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Basal serum levels of FSH and estradiol in ovulatory and anovulatory women undergoing treatment by in-vitro maturation of immature oocytes

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BACKGROUND: The study aim was to establish whether basal serum levels of FSH and estradiol are predictive of outcome in women undergoing treatment by in-vitro maturation (IVM) of immature oocytes. METHODS: Data were obtained from 123 unstimulated IVM cycles. Serum was taken between cycle days 2–4 for analysis. Patients received 10 000 IU of HCG 36 h before immature oocyte recovery that was performed between cycle days 9–14. IVM was performed and mature oocytes fertilized by ICSI, followed 2–3 days later by embryo transfer. Outcome measures included the number of immature oocytes retrieved, and the rates of oocyte maturation, fertilization, cleavage and pregnancy. RESULTS: A median (range) of 8 (0–36) immature oocytes was retrieved per patient. Oocyte maturation, fertilization, cleavage and pregnancy rates were 83, 76, 93 and 17.9% respectively. Serum FSH levels and the presence of polycystic ovary were significant independent predictors of the number of immature oocytes retrieved, whilst patient age and basal estradiol level were not. A basal serum estradiol level >100 pmol/l was associated with a significantly higher pregnancy rate (26 versus 11% for estradiol <100 pmol/l; $P = 0.032$). CONCLUSIONS: Measurement of basal serum levels of FSH and estradiol are useful in predicting the number of immature oocytes retrieved and the pregnancy rate in women undergoing unstimulated IVM treatment.

Keywords: estradiol/FSH/in-vitro maturation/oocytes/predictive variables

Introduction

The in-vitro maturation (IVM) of immature oocytes retrieved from unstimulated ovaries is an assisted conception technology of increasing interest. Treatment by IVM has many advantages over routine stimulated IVF. These are mainly due to the absence of ovarian stimulation in IVM as gonadotrophins need neither be purchased nor administered. Consequently, IVM treatment is associated with increased patient acceptability, reduced cost and fewer potential side effects, in particular the risk of developing ovarian hyperstimulation syndrome.

The pregnancy rate in IVF is related to the numbers of oocytes retrieved and numbers of embryos available for transfer (Templeton and Morris, 1998). As ovarian reserve decreases, the follicular response to gonadotrophin stimulation also reduces, and fewer oocytes are retrieved. A number of studies have attempted to assess ovarian reserve and therefore predict follicular response prior to commencing IVF treatment. Early follicular phase serum concentrations of FSH and estradiol are generally recognized as markers for ovarian reserve, albeit not entirely satisfactory ones. Raised serum levels of FSH (Scott *et al*., 1989) or estradiol (Licciardi *et al*., 1995) are predictive of a poor response to ovarian stimulation and consequently a reduced pregnancy rate in IVF. Other predictors of ovarian response include baseline ultrasound measurement of the ovarian volume (Syrop *et al*., 1995), the ovarian antral follicle count (Tomas *et al*., 1997), and the maximum velocity of ovarian stromal blood flow measured with colour and pulsed Doppler (Zaidi *et al*., 1996; Engmann *et al*., 1999).

Pretreatment predictors of success in IVM treatment remain to be fully defined, however. It has been shown that, as for IVF, the pregnancy rate in IVM is related to the number of immature oocytes retrieved (Child *et al*., 2001a) and embryos produced (Child *et al.*, 2002). In addition, the number of immature oocytes retrieved from unstimulated ovaries may be predicted by pretreatment assessment of the antral follicle count, ovarian volume and maximum stromal blood flow velocity (Child *et al*., 2001a). The IVM pregnancy rate is higher in women with polycystic ovaries (PCO) since, by definition, they have more antral follicles than do women with normal ovaries (Child *et al*., 2001b).

