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High serum oestradiol concentrations in fresh IVF cycles do not impair implantation and pregnancy rates in subsequent frozen–thawed embryo transfer cycles

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High oestradiol concentrations may be detrimental to the success of in-vitro fertilization (IVF) treatment. A total of 1122 women aged <40 years who were undergoing their first IVF cycle were evaluated retrospectively. Serum oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) administration were categorized into three groups: group A <10 000 pmol/l; group B 10 000–20 000 pmol/l and group C >20 000 pmol/l. In fresh cycles, group A had significantly lower pregnancy rates per transfer (16.2 versus 23.7% respectively, $P = 0.005$, χ^2) and implantation rates (8.7 versus 11.7% respectively, $P = 0.037$, χ^2), when compared with group B. The pregnancy rate per transfer in group C was significantly lower than that in group B (12.1 versus 23.7%, $P = 0.049$, χ^2) and group C had the lowest implantation rate (6.4%). In frozen–thawed embryo transfer cycles, implantation rates in groups A, B and C were similar (7.5, 8.1 and 9.6% respectively) and the pregnancy rates were also comparable in all groups. In conclusion, high serum oestradiol concentrations in fresh IVF cycles may adversely affect implantation and pregnancy rates. Embryo quality seemed unaffected as excess embryos from different groups had similar implantation and pregnancy rates in frozen–thawed embryo transfer cycles. The reduced implantation was probably due to an adverse endometrial environment resulting from high serum oestradiol concentrations.

Key words: frozen embryo transfer/implantation rate/IVF/pregnancy rate/oestradiol

Introduction

Recent advances in the understanding of ovarian stimulation, the techniques of oocyte retrieval, the handling of gametes, the methods of assisted fertilization and improved conditions of culture media have steadily increased the fertilization rate. Fertilization rates of 60–70% can now be expected when conventional insemination or intracytoplasmic sperm injection (ICSI) are carried out. However, there has not been a corresponding increase in implantation rates, which have remained steady at 10–15% for a long time. The success of implantation depends upon a perfect relationship between good quality embryos and a receptive endometrium.

Several studies (Forman *et al*., 1988; Simón *et al*., 1995) have shown significantly lower implantation and pregnancy rates in cycles with high serum oestradiol concentrations, whereas others (Chenette *et al*., 1990) have found no adverse effects. The impact of high oestradiol concentrations on the outcome of in-vitro fertilization (IVF)/embryo transfer treatment remains controversial. Moreover, the effects of high oestradiol concentrations in fresh cycles on the outcome of frozen–thawed embryo transfer are not well documented. One study (Schalkoff *et al*., 1993) found that the oestradiol concentrations in the fresh cycle were not related to the success of frozen–thawed embryo transfer cycles.

The purpose of this study was to examine the effects of high serum oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) administration on implantation and pregnancy rates in fresh IVF/embryo transfer cycles. Implantation and pregnancy rates in subsequent frozen–thawed embryo transfer cycles were evaluated in those who did not become pregnant in the fresh cycles.

Materials and methods

A retrospective study was undertaken of infertile patients attending the Assisted Reproduction Unit at Department of Obstetrics & Gynaecology, Queen Mary Hospital, Hong Kong, for conventional IVF or ICSI treatment from early 1993 to December 1998. Ethical approval was not required for this retrospective analysis. The indications for conventional IVF included tubal, male, endometriosis, unexplained and mixed factors. ICSI was carried out in couples with severe semen abnormalities (<100 000 motile spermatozoa recovered after sperm preparation) and surgically retrieved spermatozoa from epididymis or testis in cases of obstructive azoospermia.

Couples had to fulfil the following criteria before they were included in this study so as to control for all confounding factors as far as possible: (i) only the first treatment cycle was considered as less fertile couples due to unknown reasons might require repeated attempts and would be over-represented in pooled data of all IVF cycles carried out within a certain period of time (Templeton *et al*., 1996); (ii) the women had to be aged <40 years; and (iii) a maximum of three embryos were replaced in the fresh and frozen–thawed embryo transfer cycles.

