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Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients

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This study was undertaken to investigate an empirical observation that 'high responder patients have poorer in-vitro fertilization (IVF) outcome than normal responder patients'. The aim of our study was to analyse the effect of high serum oestradiol and progesterone concentrations at the day of human chorionic gonadotrophin (HCG) administration on endometrial receptivity and oocyte–embryo quality in high and normal responder patients. The IVF patients were divided into two groups: 59 high responder patients who voluntarily donated some of their oocytes, and a control group consisting of 105 normal responder patients. Both groups were compared in terms of the number and quality of oocytes retrieved, embryos transferred, fertilization, implantation and gestation rates, serum oestradiol and progesterone concentrations and the oestradiol:progesterone ratio on the day of HCG injection. To ascertain oocyte–embryo quality, a second control group of 96 women undergoing oocyte donation (receiving oocytes from high responder patients) was considered. To assess the impact of steroid concentrations on endometrial receptivity, high responder patients were divided into two subgroups according to oestradiol concentration, above or below the minimal oestradiol and progesterone concentrations (mean – SD) in this group. The normal responder patients were divided into two subgroups according to oestradiol concentration, above or below the maximal oestradiol and progesterone concentrations (mean + SD) in this group. To assess further the relevance of oestradiol concentration on endometrial receptivity, patients were divided into different subgroups according to increasing oestradiol concentration, regardless of whether they were high or normal responders. High responder patients had significantly decreased implantation and pregnancy rates per cycle compared with normal responder patients (33.3 versus 16.3 and 11.1 versus 5.4% respectively; $P < 0.05$). The results of 108 embryo transfers in 91 recipients who received oocytes from the high responder group showed normal embryo quality. Implantation rates and pregnancies per cycle were significantly lower in high responder patients with serum oestradiol concentrations >1700 pg/ml compared with those having oestradiol concentrations ≤1700 pg/ml, as well as in normal responder patients with serum oestradiol concentrations >2200 pg/ml compared with those having oestradiol concentrations ≤2200 pg/ml. Considering all the patients together, significant decreases in pregnancy and implantation rates were observed when oestradiol concentrations were >2500 pg/ml compared with patients having lower oestradiol concentrations. Our clinical results demonstrate that high serum oestradiol concentrations on the day of HCG injection in high and normal responder patients, regardless of the number of oocytes retrieved and the serum progesterone concentration, are detrimental to uterine receptivity without affecting embryo quality.

Key words: high responders/implantation/oestradiol/oocyte donation

Introduction

Although controversial, there is general agreement about the poor in-vitro fertilization (IVF) outcome in high responder patients compared with normal responder patients treated with gonadotrophins and gonadotrophin-releasing hormone analogues (GnRHa). We and other authors have shown that fertilization (Pellicer *et al.*, 1989a; Testart *et al.*, 1989a; Toner *et al.*, 1991), implantation (Forman *et al.*, 1988; Pellicer *et al.*, 1989a;) and pregnancy rates (Forman *et al.*, 1988; Pellicer *et al.*, 1989a; Testart *et al.*, 1989a; Toner *et al.*, 1991) decrease as the number of oocytes retrieved increases.

The search for possible answers to the lower ability to fertilize and implant in high responder patients has led to two main hypotheses: (i) decreased oocyte and embryo quality (Testart *et al.*, 1989a,b), indicated by a higher incidence of chromosomal anomalies characteristic of cytoplasmic immaturity (Tarín and Pellicer, 1990); and (ii) the anti-implantation endometrial effects of a high pre-ovulatory serum oestradiol concentration subsequent to ovarian superovulation in high responder patients. Some authors have demonstrated that ovarian stimulation inhibits embryo implantation in both mice (Fossum *et al.*, 1989) and humans after IVF (Forman *et al.*, 1988; Pellicer *et al.*, 1989a; Paulson *et al.*, 1990) by decreasing endometrial receptivity. Others reported no correlation between oestradiol concentration and pregnancy or implantation rates (Haning *et al.*, 1984), while Chenette *et al.* (1990) showed that high serum oestradiol concentrations were not detrimental to implantation in humans after IVF. Therefore, the clinical relevance of high oestradiol concentrations on endometrial receptivity in high responder patients requires further investigation. In addition, ovarian