It remains to be established whether early follicular phase serum levels of FSH and estradiol are related to outcome in IVM treatment. In a recent study conducted in regularly menstruating women with normal ovaries undergoing IVM, it was determined whether the number of retrieved oocytes, maturation and cleavage rates could be predicted by the

Table I. Patient demographics of 107 women undergoing 123 in-vitro maturation aspirations

Parameter	Median (range)
Age (years)	34 (18–42)
Duration of infertility (years)	4 (1–18)
Births (*n*)	0 (0–2)
Previous IVF cycles (*n*)	0 (0–6)
Previous ovulation induction cycles (*n*)	3 (0–20)
Serum FSH level, days 2–4 (IU/l)	5.7 (2.2–16.2)
Serum estradiol level, days 2–4 (pmol/l)	91 (37–266)
Primary diagnosis [*n* (%)]	
Unexplained	53 (43)
Male factor	42 (34)
Endometriosis	8 (7)
Tubal factor	18 (15)
Genetic disease	2 (2)
Ovarian morphology and ovulatory status [*n* (%)]	
Normal ovaries-ovulatory cycles	34 (28)
PCO-ovulatory cycles	45 (37)
PCOS-anovulatory	44 (36)

measurement of day 3–4 levels of serum FSH, estradiol and inhibin, and the antral follicle count (Mikkelsen *et al*., 2001). These authors found that a low serum level of estradiol (<200 pmol/l) was associated with a higher pregnancy rate (14%) than when the level was >200 pmol/l (0%). However, this study excluded women with PCO, and the treatment protocol did not include HCG priming prior to immature oocyte retrieval. The administration of HCG 10 000 IU 36 h prior to oocyte retrieval increases the maturation rate and final number of metaphase II stage oocytes in women undergoing IVM treatment (Chian *et al*., 1999, 2000).

The aim of the present study was to investigate whether the measurement of basal serum levels of FSH and estradiol in women with normal or polycystic ovaries undergoing IVM treatment predicts the number of immature oocytes retrieved, and the rates of oocyte maturation, fertilization, cleavage and pregnancy. If so, the ability to select women who will benefit most from IVM therapy would be improved, as would the ability to counsel women with regard to their prognosis.

Materials and methods

Data for the study were obtained from 107 women undergoing 123 immature oocyte retrieval cycles. Ninety-three women underwent one IVM aspiration, 12 underwent two, and two patients had three aspiration cycles. Demographics of the study population are shown in Table I. For the purposes of the study women were allocated to one of three groups before treatment depending on the presence or absence of ovaries of polycystic morphology, and whether or not there were features of polycystic ovarian syndrome (PCOS). An ovary was defined as polycystic when more than 10 antral follicles were visualized spread around or scattered through an enlarged echodense ovarian stroma on early follicular phase transvaginal ultrasound scan (Adams *et al*., 1986). Whilst some women had regular ovulatory cycles in the presence of ovaries of polycystic morphology (ovulatory PCO), others had PCOS, which was defined as polycystic ovaries on ultrasound along with chronic anovulation and/or clinical or biochemical evidence of hyperandrogenism. Thirty-four cycles were for women with normal ovaries and regular ovulatory cycles, 45 for women with ovulatory PCO, and 44 for patients with anovulatory PCOS.

Assays

Immunoreactive FSH and estradiol concentrations were determined using the ACS:180 assay run on an autoanalyser (Advia Centaur, Bayer). The detection limit for FSH was 0.3 mIU/l and the inter-assay coefficients of variation (CV) were 4.6% at a mean concentration of 4.3 mIU/l and 4.5% at a mean concentration of 20.9 mIU/l. The detection limit for estradiol was 37 pmol/l, and the inter-assay CV 9.8% at a mean concentration of 250 pmol/l.