Fresh IVF or ICSI cycles

The details of the long protocol of ovarian stimulation regimen used at our centre have been published previously (Ng *et al*., 1997). Briefly, women were pre-treated with a gonadotrophin-releasing hormone (GnRH) analogue, buserelin (Suprecur®; Hoechst, Frankfurt, Germany) nasal spray 150 μg four times a day from the mid-luteal phase of the cycle preceding the treatment cycle. Pituitary down-

regulation was confirmed by both transvaginal scanning and serum oestradiol determination performed on the second day of the treatment cycle. Human menopausal gonadotrophin (HMG; Pergonal, Serono, Switzerland or Humegon, Organon, The Netherlands) injections were then started. The ovarian response was monitored by serial transvaginal scanning and serum oestradiol concentrations. Oestradiol was measured using a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The inter-assay and intra-assay coefficients of variation on high concentration control (oestradiol 1082 pg/ml) were 4.2 and 4.0% respectively.

HCG (Profasi®; Serono, Switzerland) 10 000 IU was given i.m. when the leading follicle reached 18 mm in diameter and there were at least three follicles of >15 mm in diameter. Transvaginal ultrasound-guided oocyte retrieval was scheduled 36–38 h after the HCG injection. The oocyte retrieval rate was the proportion of punctured follicles that contained an oocyte.

Spermatozoa were prepared by a discontinuous Percoll (Pharmacia Biotech AB, Uppsala, Sweden) or Isolate (Irvine Scientific, Santa Ana, CA, USA) gradient separation. Insemination was performed ~4–6 h after oocyte retrieval in conventional IVF cycles. ICSI was carried out using metaphase II (MII) oocytes, which were denuded of their surrounding cumulus and corona radiata cells by hyaluronidase (~100 IU/ml) and then aspirating the oocytes through a fine needle 2 h after oocyte collection. The fertilization rate was defined as the proportion of oocytes resulting in two pronuclei (2PN) formation; only MII oocytes were counted in ICSI cycles.

A maximum of three normally cleaved embryos were replaced into the uterine cavity 48 h after the retrieval. Excess good quality embryos were frozen for subsequent transfer if the woman was not pregnant in that cycle. All fresh embryos were cryopreserved if the serum oestradiol concentration on the day of ovulatory HCG injection was >30 000 pmol/l, in order to reduce the risks of ovarian hyperstimulation syndrome (OHSS), which was graded into mild, moderate and severe degrees (Royal College of Obstetricians and Gynaecologists, 1995).

The luteal phase was supported by 1500 IU HCG injections on the day of embryo transfer and 6 days later. Progesterone injections (100 mg i.m. daily; Weimer Pharma, Germany) or vaginal pessaries (Cyclogest 400 mg twice daily, Cox Pharmaceuticals, Barnstaple, UK) were used instead from the day of embryo transfer for 10 days when the serum oestradiol concentration on the day of ovulatory HCG was >18 000 pmol/l. Serum oestradiol and progesterone concentrations were measured 6 days after embryo transfer. Progesterone was measured using a commercially available radioimmunoassay kit (Chiron Diagnostics Corporation, MA, USA) and the inter-assay and intra-assay coefficients of variation were 9.4 and 8.4% respectively. A urine pregnancy test was carried out 16 days after embryo transfer. If it was positive, ultrasound examination was performed 10–14 days later to confirm intrauterine pregnancy and to determine the number of gestation sacs present.

Frozen–thawed embryo transfer cycles

Embryos were cryopreserved using a programmable freezer with 1,2-propanediol as cryoprotectant. Frozen embryos were thawed at room temperature for 40 s and then at 30°C in a water bath for 40 s. Subsequently, the cryoprotectant was removed by washing the embryos successively through phosphate buffer saline (Sigma, St Louis, MO, USA) with decreasing concentration of propanediol and the embryos were cultured in the CO_2 incubator for a short period before transfer. Any embryo with equal to or more than half of the number of blastomeres surviving was transferred.

After thawing, frozen embryos were transferred in natural, clomiphene-induced or hormone replacement cycles. Those patients with regular ovulatory cycles underwent frozen–thawed embryo transfer in natural cycles. Clomiphene citrate (Clomid, Merrell, Staines, UK) 50–150 mg was given daily for 5 days from days 3–7 to patients with irregular/long cycles or an absence of oestradiol rise/luteinizing hormone (LH) surge in previous natural cycles. During natural or clomiphene-induced cycles, patients were monitored daily for serum oestradiol and LH concentrations from 18 days before the expected date of next period. The transfer was performed on the third day after the LH surge. The luteal phase was supported by two HCG injections as in fresh embryo transfer.

