vitro fertilization. *Fertil Steril* 2012;**97**:1374–1379.

Imudia AN, Awonuga AO, Kaimal AJ, Wright DL, Styer AK, Toth TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: a preliminary study. *Fertil Steril* 2013;**99**:168–173.

Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;**103**:551–563.

Järvelä IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nargund G. Quantification of ovarian power Doppler signal with three-dimensional ultrasonography to predict response during in vitro fertilization. *Obstet Gynecol* 2003;**102**:816–822.

Järvelä IY, Niinimäki M, Martikainen H, Ruokonen A, Tapanainen JS. Ovarian response to the human chorionic gonadotrophin stimulation test in normal ovulatory women: the impact of regressing corpus luteum. *Fertil Steril* 2007;**87**:1122–1130.

Järvelä IY, Ruokonen A, Tekay A. Effect of rising hCG levels on the human corpus luteum during early pregnancy. *Hum Reprod* 2008;**23**:2775–2781.

Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. *Hum Reprod Update* 2006;**12**:747–755.

Johnson MR, Abbas AA, Irvine R, Riddle AF, Norman-Taylor JQ, Grudzinskas JG, Collins WP, Nicolaides KH. Regulation of corpus luteum function. *Hum Reprod* 1994;**9**:41–48.

Kalra SK, Ratcliffe SJ, Coutifaris C, Molinaro T, Barnhart KT. Ovarian stimulation and low birth weight in newborns conceived through in vitro fertilization. *Obstet Gynecol* 2011;**118**:863–871.

Kansal Kalra S, Ratcliffe SJ, Milman L, Gracia CR, Coutifaris C, Barnhart KT. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertil Steril* 2011;**95**:548–553.

Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol* 1986;**93**:1049–1059.

Labarrere CA, Althabe OH. Inadequate maternal vascular response to placentation in pregnancies complicated by preeclampsia and by small-for-gestational-age infants. *Br J Obstet Gynaecol* 1987; **94**:1113–1116.

Liu XR, Mu HQ, Shi Q, Xiao XQ, Qi HB. The optimal duration of progesterone supplementation in pregnant women after IVF/ICSI: a meta-analysis. *Reprod Biol Endocrinol* 2012;**10**.107-7827-10-107.

Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2012;**98**:368–377.e1–9.

Makinen S, Soderstrom-Anttila V, Vainio J, Suikkari AM, Tuuri T. Does long in vitro culture promote large for gestational age babies? *Hum Reprod* 2013; **28**:828–834.

Martikainen H, Tiitinen A, Tomas C, Tapanainen J, Orava M, Tuomivaara L, Vilska S, Hyden-Granskog C, Hovatta O. One versus two embryo transfer after IVF and ICSI: a randomized study. *Hum Reprod* 2001; **16**:1900–1903.

Moriyama I, Sugawa T. Progesterone facilitates implantation of xenogenic cultured cells in hamster uterus. *Nat New Biol* 1972;**236**:150–152.

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.

Ombelet W, Martens G, De Sutter P, Gerris J, Bosmans E, Ruyssinck G, Defoort P, Molenberghs G, Gyselaers W. Perinatal outcome of 12,021 singleton and 3108 twin births after non-IVF-assisted reproduction: a cohort study. *Hum Reprod* 2006;**21**:1025–1032.

Opitz JM. Blastogenesis and the 'primary field' in human development. *Birth Defects Orig Artic Ser* 1993;**29**:3–37.

Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* 2012; **18**:485–503.

Pelinck MJ, Hadders-Algra M, Haadsma ML, Nijhuis WL, Kiewiet SM, Hoek A, Heineman MJ, Middelburg KJ. Is the birthweight of singletons born after IVF reduced by ovarian stimulation or by IVF laboratory procedures? *Reprod Biomed Online* 2010a;**21**:245–251.

Pelinck MJ, Keizer MH, Hoek A, Simons AH, Schelling K, Middelburg K, Heineman MJ. Perinatal outcome in singletons after modified natural cycle IVF and standard IVF with ovarian stimulation. *Eur J Obstet Gynecol Reprod Biol* 2010b;**148**:56–61.

Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, Martikainen H, Tiitinen A, Hartikainen AL. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. *Hum Reprod* 2010;**25**:914–923.

Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, van Assche A. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol* 1991;**98**:648–655.

Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. *Fertil Steril* 2010; **94**:1320–1327.

Pinborg A, Henningsen AK, Malchau SS, Loft A. Congenital anomalies after assisted reproductive technology. *Fertil Steril* 2013;**99**:327–332.

Resnik R, Killam AP, Battaglia FC, Makowski EL, Meschia G. The stimulation of uterine blood flow by various estrogens. *Endocrinology* 1974; **94**:1192–1196.

Romundstad LB, Romundstad PR, Sunde A, von During V, Skjaerven R, Gunnell D, Vatten LJ. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. *Lancet* 2008;**372**:737–743.

Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012;**27**:1343–1350.

Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstet Gynecol* 2004; **103**:1144–1153.

Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, Baker HW. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum Reprod* 2008;**23**:1644–1653.

Siiteri PK, Febres F, Clemens LE, Chang RJ, Gondos B, Stites D. Progesterone and maintenance of pregnancy: is progesterone nature's immunosuppressant? *Ann N Y Acad Sci* 1977;**286**:384–397.

Thomson F, Shanbhag S, Templeton A, Bhattacharya S. Obstetric outcome in women with subfertility. *BJOG* 2005;**112**:632–637.

Tulchinsky D, Hobel CJ. Plasma human chorionic gonadotropin, estrone, estradiol, estriol, progesterone, and 17 alpha-hydroxyprogesterone in human pregnancy. 3. Early normal pregnancy. *Am J Obstet Gynecol* 1973; **117**:884–893.

Tulchinsky D, Hobel CJ, Yeager E, Marshall JR. Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *Am J Obstet Gynecol* 1972; **112**:1095–1100.

Tunon K, Eik-Nes SH, Grottum P, Von During V, Kahn JA. Gestational age in pregnancies conceived after in vitro fertilization: a comparison between age assessed from oocyte retrieval, crown-rump length and biparietal diameter. *Ultrasound Obstet Gynecol* 2000;**15**:41–46.

Vaisbuch E, Leong M, Shoham Z. Progesterone support in IVF: is evidence-based medicine translated to clinical practice? A worldwide web-based survey. *Reprod Biomed Online* 2012;**25**:139–145.

Wald NJ, White N, Morris JK, Huttly WJ, Canick JA. Serum markers for Down's syndrome in women who have had in vitro fertilisation: implications for antenatal screening. *Br J Obstet Gynaecol* 1999; **106**:1304–1306.

Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, Selbing A, Loft A. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod* 2009;**24**:2158–2172.

Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A. Perinatal outcomes of children born after frozen–thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. *Hum Reprod* 2013; **28**:2545–2553.

Wright D, Spencer K, Kagan KK, Torring N, Petersen OB, Christou A, Kallikas J, Nicolaides KH. First-trimester combined screening for trisomy 21 at 7–14 weeks' gestation. *Ultrasound Obstet Gynecol* 2010; **36**:404–411.