stimulation produces elevated concentrations of other important gonadal steroids, such as progesterone. Specifically, midcycle serum progesterone concentrations in ovarian stimulation cycles, defined as premature luteinization [>0.5 ng/ml (Schoolcraft *et al.*, 1991) or >0.9 ng/ml (Silverberg *et al.*, 1991)], are associated with reduced pregnancy rates in IVF and embryo transfer cycles.

This clinical study was undertaken to analyse the effect of gonadal steroid concentrations on oocyte–embryo quality and endometrial receptivity in high responder as well as normal responder patients. To this end, we have compared the reproductive outcomes of normal versus high responder patients who underwent IVF, relating the results to oestradiol and progesterone concentration cut-offs for both groups. We have also used our oocyte donation programme to assess the embryonic quality of the oocytes obtained from the high responder group who donated some of their oocytes. Finally, we have demonstrated that high oestradiol concentrations in both groups have a deleterious effect on embryonic implantation.

Materials and methods

Subjects

The study included a total of 63 IVF cycles, corresponding to 59 high responder patients who voluntarily donated some of their oocytes. The aetiology of infertility in this group was tubal factor in 47 patients, and 12 patients with polycystic ovarian disorder. We have defined previously the concept of a high response based on a regression analysis of the proportion of unfertilized oocytes and the number of oocytes retrieved per patient (Tarín *et al.*, 1992). According to our data, we had considered previously a high response as the retrieval of ⩾11 follicles after ovarian stimulation (Pellicer *et al.*, 1989a). We have reviewed the two parameters above from the past 2 years (January 1992 to January 1994), which showed an increase in the number of oocytes retrieved in our IVF programme. Therefore, using the same concept of high response (Tarín *et al.*, 1992), the definition in our institution has changed to ⩾15 follicles retrieved as a result of more aggressive treatments which have increased the number of oocytes collected.

An initial control group consisted of 114 cycles, corresponding to 105 normal responder patients (<15 oocytes retrieved) for whom tubal factor was the only apparent cause of infertility. To rule out time variations, we included only those patients who underwent IVF simultaneously with the high responder patients.

A second control group of patients was composed of 108 cycles, corresponding to 96 women undergoing oocyte donation because of premature ovarian failure or low response. These patients received oocytes from the high responder group.

High responder patients were divided into two oestradiol and progesterone concentration subgroups above or below the minimal oestradiol concentration (mean – SD) in this group. Normal responder patients were divided into two subgroups according to oestradiol and progesterone concentrations above or below the maximal oestradiol concentration (mean + SD) in this group.

Stimulation protocol

The ovarian stimulation protocol using GnRHa and gonadotrophins has been described previously (Pellicer *et al.*, 1989b). Briefly, a long protocol was used for pituitary desensitization with s.c. administration of leuprolide acetate (1 mg/day; Procrin; Abbot Scientific SA, Madrid, Spain), commencing in the luteal phase of the previous cycle. Serum oestradiol concentrations <60 pg/ml (conversion factor to SI unit = 3.671) and negative vaginal sonographic scans were used to define ovarian quiescence. On days 1 and 2 of ovarian stimulation, two ampoules per day of human menopausal gonadotrophin (HMG; Pergonal; Serono Laboratories, Madrid, Spain) together with two ampoules per day of follicle stimulating hormone (FSH; Fertinorm; Serono Laboratories) were administered. On days 3, 4 and 5 of ovarian stimulation, three ampoules per day of HMG were administered to each patient. Commencing on day 6, HMG was administered on an individual basis according to serum oestradiol concentration and transvaginal ovarian ultrasound scans. The criteria for human chorionic gonadotrophin (HCG) administration (10 000 IU; Profasi; Serono Laboratories) were the presence of two or more follicles >19 mm in diameter and a serum oestradiol concentration >800 pg/ml (2.94 nmol/l). Leuprolide acetate and HMG were discontinued on the day of HCG administration. Oocyte retrieval was scheduled 36–38 h after HCG administration. Intravaginal micronized progesterone (400 mg/day; Utrogestan; Laboratoires Besins-Iscovesco, Paris, France) was administered as luteal support.