IVM cycle

Women with amenorrhoea received vaginal progesterone (Prometrium; Schering, Pointe-Claire, Quebec, Canada) 300 mg once daily for 10 days in order to induce a withdrawal bleed. All women underwent a baseline ultrasound scan on day 2–4 of menstrual bleeding to ensure that no ovarian cysts were present. Serum was taken for measurement of FSH and estradiol concentrations. The results of these tests did not affect the management of the treatment cycle. Transvaginal ultrasound scans were repeated on either cycle day 8 or the day of HCG administration to exclude the development of a dominant follicle. All follicles had to be <10 mm diameter in order to proceed to oocyte retrieval, which was performed between days 9–14. Women with ovulatory cycles had their immature oocyte retrieval planned between days 9 and 11 to reduce the chance of observing a dominant follicle >10 mm in diameter at the ultrasound scan performed on the day of HCG (2 days before oocyte retrieval). Data suggested that the presence of a dominant follicle at the time of immature oocyte retrieval was deleterious to outcome in IVM (Trounson *et al*., 1998; Cobo *et al*., 1999).

All patients received 10 000 IU HCG (Profasi; Serono, Oakville, Ontario, Canada) s.c. 36 h before oocyte retrieval. It had been shown previously, in a randomized controlled trial, that HCG priming increased the percentage of oocytes that mature by 24 and 48 h of in-vitro culture (Chian *et al*., 1999, 2000). Transvaginal ultrasound-guided oocyte collection was performed using a specially designed 17G single-lumen aspiration needle (K-OPS-1235-Wood, Cook, Australia) with a reduced aspiration pressure of 7.5 kPa. Aspiration of all small follicles was performed using either spinal anaesthesia or, more recently, intravenous fentanyl and midazolam with a paracervical block of 10 ml of 1% lidocaine. Follicular flushing was not performed.

The oocyte IVM technique used in this study has been reported in detail previously (Chian *et al*., 2000). Oocytes were collected in culture tubes containing warm 0.9% saline with 2 IU/ml heparin. The oocytes were evaluated for the presence or not of a germinal vesicle (GV) in the cytoplasm of the oocyte, after which the immature oocytes were transferred into maturation medium for culture. The immature oocytes were incubated in culture dishes containing 1 ml of maturation medium, TC-199 medium supplemented with 20% heat-inactivated maternal serum, 0.25 mmol/l pyruvic acid (Sigma Chemical Co.), penicillin 50 mg/ml, streptomycin 75 mg/ml, 75 mIU/ml each of FSH and LH (Humegon; Organon; Scarborough, Ontario, Canada) at 37°C in an atmosphere of 5% CO_2 and 95% air with high humidity. Following culture, the maturity of the oocytes was determined by microscopic examination at 24 h and 48 h. Oocytes that were mature at the time of checking were denuded of cumulus cells ready for ICSI. A single spermatozoon was injected into each metaphase II oocyte. Following ICSI, each oocyte was transferred into a 20 µl micro droplet of G 1.2 medium (Vitrolife). Fertilization was assessed 18 h after ICSI for the appearance of two distinct pronuclei and two polar bodies.

Embryos were transferred on day 2 or 3 after ICSI. As the oocytes

were not matured and inseminated at the same time following maturation in culture, the developmental stages of embryos at the time of embryo transfer were often variable. Before transfer, all embryos for each patient were pooled and selected for transfer based on standard embryological criteria such as cleavage stage and morphological quality (Steer *et al*., 1992; Child *et al*., 2002). The Cumulative Embryo Score (CES) at each embryo transfer procedure was calculated as follows. On the day of transfer the embryos were scored as: grade 4, equal-sized symmetrical blastomeres; grade 3, uneven blastomeres with <10% fragmentation; grade 2, 10–50% blastomeric fragmentation; and grade 1, >50% blastomeric fragmentation. The grade of each embryo was multiplied by the number of blastomeres to produce a quality score of each embryo. The scores of all embryos transferred per patient were added to obtain the CES (Steer *et al*., 1992).