Hormone replacement cycles were offered to those patients who showed no ovulatory response after taking clomiphene citrate 150 mg daily for 5 days. No information was recorded on cycle pattern or length for patients in the different groups. After down-regulation by buserelin nasal spray (150 μg four times a day) starting in the mid-luteal phase, Estrofem (Novo Nordisk, Surrey, UK) was started on the second day of the next menstrual cycle in incremental dosage (2 mg daily for 5 days, 4 mg daily for 4 days and then 6 mg daily for 4 days). Estrofem was then reduced to 4 mg daily and progesterone injections (100 mg daily) or Cyclogest vaginal pessaries (400 mg twice daily) were started if endometrial thickness measured by ultrasound scanning reached ⩾8 mm. Embryo transfer was carried out on the fourth day after starting progesterone.

Statistical analysis

Only clinical pregnancies were considered. A clinical pregnancy is defined by the presence of one or more gestation sacs, including ectopic pregnancy or the demonstration of gestational product in the uterine evacuate. On-going pregnancies were those pregnancies of >10–12 weeks gestation, when the patients were referred out for antenatal care. The mean implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac.

Serum oestradiol concentrations on the day of ovulatory dose of HCG were categorized into three groups: group A <10 000 pmol/l; group B 10 000–20 000 pmol/l and group C >20 000 pmol/l. Data on the age of women, type of treatment (IVF/ICSI), the duration/dosage of HMG used, the number of follicles aspirated, the number of oocytes obtained/fertilized, the egg retrieval rate, the fertilization rate, the OHSS rate, the pregnancy rate, the implantation rate and the pregnancy outcomes were compared between the groups. Continuous data were expressed as mean ± SD. Statistical tests were carried out using one-way analysis of variance (ANOVA) with multiple comparisons (Tukey HSD) for continuous data and χ^2 test for categorical data, where appropriate. Correlation was assessed by the Pearson method. $P < 0.05$ was considered to be statistically significant.

Results

A total of 1122 cycles were included in this study: 899 conventional IVF cycles and 223 ICSI cycles. The women were aged 33.1 ± 3.2 years. The serum oestradiol concentrations on the day of ovulatory dose of HCG were 10 509 ± 7018 pmol/l (median 8567 pmol/l; range 1145–58 590 pmol/l) and the number of oocytes obtained was 10.2 ± 6.9 (median 8.0; range 0–50). The retrieval and fertilization rates were 72.5 ± 24.7% and 70.8 ± 30.1% respectively. In all, 58 (5.2%) initiated cycles were complicated by moderate or severe OHSS and they all responded to conservative management.

Embryo transfer was carried out in 1005 cycles and 184 clinical pregnancies resulted. Oocytes were not obtained in two retrievals and fertilization failure was encountered in 66 cycles. Embryos failed to cleave in three cycles and embryos

Table I. Ovarian responses, fertilization rates and moderate or severe ovarian hyperstimulation syndrome (OHSS). Results are given as mean ± SD; values in parentheses are percentages

	Group A (n = 680)	Group B (n = 335)	Group C (n = 107)	Significance (ANOVA)
Type of treatment (no. of cycles)				$P = 0.034^{c,d}$
IVF	556 (81.8)	267 (79.7)	76 (71.0)	
ICSI	124 (18.2)	68 (20.3)	31 (29.0)	
Age of women (years)	33.4 ± 3.2	32.7 ± 2.9	32.0 ± 3.1	$P < 0.001^{a,c}$
HMG dosage (ampoules)	32.0 ± 14.3	27.5 ± 10.6	25.9 ± 8.4	$P < 0.001^{a,c}$
HMG duration (days)	12.5 ± 3.4	11.4 ± 2.7	11.3 ± 2.0	$P < 0.001^{a,c}$
No. of follicles aspirated	10.8 ± 6.9	17.7 ± 8.1	26.1 ± 9.8	$P < 0.001^{a,b,c}$
No. of eggs obtained	7.1 ± 4.3	13.4 ± 6.4	19.9 ± 8.5	$P < 0.001^{a,b,c}$
No. of eggs fertilized	4.8 ± 3.4	9.0 ± 5.8	13.4 ± 8.4	$P < 0.001^{a,b,c}$
No. of embryos cleaved	4.7 ± 3.3	8.8 ± 5.8	12.9 ± 8.3	$P < 0.001^{a,b,c}$
No. of embryos frozen	1.4 ± 2.3	4.2 ± 4.3	7.9 ± 6.6	$P < 0.001^{a,b,c}$
Fertilization failure (no. of cycles)	41 (6.0)	19 (5.7)	6 (5.6)	NS
Oocyte retrieval rate (%)	69.3 ± 25.7	77.5 ± 23.3	77.5 ± 18.7	$P < 0.001^{a,c}$
Fertilization rate (%)	71.5 ± 31.0	69.8 ± 29.3	69.1 ± 27.1	NS
Cleavage rate (%)	90.2 ± 28.8	92.0 ± 23.7	90.5 ± 25.0	NS
Moderate or severe OHSS (no. of cycles)	13 (1.9)	24 (7.2)	21 (19.6)	$P < 0.0001^{a,b,c,d}$