Patients undergoing oocyte donation received steroid replacement therapy as described previously (Remohí *et al.*, 1993), with the following modifications. Patients with ovarian function were desensitized with leuprolide acetate in the secretory phase of the previous cycle. The hormone replacement protocol commenced on day 1 of the cycle, with the administration of oestradiol valerate (Progynova; Schering, Madrid, Spain) at 2 mg/day on days 1–8, 4 mg/day on days 9–11 and 6 mg/day from day 12 onwards. After 13 days of oestradiol valerate administration, recipients were ready to receive donated oocytes and they waited until a donation became available. On the day of recovery of donated oocytes, 100 mg/day i.m. of natural oil progesterone were administered. Embryo transfer was performed on day 3, after oocyte recovery. The regimen of 6 mg/day of oestradiol valerate and 100 mg/day of progesterone was maintained for 15 days, and then a urinary β-HCG analysis was performed. In cases of positive results, oestradiol valerate was increased to 8 mg/day and progesterone was maintained at the same dose until day 100 of pregnancy.

IVF procedure

The standard IVF procedure has been described previously (Tarín *et al.*, 1992). Oocyte–cumulus complexes were evaluated under the dissecting microscope and classified according to Laufer *et al.* (1983). The oocyte–cumulus complexes were incubated at 37°C under 5% CO_2 in air. Four hours later oocytes were inseminated. Embryos were scored on the transfer day according to their morphology under the dissecting microscope. Four types of embryo were established, ranging from types I to IV (Conaghan *et al.*, 1993). Type I embryos were the best and were defined as round well-shaped blastomeres without fragments. Our policy for embryo transfer was to select as many type I embryos as possible, while the remaining embryos were cryopreserved with 1,2-propanediol and sucrose. Only patients with freshly transferred embryos were included in this study.

Steroid hormone assays

Oestradiol and progesterone were measured in serum using commercially available kits. Oestradiol was analysed using an immunoenzymatic assay (MEIA; IMx; Abbott Scientific SA). The inter-assay and intra-assay variabilities for oestradiol at a concentration of 140 pg/ml (513.8 pmol/l) were 6.7 and 6.3% respectively. Progesterone was measured using a radioimmunoassay (Biomerieux, Charbonnieres Les Bains, France). Coefficients of variation at a concentration of 0.6 ng/ml (1.9 nmol/l) were 9 and 10% respectively.

Table I. In-vitro fertilization outcome in normal responder versus high responder patients. Values are mean ± SD

	Normal responder	High responder
No. of cycles	114	63
No. of patients	105	59
Age (years)	33.1 ± 3.8[a]	30.3 ± 2.8
No. of days stimulation	9.8 ± 2.6	9.7 ± 2.5
Total dose of gonadotrophins (ampoules)	25.5 ± 6.8	24.7 ± 5.5
Oestradiol day of HCG (pg/ml)[b]	1410 ± 780[a]	3194 ± 1637
Progesterone day of HCG (ng/ml)	0.5 ± 0.4[a]	0.9 ± 0.7
Oestradiol:progesterone ratio[c]	11.3 ± 39.3	14.5 ± 43.2
No. of oocytes retrieved	8.5 ± 2.8[a]	25.7 ± 9.6
Type I	(53.7 ± 26.6)	(52.2 ± 21.0)
Type II	(36.2 ± 24.3)	(35.9 ± 17.8)
Type III	(8.7 ± 13.9)	(11.9 ± 12.0)
Fertilization rate (%)	65.5 ± 24.1	59.6 ± 25.2
No. of embryos transferred per cycle	3.7 ± 1.2[a]	4.1 ± 0.9
No. of types I and II embryos transferred per cycle	3.1 ± 1.4	3.3 ± 1.2
No. of pregnancies per cycle (%)	38/114 (33.3)[a]	10/61 (16.4)
Implantation rate (%)	48/432 (11.1)[a]	14/258 (5.4)

HCG = human chorionic gonadotrophin. Values in parentheses are percentages.
[a]Significantly different when compared with the high responder group ($P < 0.05$).
[b]Conversion factor to SI units = 3.671.
[c]All progesterone values were converted to pg/ml to calculate the oestradiol:progesterone ratio.