For endometrial preparation, patients received estradiol valerate (Estrace; Roberts Pharmaceutical, Mississauga, Canada), starting on the day of oocyte retrieval, depending on the endometrial thickness on that day. If the endometrial thickness was <6 mm, a 10 mg dose was given; if it was >6 mm, a 6 mg dose was administered. If the endometrial thickness was <7 mm on the day of embryo transfer, the patient was recommended to cryopreserve all embryos for replacement in a later cycle. Luteal support was provided by 200 mg intravaginal progesterone (Prometrium) twice daily starting on the day of ICSI and continued, along with estradiol, until 12 weeks of gestation.

Statistical analysis

Multiple linear regression analysis was used to assess the predictive values of the independent variables age, serum FSH, and estradiol concentration on the dependent variable number of oocytes retrieved. Included in the regression model, as an independent variable, was the presence or absence of ovaries of polycystic morphology. The number of oocytes retrieved was square-root transformed in order to satisfy the requirements of linear regression analysis.

Differences in the rates of maturation, fertilization, cleavage and pregnancy were analysed with Fisher's exact test or χ^2 test as appropriate. Unpaired data were compared using the non-parametric Mann–Whitney *U*-test. The comparison of basal serum estradiol levels between women with normal ovaries, ovulatory PCO or anovulatory PCOS was performed using one-way ANOVA after data normalization. SPSS (version 9.0) was used for all statistical analyses.

Results

A total of 123 cycles performed in 93 patients was included for analysis. A total of 1136 [8 (0–36); median (range) per aspiration] oocytes were retrieved, of which 1032 were viable and cultured *in vitro*. By 48 h of culture, 858 (83%) oocytes had matured to metaphase II stage and underwent ICSI. Consequently, 652 fertilized with two pronuclei (76%) and 606 cleaved (93%). There were 118 embryo transfer procedures of a median (range) 3 (1–5) embryos per transfer. Twenty-two pregnancies were obtained (17.9% per oocyte retrieval and 18.6% per embryo transfer procedure), and 16 healthy babies were born including one set of triplets and two sets of twins. No congenital malformations were reported. The pregnancy rate in women with PCO (ovulatory and anovulatory) was 22.5% (20/89) compared with 5.9% (2/34) in women with normal ovaries ($P < 0.05$).

Multiple linear regression analysis, controlling for patient age and the presence of PCO, was performed (Table II). With univariate analysis, basal FSH level, patient age and the presence of PCO were all significant predictors of the number of immature oocytes retrieved. However, after controlling for each of the other factors, only basal FSH and presence of PCO were significant independent predictors ($P < 0.001$).

Table II. Multiple linear regression analysis of predictive value of basal serum FSH and estradiol, female age, and presence of PCO on numbers of oocytes retrieved in 123 unstimulated IVM cycles

Variable	*P* (Model 1)[a]	*P* (Model 2)[b]
Serum FSH day 2–4	<0.001	<0.001
Serum estradiol day 2–4	0.385 (NS)	–
Female age	0.010	0.367 (NS)
Polycystic ovaries	<0.001	<0.001

[a]Based on univariate analysis.
[b]Based on analysis adjusted for all other variables in the model.
Regression equation: [α(no. of immature oocytes retrieved) = 2.673 + 0.410×Ovaries – 0.285×FSH]. If the ovaries are normal, then the value of Ovaries = 1; if the ovaries are polycystic, then Ovaries = 2.
NS = not significant.

Different estradiol thresholds were explored to examine whether there were differences in IVM outcome between cycles with low or high estradiol values. There was a significantly increased pregnancy rate in the high estradiol level group when thresholds of 100 pmol/l ($P = 0.032$) or 125 pmol/l ($P = 0.025$) were used, and a borderline increase when a 150 pmol/l ($P = 0.06$) threshold was applied. No difference between the two groups was found with a threshold estradiol value of 200 pmol/l. The 100 pmol/l threshold was associated with the best performance as a pretreatment predictor of pregnancy. The sensitivity and specificity for pregnancy was 64 and 61%, with positive and negative predictive values of 26 and 78% respectively. For the 100 pmol/l threshold, there were no differences in patient age or numbers of oocytes retrieved or matured, or embryos produced or transferred (Table III). In particular, the CES was comparable between the groups. However, the endometrial thickness on the day of embryo transfer was significantly greater (median of 9.6 mm versus 8.7 mm) in the high-estradiol group ($P = 0.035$). Endometrial thickness on the day of oocyte recovery was available in 93 cycles. The median (range) thickness in the low- and high-estradiol groups was 6.7 (4–10) and 6.7 (4–12) mm respectively (P = NS). There were no differences in the rates of oocyte maturation, fertilization or cleavage between the low- and high-estradiol groups (data not shown).