ANOVA = analysis of variance; IVF = in-vitro fertilization; ICSI = intracytoplasmic sperm injection; HMG = human menopausal gonadotrophin; NS = not significant; for definition of groups, see text.
[a]Significant difference between groups A and B ($P < 0.05$).
[b]Significant difference between groups B and C ($P < 0.05$).
[c]Significant difference between groups C and A ($P < 0.05$).
[d]χ^2 test.

were not replaced in another 46 cycles because of the risk of OHSS. The pregnancy rate was 16.4% per initiated cycle and 18.3% per transfer. IVF and ICSI cycles had similar pregnancy rates per transfer (18.7 versus 16.8% respectively, $P = 0.662$, χ^2). The pregnancy rate per transfer was 2.0% (2/98), 11.2% (22/197) and 22.5% (160/710) respectively when one, two and three embryos were replaced. These differences were statistically significant ($P < 0.0001$, χ^2).

Table I summarizes the ovarian responses, fertilization rates and incidence of moderate or severe degree of OHSS in different groups according to the concentrations of serum oestradiol on the day of HCG administration. Women in group C had more ICSI cycles compared with group A ($P = 0.009$, χ^2). The indications for conventional IVF were similarly distributed in all groups (data not shown). Women in groups B and C were significantly younger and required significantly fewer ampoules of HMG over a shorter duration, when compared with group A. Significantly more follicles developed on the day of HCG and resulted in a higher number of follicles being punctured in groups B and C. The retrieval rate was significantly lower in group A than in groups B or C. The mean number of oocytes obtained in groups A, B and C was 7.1, 13.4 and 19.9 respectively ($P < 0.001$, analysis of variance) and a statistically significant difference was found amongst all three groups.

More oocytes were fertilized and cleaved in groups B and C compared with group A. This resulted in more frozen–thawed embryo transfer cycles per patients being performed in groups B and C [1.87 (290/155) and 2.53 (185/73) respectively] than in group A [1.3 (227/175)]. However, fertilization and cleavage rates were similar for the three groups and the incidence of fertilization failure did not differ between them (Table I). Significantly more cycles were complicated by moderate or severe degree of OHSS in group C than groups A or B.

Women in group A had fewer transfer cycles with three embryos replaced and a significantly lower pregnancy rate per transfer than those in group B (16.2 versus 23.7% respectively, $P = 0.005$, χ^2) (Table II). Despite a similar number of transfers with three embryos replaced, the pregnancy rate per transfer was significantly lower in group C than group B (12.1 versus 23.7% respectively, $P = 0.049$, χ^2). The implantation rate in group A was also significantly lower than in group B (8.7 versus 11.7% respectively, $P = 0.037$, χ^2). Group C had the lowest implantation rate (6.4%) but no significant difference was observed between groups B and C.

No differences were found between groups A and C with regard to the number of embryos replaced, the pregnancy and implantation rates. Multiple pregnancy rates and the outcome of pregnancy were comparable in all three groups (Table II). In frozen–thawed embryo transfer cycles, the pregnancy and implantation rates were similar and the percentages of frozen embryos showing lysis on thawing were not different between the three groups (Table III). The total number of cycles in which embryos were frozen was 622, but only 403 patients came back for frozen–thawed embryo transfer cycles up to the end of December 1998. There are two possible reasons for this: (i) if only one frozen embryo is available, we prefer to begin another stimulation cycle; and (ii) the stress of treatment or pressure at work may cause patients to delay returning, especially as the maximum duration for storing frozen embryos is 5 years. The pregnancy rates per transfer were similar in frozen–thawed embryo transfer using natural, clomiphene-induced or hormone replacement cycles (data not shown). When pregnancy and implantation rates in fresh cycles were compared with respect to the number of oocytes obtained,