Statistical evaluation

Data were expressed as mean ± SD. For statistical comparison among groups, Student's *t*-test, the χ^2 test and an analysis of variance were applied. A value of $P < 0.05$ was considered to be statistically significant. The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS).

Results

Table I compares the IVF outcome of normal responder with high responder patients. As expected, there was a significant increase in the oestradiol ($P < 0.001$) and progesterone ($P < 0.01$) concentrations on the day of HCG injection in the high responder group. Interestingly, although the high responder patients were significantly younger (30.3 ± 2.8 versus 33.1 ± 3.8 years) and the number of embryos transferred was increased (4.1 ± 0.9 versus 3.7 ± 1.2), this group had significantly decreased implantation ($P < 0.01$) and pregnancy ($P < 0.02$) rates per cycle. The quality of the oocytes retrieved, the oestradiol:progesterone ratio and the fertilization rate, as well as the number of types I and II embryos transferred, were not different between the groups.

From this first approach to the evaluation of our results, we observed that implantation was impaired significantly in high responder patients. Whether the endometrial receptivity or the quality of the embryos replaced, or both, were responsible for such an observation was addressed by analysing the results of our oocyte donation programme.

Table II shows the results of 108 embryo transfers in 96 recipients who received oocytes from the high responder group considered in this study versus our standard oocyte donation. There was no difference between groups in the fertilization, implantation or pregnancy rates per cycle, despite the origin of the embryos replaced in each group. These clinical data indicate that oocyte and embryo quality is not affected in high responder patients.

To demonstrate that higher concentrations of oestradiol, progesterone or both, rather than the number of oocytes retrieved, are important to the endometrial receptivity, we analysed the cut-off concentrations of both steroids in normal and high responder patients. Two critical break-points were used: (i) the maximal (mean + SD) serum oestradiol (1410 + 780 pg/ml) and progesterone (0.5 + 0.4 ng/ml) concentrations in normal responders (Table I); we chose as maximal concentrations, cut-offs of 2200 pg/ml and 0.9 ng/ml for oestradiol and progesterone respectively; and (ii) in the high responder group (Table I), minimal (mean − SD) serum oestradiol (3194 − 1637 pg/ml) and progesterone (0.9 − 0.7 ng/ml) concentrations of 1700 pg/ml and 0.2 ng/ml were selected respectively.

The serum progesterone cut-off above or below 0.9 ng/ml in the normal responder group showed two subgroups with no differences in terms of implantation or pregnancy rates. Similarly, the serum progesterone cut-off above or below 0.2 ng/ml in the high responder group showed no differences in terms of implantation or pregnancy rates.

Table III shows the IVF outcomes of high responder

Table II. Reproductive outcome in recipients of high responder oocytes versus recipients of standard population oocytes. Values are mean ± SD.

	Recipients of high responder oocytes	Recipients of ovum donation programme[a]
No. of cycles	108	216
Age (years)[b]	36.1 ± 5.5	36.7 ± 0.3
No. of oocytes inseminated[b]	8.2 ± 2.9	8.1 ± 0.1
Fertilization rate (%)[b]	76.5 ± 20.2	71.3 ± 17.2
No. of embryos transferred per cycle[b]	4.1 ± 1.0	4.0 ± 0.1
No. of pregnancies/cycle[b]	58/108 (53.7)	114/216 (52.8)
Implantation rate (%)[b]	77/517 (14.9)	157/880 (17.8)

[a]In this group, high responder patients considered for this study were excluded.
[b]Inter-group differences were not statistically significant.