When the same estradiol threshold of 100 pmol/l was applied solely to the 89 cycles performed for women with PCO (including ovulatory PCO and anovulatory PCOS), the test performance improved somewhat: sensitivity 70%, specificity 58%, positive and negative predictive values 33 and 87% respectively. The pregnancy rate for women with PCO and low (<100 pmol/l) estradiol levels was 13% (6/46) compared with 33% (14/43) in the high (≥100 pmol/l) estradiol group ($P = 0.028$). There were only two pregnancies among the 34 cycles performed for women with normal ovaries. Meaningful

Table III. In-vitro maturation outcome according to serum estradiol level on day 2–4 in 123 cycles for women with normal or polycystic ovaries

Parameter	Serum estradiol level	
	<100 pmol/l (Group 1)	⩾100 pmol/l (Group 2)
No. of cycles	70	53
No. of transfers	67	51
Age (years)	34 (18–42)	34 (24–42)
Serum FSH day 2–4 (IU/l)	5.9 (2.2–13.3)	5.6 (2.2–16.2)
No. of immature oocytes	7.5 (1–36)	9 (0–32)
No. of metaphase II oocytes	6 (1–20)	7 (0–23)
No. of 2PN embryos	5 (0–16)	5 (0–19)
No. of cleaving embryos	4 (0–14)	5 (0–19)
No. of embryos transferred	3 (1–5)	3 (1–5)
Cumulative Embryo Score	30 (7–80)	32 (4–82)
Endometrial thickness at transfer	8.7 (6.1–13.4)	9.6 (5.1–15.5)[a]
No. of pregnancies (%)	8 (11)	14 (26)[b]

Data shown are median number (range) per cycle unless otherwise indicated.
[a]$P = 0.035$ (Mann–Whitney *U*-test).
[b]$P = 0.032$ (χ^2 test).
2PN = two pronuclei.

Table IV. In-vitro maturation outcome according to day 2–4 serum FSH level in 123 cycles for women with normal or polycystic ovaries

Parameter	Serum FSH level	
	<7.5 IU/l (Group 1)	⩾7.5 IU/l (Group 2)
No. of cycles	100	23
No. of transfers	96	22
Age (years)	33 (24–42)	34 (18–42)
Serum estradiol day 2–4 (pmol/l)	89 (37–266)	91 (37–234)
No. of immature oocytes collected	9 (1–36)	7 (0–24)[a]
No. of metaphase II oocytes	7 (1–23)	6 (0–20)
No. of 2PN embryos	5 (0–19)	5 (0–16)
No. of cleaving embryos	5 (0–19)	4 (0–14)
No. of embryos transferred	3 (1–5)	3 (1–4)
Cumulative Embryo Score	32 (4–82)	31.5 (5–68)
No. of pregnancies (%)	16 (16)	6 (26)

Data shown are median number (range) per cycle unless indicated.
[a]$P = 0.003$ (Mann–Whitney *U*-test).
2PN = two pronuclei.

subgroup analysis of the predictive value of basal estradiol levels could not therefore be performed for these cycles.

After data normalization, one-way ANOVA was used to evaluate differences in mean basal estradiol level between women with normal ovaries, ovulatory PCO or anovulatory PCOS. No significant differences in basal estradiol level were found between the three groups.