Table II. Pregnancy and implantation rates in fresh embryo transfer cycles. Results are given as mean ± SD

	Group A	Group B	Group C	*P* value (χ^2)
Total no. of embryos transferred	635	312	58	
No. of embryos transferred				< 0.001[a]
One (%)	76 (12.0)	20 (6.4)	2 (3.4)	
Two (%)	139 (21.9)	44 (14.1)	14 (24.1)	
Three (%)	420 (66.1)	248 (79.5)	42 (72.4)	
Oestradiol:progesterone ratio (6 days after embryo transfer)	21.6 ± 14.2	22.7 ± 16.2	19.5 ± 11.0	NS
Pregnancy rate per transfer (%)	16.2 (103/635)	23.7 (74/312)	12.1 (7/58)	0.009[a,b]
Implantation rate (%)	8.7 (141/1614)	11.7 (100/852)	6.4 (10/156)	0.040[a]
Multiple pregnancy rate	29.1 (30/103)	31.1 (23/74)	42.9 (3/7)	NS
Outcome of pregnancy				NS
Abortion (%)	12 (11.7)	8 (10.8)	0	
Ectopic (%)	6 (5.8)	3 (4.1)	0	
On-going (%)	85 (82.5)	63 (85.1)	7 (100)	

See text for definition of groups; NS = not significant.
[a]Significant difference between groups A and B ($P < 0.05$).
[b]Significant difference between groups B and C ($P < 0.05$).

Table III. Pregnancy and implantation rates in frozen–thawed embryo transfer cycles[d]

	Group A	Group B	Group C	*P* value (χ^2)
No. of frozen embryos per cryo-cycle[e]	3.2 (945/297)	6.0 (1408/235)	9.3 (840/90)	< 0.001[a,b,c]
No. of frozen–thawed embryo transfer cycles performed	227	290	185	
No. of patients	175	155	73	
Lysis of embryos on thawing (%)	13.8 (81/587)	12.8 (107/839)	16.0 (91/569)	NS
Mean no. of embryos per transfer	2.23	2.52	2.58	NS
Pregnancy rate per transfer (%)	14.5 (33/227)	17.9 (52/290)	18.9 (35/185)	NS
Implantation rate (%)	7.5 (38/506)	8.1 (59/732)	9.6 (46/478)	NS

NS = not significant.
[a]Significant difference ($P < 0.05$) between groups A and B.
[b]Significant difference ($P < 0.05$) between groups B and C.
[c]Significant difference ($P < 0.05$) between groups C and A.
[d]Any patients who became pregnant after a fresh cycle and returned for a frozen–thawed embryo transfer cycle were excluded from this analysis.
[e]Significantly more embryos were frozen per cryo-cycle in groups B and C since more oocytes were fertilized and cleaved in these groups (see text).

they were similar for those cycles with <15 oocytes and ⩾15 oocytes obtained (Table IV).

Serum oestradiol concentrations were found to be negatively correlated with the age of women ($r = -0.134$, $P < 0.001$), the dosage ($r = -0.181$, $P < 0.001$) and duration ($r = -0.157$, $P < 0.001$) of HMG stimulation, but positively correlated with the number of oocytes obtained ($r = 0.670$, $P < 0.001$).

Discussion

High serum oestradiol concentrations may be detrimental to the implantation and pregnancy rates in IVF treatment. In 825 conventional IVF cycles using clomiphene and HMG as the stimulation regimen an adverse effect of excessive oestradiol concentrations (>2320 pg/ml; conversion factor to SI unit, 3.671) was found on implantation when one or two embryos were replaced (Forman *et al*., 1988). This impairment in implantation was overcome by transferring three embryos, i.e. it appeared that a low embryo number was confounded by the oestradiol concentration. Recently, a significant decrease was shown (Simón *et al*., 1995) in implantation and pregnancy rates in IVF when serum oestradiol concentrations were >2500 pg/ml (conversion factor to SI unit, 3.671) in 105 normal and 59 high responders (i.e. ⩾15 oocytes). However, a higher pregnancy rate was found in patients with serum oestradiol concentrations >2777 pg/ml (conversion factor to SI unit, 3.671) in 141 IVF cycles after pituitary down-regulation (Chenette *et al*., 1990). The impact of high serum oestradiol concentrations on the outcomes of IVF/embryo transfer treatment remains controversial.