Table III. In-vitro fertilization outcome in high responders by oestradiol concentration. Values are mean ± SD.

	≤1700 pg/ml[a]	>1700 pg/ml[a]
No. of cycles	11	52
Age (years)	30.9 ± 2.7	30.2 ± 2.9
No. of oocytes retrieved	21.9 ± 8.1	26.2 ± 10.2
No. of oocytes inseminated	14.1 ± 5.2	13.7 ± 3.5
Progesterone day of HCG (ng/ml)	0.6 ± 0.4	0.9 ± 0.7
Oestradiol:progesterone ratio[b]	6.0 ± 12.7	16.8 ± 48.2
Fertilization rate (%)	56.6 ± 23.1	59.9 ± 25.2
No. of embryos transferred per cycle	4.4 ± 0.8	4.0 ± 0.9
No. of types I and II embryos transferred per cycle	3.6 ± 0.8	3.3 ± 1.2
No. of pregnancies per cycle (%)	5/11 (45.5)[c]	5/52 (9.6)
Implantation rate (%)	6/49 (12.2)[c]	8/209 (3.8)

HCG = human chorionic gonadotrophin. Values in parentheses are percentages.
[a]Conversion factor to SI units = 3.671.
[b]All progesterone values were converted to pg/ml to calculate the oestradiol:progesterone ratio.
[c]Significantly different when compared with the oestradiol concentration >1700 pg/ml subgroup ($P < 0.05$).

Table IV. In-vitro fertilization outcome in normal responders by oestradiol concentration. Values are mean ± SD.

	≤2200 pg/ml[a]	>2200 pg/ml[a]
No. of cycles	90	24
Age (years)	33.5 ± 4.5	33.0 ± 3.7
No. of oocytes retrieved	8.4 ± 2.9	8.5 ± 2.6
Progesterone day of HCG (ng/ml)	0.5 ± 0.3	0.5 ± 0.4
Oestradiol:progesterone ratio[b]	6.8 ± 21.7[c]	28.8 ± 74.7
Fertilization rate (%)	67.7 ± 23.6	59.8 ± 25.1
No. of embryos transferred per cycle	3.9 ± 1.2	3.3 ± 1.0
No. of types I and II embryos transferred per cycle	3.2 ± 1.5	2.9 ± 1.3
No. of pregnancies per cycle (%)	36/90 (40.0)[c]	2/24 (8.3)
Implantation rate (%)	46/351 (13.1)[c]	2/81 (2.5)

HCG = human chorionic gonadotrophin. Values in parentheses are percentages.
[a]Conversion factor to SI units = 3.671.
[b]All progesterone values were converted to pg/ml to calculate the oestradiol:progesterone ratio.
[c]Significantly different when compared with the oestradiol concentration >2200 pg/ml subgroup ($P < 0.05$).

patients divided into two subgroups according to oestradiol concentrations above or below the minimal oestradiol concentration (1700 pg/ml) in this group. There were no differences between the two subgroups in the number and quality of oocytes retrieved, fertilization rates, oestradiol:progesterone ratios and the total number and types I and II of embryos transferred. However, implantation and pregnancy rates per cycle were significantly ($P < 0.05$) lower in high responder patients with serum oestradiol concentrations >1700 pg/ml compared with those having oestradiol concentrations ≤1700 pg/ml.

Table IV shows the IVF outcome of normal responder patients divided into two subgroups according to oestradiol concentration above or below the maximal oestradiol concentration (2200 pg/ml) in this group. Interestingly, implantation and pregnancy rates per cycle were significantly ($P < 0.05$) reduced in normal responder patients with serum oestradiol concentrations >2200 pg/ml compared with those having oestradiol concentrations ≤2200 pg/ml.