The day 2–4 FSH concentration was not found to be discriminatory for pregnancy (Table IV). Using a threshold FSH of 7.5 IU/l, as proposed previously (Mikkelsen *et al*., 2001), the lower FSH concentration group had significantly more immature oocytes retrieved than the higher group. Other parameters were, however, similar between the groups. In particular, there were no differences in the rates of oocyte maturation, fertilization, cleavage or pregnancy.

Discussion

It has been shown previously that the pregnancy rate in IVM is related to the numbers of immature oocytes retrieved (Child *et al.*, 2001a), as this dictates the numbers of embryos available for transfer (Child *et al.*, 2002). It has also been shown that the numbers of oocytes retrieved from unstimulated ovaries may be predicted by early follicular phase transvaginal ultrasound measurement of the antral follicle count, ovarian volume and maximum ovarian stromal blood flow velocity (Child *et al.*, 2001a). Following multiple linear regression analysis, the antral follicle count was shown to be the most important independent predictor of the numbers of oocytes retrieved. In that study, no consideration was made of either patient age or basal hormone levels. The present data show that increasing basal FSH level and increasing patient age are both associated with a reduction in the number of immature oocytes collected. However, after controlling for other factors in the regression model, FSH rather than age was the significant independent predictor. Others (Mikkelsen *et al*., 2001) also found FSH to be a predictor of numbers of oocytes collected in IVM, though these authors did not perform multiple regression analysis, nor did they control for other variables such as age or number of antral follicles.

It was found that higher basal levels of estradiol (⩾100 pmol/l) were associated with higher pregnancy rates in IVM. This was not due to differences in basal estradiol concentrations, since none existed between ovulatory and anovulatory women or between those with normal or polycystic ovaries. Interestingly, others (Mikkelsen *et al.*, 2001) found that high, rather than low, estradiol levels were associated with poor IVM outcome. In this retrospective study of 132 unstimulated IVM cycles in women with normal ovaries and ovulatory cycles, it was found that all 15 pregnancies occurred in the 106 cycles with basal estradiol levels <200 pmol/l. There were no pregnancies in the 26 cycles with estradiol levels ⩾200 pmol/l. However, the present data might be consistent with this observation if the majority of pregnancy cycles in Mikkelsen's study had basal estradiol levels between 100 and 200 pmol/l, and a number of non-pregnancy cycles had lower estradiol levels. This cannot be determined from the data presented. In the present study, 10 cycles had basal estradiol levels >200 pmol/l, and three (30%) resulted in pregnancy. Other explanations for the different findings include different patient populations, as all women in Mikkelsen's study had normal ovaries with ovulatory cycles, while suspected poor ovarian reserve was an exclusion criteria. In addition, different estradiol assays were used. Finally, the present patients were primed with HCG prior to oocyte retrieval in order to increase the rate of oocyte maturation (Chian *et al*., 2000). The present data confirm Mikkelsen's observations that basal estradiol and FSH levels are not related to oocyte quality as determined by rates of oocyte maturation, fertilization and cleavage.

Women in the low- and high-estradiol groups were of similar age, and their treatment cycles resulted in comparable numbers of oocytes retrieved, embryos produced and transferred, as well as CES values (Table III). The increased pregnancy rate in the high-estradiol group was also found when only cycles

for women with PCO were considered. Cycles for women with ovulatory PCO or anovulatory PCOS were grouped together for analysis as they are known to have similar outcomes following IVM in terms of numbers of oocytes collected, and rates of maturation, fertilization, pregnancy and live birth (Child *et al.*, 2001b). It is clinically most useful, therefore, to consider these women as one patient group. Furthermore, in the present study no difference was found in terms of mean basal serum estradiol level between women with normal ovaries, ovulatory PCO or PCOS.

Raised basal levels of estradiol have been associated with a reduced ovarian response to ovulation induction (Licciardi *et al*., 1995). Interestingly, it was found that women with a low estradiol level had a poorer outcome compared with those with high estradiol levels. As there was no difference between the groups in terms of the numbers and apparent quality of oocytes and embryos, the explanation must lie elsewhere. Endometrial thickness on the day of transfer was found to be significantly greater in the high-estradiol group, and it has been noted recently that the clinical pregnancy rate in IVM increases with increasing endometrial thickness (unpublished data). This difference in endometrial thickness is the most likely explanation of a higher pregnancy rate associated with raised serum estradiol levels.