This retrospective study of more than 1000 first IVF cycles revealed that high serum oestradiol concentrations i.e. >20 000 pmol/l on the day of HCG administration impaired implantation and pregnancy rates in fresh IVF cycles. Group A had a significantly lower pregnancy rate than group B probably because of more transfers having one to two embryos

Table IV. Pregnancy and implantation rates in fresh cycles with respect to the number of eggs obtained

	Number of eggs obtained		*P* value χ^2)
	<15	⩾15	
No. of embryo transfers	884	161	
No. of embryos replaced			0.041*
One (%)	91 (10.8)	7 (4.4)	
Two (%)	163 (19.3)	34 (21.1)	
Three (%)	590 (69.9)	120 (74.5)	
Pregnancy rate per transfer (%)	18.0 (152/844)	19.9 (32/161)	NS
Implantation rate (%)	9.2 (202/2187)	11.3 (49/435)	NS

NS = not significant.
*Significantly different ($P < 0.05$).

replaced and a lower implantation rate. The pregnancy rate in group C was significantly lower than group B, despite similar percentage of transfers with three embryos replaced in these two groups. The reduced pregnancy rate in group C was most likely due to impaired implantation as shown by the lowest implantation rate in this group. No significant difference in implantation rates was found between groups B and C and this was probably due to the small number of cycles in group C. A wide range of oestradiol concentrations was found to be detrimental to implantation and pregnancy rates and the differences in the studies may be explained by different patient populations, stimulation regimens and oestradiol assay methods (Tummon *et al*., 1999).

The results of the current study did not show any difference in implantation and pregnancy rates between cycles with <15 oocytes and with ⩾15 oocytes obtained. Similar findings were also demonstrated previously (Toner *et al*., 1991) in 327 women undergoing IVF treatment. This contrasts with previous results (Pellicer *et al*., 1989; Simón *et al*., 1995). The threshold value of the number of oocytes for high responders was derived using a regression model from cycles with fertilization rates of <50% (Tarìn *et al*., 1992) and has been changed from ⩾11 (Pellicer *et al*., 1989) to ⩾15 (Simón *et al*., 1995). The fertilization rate was found to be unrelated to the serum oestradiol concentrations (Chenette *et al*., 1990) or the number of oocytes retrieved (Simón *et al*., 1995).

Simón *et al*. (1995) also concluded that implantation impairment was related only to higher serum oestradiol concentration subgroups in both normal and high responder patients, regardless of the number of oocytes collected. This reinforced the concept of a direct adverse effect of higher oestradiol concentration (rather than the number of oocytes retrieved) on endometrial receptivity. As shown in this study, serum oestradiol concentrations were significantly correlated with the number of oocytes obtained ($r = 0.670$, $P < 0.001$).

Implantation depends on the synchronized development of both embryos and the endometrium. The detrimental effects of very high oestradiol concentrations on implantation may result from poor embryo quality, lower endometrial receptivity or a combination of both. Pellicer *et al*. had suggested that retrieval of >10 oocytes in women was correlated with oocytes of lower quality, as manifested by a decrease in the fertilization rate (Pellicer *et al*., 1989). The same group (Tarìn and Pellicer, 1994) had further demonstrated lower oestradiol concentrations per follicle, lower follicular volume and a higher incidence of diploid oocytes and cytoplasmic immaturity in high responders.

Women in groups B and C were of similar age and were significantly younger than those in group A although the difference was small. The quality of oocytes appeared not to be affected by the very high oestradiol concentrations in group C as the incidence of fertilization failure, the fertilization and cleavage rates were similar for all three groups. Moreover, frozen embryos from different groups had a similar percentage of lysis on thawing and comparable implantation and pregnancy rates in frozen–thawed embryo transfer cycles. These clinical data indicated that oocyte and embryo quality were not affected by the high serum oestradiol concentrations. Another author (Schalkoff *et al*., 1993) also concluded that high concentrations of serum oestradiol (>11 000 pmol/l) in fresh cycles did not adversely affect the pregnancy rate during frozen–thawed embryo transfer cycles. However, that study examined only 185 consecutive frozen–thawed embryo transfer cycles in 161 patients. Using oocyte donation cycles, similar implantation and pregnancy rates were also shown in recipients of high responder oocytes and recipients of normal responder oocytes (Simón *et al*., 1995).