Finally, to assess further the relevance of oestradiol concentration on endometrial receptivity, we compared the IVF outcome of all patients (high and normal responders together) according to increasing oestradiol concentrations (Table V). Again, a significant decrease in pregnancy and implantation rates was observed when oestradiol concentrations increased. Specifically, there was a drop in endometrial receptivity when oestradiol concentrations were >2500 pg/ml compared with those having lower oestradiol levels.

Discussion

The process of implantation is crucial in determining the outcome of assisted reproductive technology treatments. Specifically, IVF and embryo transfer, in which a low implantation rate is considered to be the limiting factor for a successful pregnancy, have directed attention towards embryonic implantation.

This study was undertaken to investigate an empirical observation that 'high responder patients have poorer IVF outcomes than normal responder patients'. We have presented clinical evidence for a deleterious effect of high serum oestradiol concentrations at the day of HCG administration on implantation, not only in high responder patients but also in normal responder patients treated with gonadotrophins and GnRHa. Furthermore, the data obtained from our oocyte donation programme indicate that oocyte–embryo quality is not affected in these patients.

Our clinical data concerning oocyte donation are in agreement with previous studies demonstrating that oocyte and embryo quality are independent of the donor serum oestradiol (Diamond *et al.*, 1985; Chenette *et al.*, 1990) and progesterone concentrations (Legro *et al.*, 1993). These results appear to differ from those reported by Toner *et al.* (1991), Testart *et al.* (1989a) and our previously reported results (Pellicer *et al.*, 1989a). However, these previous studies are different in concept, in GnRHa used and in experimental design. In addition, the concept of high response has changed in this study.

Our results provide evidence for an alteration of uterine receptivity in high responder patients compared with normal responder patients. It is of interest to note 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 and the progesterone concentration. This observation reinforces the concept of a direct adverse effect of higher oestradiol concentrations at the moment of HCG administration, rather than the number of oocytes retrieved on endometrial receptivity. The oestradiol:progesterone ratio has been proposed as a better indicator of pregnancy failure than the absolute concentration of either hormone (O'Neill *et al.*, 1985; Gidley-Baird *et al.*, 1986). However, our study is in agreement with that of Forman *et al.* (1988) who demonstrated that the ratio of these two hormones on the day of HCG administration is not important in predicting implantation.

The clinical relevance of high oestradiol concentrations on endometrial receptivity is controversial. Some authors have

Table V. In-vitro fertilization outcome by oestradiol concentration in both normal and high responders combined. Values are mean ± SD

	Oestradiol concentration (pg/ml)[a]							
	<500	500–1000	1000–1500	1500–2000	2000–2500	2500–3000	3000–3500	>3500
No. of cycles	11	31	37	30	21	11	16	20
Age (years)	32.9 ± 4.8	32.8 ± 4.2	33.4 ± 4.2	32.1 ± 4.3	31.7 ± 2.6	32.9 ± 3.8	30.2 ± 4.0	31.6 ± 3.3
No. of oocytes retrieved	8.3 ± 6.4	9.6 ± 6.8	11.7 ± 6.6	12.9 ± 5.5	14.2 ± 6.7	21.2 ± 7.4	20.3 ± 7.9	33.1 ± 11.6
Fertilization rate (%)	62.8 ± 28.6	67.8 ± 28.4	61.0 ± 23.2	61.2 ± 2.4	63.6 ± 21.1	57.9 ± 25.8	64.1 ± 23.4	58.8 ± 19.9
No. of embryos transferred per cycle	4.3 ± 1.2	3.4 ± 1.3	4.2 ± 1.3	4.1 ± 0.9	4.0 ± 0.9	3.9 ± 1.1	3.7 ± 0.8	4.4 ± 1.0
No. of types I and II embryos transferred per cycle	3.5 ± 1.7	3.1 ± 1.5	3.2 ± 1.7	3.4 ± 1.3	3.5 ± 0.9	3.3 ± 1.4	3.6 ± 1.1	3.6 ± 1.3
No. of pregnancies per cycle (%)	4/11 (36.4)	13/31 (41.9)[b]	8/37 (21.6)	15/30 (50.0)[b]	7/21 (33.3)	1/11 (9.1)[c]	3/16 (18.8)[c]	3/20 (15.0)[c]
Implantation rate (%)	6/38 (15.8)	15/95 (15.8)	12/119 (10.1)	14/72 (19.4)[b]	8/74 (10.8)	2/36 (5.6)[c]	3/57 (5.3)[c]	5/73 (6.8)[c]