During conception *in vivo* in the natural menstrual cycle, the endometrium is partially primed for implantation by endogenous estrogen produced by granulosa cells within the dominant ovarian follicle. During a stimulated IVF cycle, estrogen is produced endogenously from the numerous mature follicles produced secondary to gonadotrophin stimulation. However, during an IVM cycle a mature dominant follicle does not develop since aspiration is performed, and so exogenous estrogen must be administered. When the role of early or late exogenous estradiol priming of the endometrium prior to immature oocyte retrieval was examined (Russell, 1998), prolonged endometrial priming prior to immature oocyte retrieval was found to be deleterious to oocyte quality and developmental potential. Hence, in the present study estrogen priming was commenced from the day of oocyte retrieval, with the dose being dependent on endometrial thickness, and this approach was found to be satisfactory (Chian *et al*., 2000).

The patient group with raised basal estradiol levels could conceivably continue to have tonically raised estradiol levels during the follicular phase, resulting in a degree of endometrial priming. However, in 93 cycles, the endometrial thickness on the day of oocyte recovery was available for analysis. The median thickness in both low- and high-estradiol groups was 6.7 mm (P = NS); hence, the two groups would have received comparable doses of estradiol valerate as the dose was dependent on endometrial thickness on the day of oocyte retrieval. However, there was a proportionately greater increase in endometrial thickness between oocyte recovery and embryo transfer in the high-estradiol group. Conceivably, low-level estradiol priming could increase the responsiveness of the endometrium to exogenous estradiol priming, though further investigation is required to test this hypothesis. If true however, women with low basal estradiol levels might benefit from very low doses of estrogen priming prior to oocyte retrieval.

An alternative explanation for the difference in pregnancy rates between the two groups was that there was a subtle difference in oocyte or embryo quality that was not immediately apparent. The groups had comparable oocyte maturation, fertilization and cleavage rates, and similar morphological embryo quality as assessed by CES. However, oocyte nuclear and cytoplasmic maturation, both of which involve a complex cascade of events, need to be closely integrated to ensure developmental competence. In IVM oocytes, nuclear maturation may have been complete, as evidenced by extrusion of the first polar body, whilst cytoplasmic maturation was incomplete. Maturation media containing FSH significantly increase fertilization and early embryo development (Schroeder *et al*., 1988; Jinno *et al*., 1989). However, cytoplasmic maturation might differ between the low- and high-estradiol groups.

In the present authors' institution, HCG 10 000 IU is routinely administered 36 h prior to immature oocyte retrieval, this being based on a randomized controlled trial in women with PCOS, in whom HCG priming was found significantly to increase the numbers of oocytes matured at 24 h (78.2 versus 4.9%) and 48 h (85.2 versus 68.0%) of culture (Chian *et al*., 2000). More recently, it has been found that similarly high rates of oocyte maturation are also obtained when HCG priming is used in women undergoing IVM who either have normal ovaries or have ovulatory PCO (Child *et al*., 2001b).

In conclusion, it has been shown that the measurement of basal serum levels of FSH and estradiol are useful in predicting outcome in ovulatory and anovulatory women undergoing unstimulated IVM treatment. After controlling for the presence of polycystic ovaries, the basal FSH level was superior to patient age in predicting the number of immature oocytes retrieved. Women with a low ($<$100 pmol/l) basal serum level of estradiol had a significantly lower pregnancy rate than those with serum estradiol levels $\geqslant$100 pmol/l. Although this difference appears to be due to a significantly thicker endometrium at the time of embryo transfer in the high basal estradiol group, further investigations are required in order to elucidate the possible mechanisms behind this observation.

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