Pellicer *et al*. (1996) noted abnormal oestradiol:progesterone ratios around the time of implantation in high responders having >15 oocytes; a critical ratio of <15 was suggested for successful implantation. In this study, the mean oestradiol: progesterone ratios 6 days after embryo transfer were 21.6 [20.3–22.9; 95% confidence interval (CI)], 22.7 (20.6–24.8 95% CI) and 19.5 (15.8–23.3 95% CI) in groups A, B and C respectively. Different luteal support (HCG and progesterone injection or vaginal pessaries) in fresh cycles did not seem to affect the oestradiol:progesterone ratio.

The endometrium undergoes a series of precisely-regulated morphological changes under the influence of serum oestrogen and progesterone. Synthetic oestrogen has been used as an effective emergency contraceptive agent to prevent implantation (Haspels, 1976). A much higher implantation rate was demonstrated when donated embryos from hyperstimulated mated mice were transferred during natural cycles than those of stimulated mice (Fossum *et al*., 1989). The supraphysiological concentrations of steroid hormone in ovarian stimulation were associated with a high incidence of dyssynchrony between endometrial glands and stroma when compared with non-stimulated cycles (Benadiva and Metzger, 1994). Impaired development of the endometrial glands (Bonhoff *et al*., 1990), advanced stromal development (Noci *et al*., 1997) and an earlier expression of pinopodes (Kolb *et al*., 1997) were also reported following ovarian stimulation. On the other hand, abnormal development of endometrial glands after ovarian stimulation was not observed in a small study (Macrow *et al*., 1994).

There may not be any detectable morphological changes in the endometrium. A significant reduction in nuclear receptors in both the glands and stroma for progesterone and oestrogen receptors was found after ovarian stimulation in the presence

of supraphysiological amounts of steroids and most of the endometrial biopsies were in phase (Hadi *et al*., 1994). The influence of high steroid concentrations on the secretory products in the endometrial glands is largely unknown and remains to be explored in further studies.

Our current practice is to cryopreserve all embryos in cycles where the serum oestradiol concentration is ⩾30 000 pmol/l in order to reduce the risk of OHSS. A reduction in implantation and pregnancy rates in cycles with concentrations of >20 000 pmol/l implied that fresh embryos arising from these stimulated cycles should also be cryopreserved for transfer later. The resulting pregnancy rates in frozen–thawed embryo transfer cycles would not be compromised as shown in this study and the risk of moderate or severe OHSS could be further reduced.

A recent multicentre study (Waldenström *et al*., 1999) showed high pregnancy rates and successful prevention of severe OHSS by 'prolonged coasting' of extremely stimulated patients. Serum oestradiol concentrations were markedly reduced from the first day of withholding gonadotrophin to the day of HCG administration. These data suggest that the high oestradiol concentrations may not have lasting adverse effects on uterine receptivity or the adverse effects on the endometrium may be reversible. Coasting can be considered as another option for those patients with serum oestradiol >20 000 pmol/l although the average number of retrieved eggs was only 10.0 (range 3–21). Frozen embryos seem to be decreased in number after coasting. Furthermore, the threshold values for serum oestradiol concentration and follicle size for initiating and ending of the coasting period, need further evaluation.

It is, of course, important to identify those at risk of developing excessive ovarian responses and not to over-stimulate them. Milder forms of ovarian stimulation, e.g. a lower starting dose of gonadotrophin (Devroey *et al*., 1998) or using GnRH antagonists (Diedrich and Felberbaum, 1998) may be of help in the next stimulated cycle. In the next cycle of the high responders, Simón *et al*. demonstrated better implantation and pregnancy rates in those using step-down protocol despite a reduction in the number of oocytes and serum oestradiol, when compared with those receiving the standard protocol (Simón *et al*., 1998).

In conclusion, this retrospective study showed an association between high serum oestradiol concentrations on the day of HCG administration and impaired implantation and pregnancy rates in fresh IVF/embryo transfer cycles, which were unrelated to the number of oocytes obtained. Embryo quality seemed unaffected as excess embryos from different groups had similar implantation and pregnancy rates in frozen–thawed embryo transfer cycles. The impairment in implantation was likely to be related to an adverse environment in the endometrium resulting from high serum oestradiol concentrations.

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