[a]Conversion factor to SI units = 3.671.
[b,c]Significantly different when compared against each other ($P < 0.05$).

shown that ovarian hyperstimulation inhibits embryo implantation in both mice (Fossum *et al.*, 1989) and humans after IVF (Forman *et al.*, 1988; Pellicer *et al.*, 1989a; Paulson *et al.*, 1990) by decreasing endometrial receptivity. Specifically, Forman *et al.* (1988) demonstrated that oestradiol concentrations >2320 pg/ml were detrimental to implantation when one or two embryos were transferred. Others reported no correlation between oestradiol concentration and pregnancy or implantation rates (Haning *et al.*, 1984), while Chenette *et al.* (1990) showed that very high serum oestradiol concentrations (>2777 pg/ml) were not detrimental to implantation in humans after IVF (Chenette *et al.*, 1990). Our results, considering both high and normal responders, indicate that there is a drop in endometrial receptivity, as demonstrated by a significant decrease in pregnancy and implantation rates when oestradiol concentrations are >2500 pg/ml (Table V).

The pre-nidatory oestradiol requirements for implantation in humans are very slight. The majority of experts in this field appear to agree with the concept that oestradiol is permissive but not essential in mammals that do not undergo diapause (Ghosh *et al.*, 1994; De Ziegler, 1995; Edgar, 1995; Ghosh and Sengupta, 1995). Another distinct concept raised by the introduction of ovulation induction techniques is the maximal requirement of oestradiol (if any) to allow successful implantation. This clinical study suggests the existence of an oestradiol window with an upper threshold at the time of HCG administration. An elevation above this threshold could be deleterious for embryonic implantation.

The possible mechanisms responsible for our clinical observations remain unknown. Moreover, this study does not consider secretory products that may be altered, other than ovarian steroids. There are numerous discrepant results showing the effect of ovulation induction on endometrial development. Previous studies have described a high incidence of endometrial glandular advancement (Garcia *et al.*, 1984; Forman *et al.*, 1988) or retardation (Sterzik *et al.*, 1988) in ovulation induction regimens. Recently, Benadiva and Metzger (1994) demonstrated an endometrial asynchrony in 57% of endometrial biopsies from women undergoing ovulation induction with HMG, versus only 13% obtained during non-stimulated cycles. Endometrial asynchrony, defined as the dating of glandular epithelium, differs by >2 days when compared with stroma. Nonetheless, there was no difference in oestradiol or progesterone concentrations in cycles with asynchrony versus those with coordinate development. On the contrary, Macrow *et al.* (1994) failed to demonstrate any adverse effect of ovulation induction using GnRHa, HMG and HCG on the endometrial structure. Additionally, Hadi *et al.* (1994) indicated the existence of a reduction in steroid receptors without detectable morphological changes in the presence of supraphysiological concentrations of steroids. To confuse this problem further, Catellbaum *et al.* (1993) demonstrated that an early luteal 'out of phase' biopsy can be overcome physiologically because a second and later (in the same cycle) biopsy showed normal maturation. We believe that these discrepancies are caused by variations in ovulation induction protocols, the timing of endometrial biopsy and the absence of morphological, functional or immunohistochemical criteria of endometrial receptivity. Nevertheless, our clinical data confirm an impairment of endometrial receptivity in patients undergoing ovulation induction and having higher concentrations of oestradiol.

In summary, by using the combination of IVF, embryo transfer and oocyte donation techniques, this study demonstrates that uterine receptivity and not oocyte–embryo quality is affected in patients undergoing ovulation induction and with high serum oestradiol concentrations on the day of HCG administration, regardless of the number of oocytes retrieved and the progesterone concentration.

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