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Percentile curves of serum estradiol levels during controlled ovarian stimulation in 905 cycles stimulated with recombinant FSH show that high estradiol is not detrimental to IVF outcome

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BACKGROUND: High, normal and poor responders are usually defined by reference to subjectively selected estradiol (E_2) levels at days 4–6 and the day of hCG administration (d-hCG). The purpose of this study was to use E_2 percentile curves from day 5 until d-hCG to determine high, normal and poor responders, and to predict IVF outcome. METHODS: In this retrospective study, 762 patients underwent 905 cycles with a GnRH agonist/recombinant FSH short protocol. They were divided into three groups according to their age. Percentile E_2 curves according to E_2 levels were plotted. High responders were those patients with E_2 levels above the 90th percentile, normal responders had E_2 between the 10th and 90th percentiles, and poor responders had E_2 below the 10th percentile. RESULTS: IVF outcome, expressed as number of oocytes, total embryos obtained and number of high grade embryos, was significantly better for patients with E_2 above the 90th percentile at d-hCG for the three age groups and at day 5 for group A (<35 years). Pregnancy rates were higher for high responders, but the difference did not reach statistical significance. CONCLUSIONS: Percentile curves can be useful in controlled ovarian stimulation cycles to define high, normal and poor responders, and also to predict IVF outcome.

Key words: estradiol/hyperstimulation/ovarian stimulation/percentiles/rFSH

Introduction

Controlled ovarian stimulation (COS) for IVF cycles is usually monitored by serum estradiol (E_2) levels and pelvic ultrasonography with two purposes: (i) to obtain an adequate number of mature oocytes, and (ii) to prevent the risk of severe ovarian hyperstimulation syndrome (OHSS).

In order to decide whether to proceed with an ongoing cycle and to define the so-called poor and high responders, Phelps *et al*. and Khalaf *et al*. used different cut-off E_2 levels at day 4–6 and on the day of hCG administration (d-hCG) of the stimulation cycle. Poor responders, defined by E_2 levels <75 pg/ml at day 4 (Phelps *et al*., 1998) or <50 pg/ml at day 6 (Khalaf *et al*., 2000), were associated with low pregnancy and high cancellation rates. Follicular aspiration in patients with E_2 levels <500 pg/ml at d-hCG yields less than five mature oocytes (Sharara and McClamrock, 1999). On the other hand, high responders, defined as having E_2 levels >3000 pg/ml at d-hCG, are more often subject to OHSS (Asch *et al*., 1991; Morris *et al*., 1995). However, the influence of high E_2 levels on the outcome of IVF cycles is still controversial. Simon *et al*. suggested a detrimental effect of high E_2 on endometrial receptivity (Simon *et al*., 1998), but others found no adverse consequences (Sharara and McClamrock, 1999; Levi *et al*., 2001).

The use of E_2 levels to differentiate poor, normal and high responders, and to predict IVF outcome, has been the subject of debate. In most studies, cut-off levels were chosen arbitrarily and depend on various factors. Percentile curves offer a more objective definition of these three categories of patients. To our knowledge, only one study has used percentile curves to adjust the doses of gonadotrophins to the ovarian response and to evaluate IVF outcome in stimulation cycles (Forman *et al*., 1988). In our centre, since 1989 we have routinely based COS on percentile E_2 curves for long and short GnRH agonist protocols combining triptorelin and hMG (J.-R.Zorn, unpublished data). Gonadotrophin dose and ovulation induction by hCG are adjusted according to the pattern of E_2 levels on the percentile curve of the corresponding protocol.

The objectives of the present study were: (i) to determine the ovarian response in COS with recombinant (r)FSH in a short GnRH agonist protocol by using E_2 percentile curves; (ii) to define low, normal and high responders; and (iii) to predict the IVF outcome by using percentile E_2 serum levels at day 5 and d-hCG.

Materials and methods

Patients

Using a computerized database, records of 2184 (COS) cycles for IVF/embryo transfer performed between January 1996 and October 2000 at the University IVF Center of Paris-Baudelocque, Cochin Hospital, Paris, were reviewed. During this period, 762 couples in an IVF programme for male, female, mixed or unexplained infertility underwent 905 conventional IVF or ICSI cycles using a GnRH agonist short (flare) protocol combining triptorelin and rFSH. Group A (women <35 years old) comprised 461 cycles, group B (35–38 years) 234 cycles and group C (>38 years) 210 cycles. There were 402 cycles of IVF and 503 cycles of ICSI. Exclusion criteria were age >43 years, day 3 FSH >15 IU/l or participation in other COS protocols. Due to the retrospective study design based on routine practice, consultation with the hospital ethics committee was not required.

Drugs and COS protocols

GnRH agonist

Triptorelin (Decapeptyl; Ipsen, Paris, France) was administered in a dose of 0.1 mg/day, starting 1–2 days after withdrawal bleeding.

rFSH

Follitropin-β (Puregon; NV Organon, Paris, France) or follitropin-α (Gonal-F; Serono, Geneva, Switzerland) were used during this study. A total of 787 cycles were stimulated with Gonal-F and 118 with Puregon.

hCG

hCG (Gonadotrophine Chorionique Endo; Organon) was used at a dose of 5000 IU/ampoule. Patients received norethisterone (NE) (Primolut Nor; Schering, Paris, France) 10 mg daily for 8–16 days starting from day 3 of a natural cycle. Triptorelin, 0.1 mg daily, was given for 6–15 days starting 4 days after the last day of NE. At day 3 of triptorelin treatment, rFSH was started at a daily dose of 3–6 ampoules (75 IU) per day. Generally, the initial dose for patients in group A was 3 ampoules, for group B, 4 ampoules and for group C, 6 ampoules. From day 5 of the stimulation cycle, doses of rFSH were adjusted according to the number of follicles found on ultrasound and to the E_2 levels. hCG 10 000 IU was injected when two or more follicles >15 mm were found. Oocyte retrieval was performed transvaginally under ultrasound guidance.

Embryology assessments

After liquefaction at 37°C, motile sperm were selected through a two-step density gradient (Puresperm; JCD, Lyon, France). The pellet was washed and resuspended in human tubal fluid (HTF) culture medium (Irvine scientific, CA, USA) supplemented with 7.5% autologous female decomplemented serum. For the IVF procedure, oocytes were inseminated with 10 000 motile sperm in microdroplets of 30 µl of HTF with 7.5% serum under mineral oil and kept under 5% CO_2 at 37°C. For ICSI, the cumulus cells around the oocytes were removed with 80 IU/ml hyaluronidase, and the oocytes were checked for their nuclear status. Only oocytes in metaphase II were microinjected and kept in HTF + 7.5% serum under 5% CO_2 at 37°C. The oocytes were checked 16–20 h after insemination or microinjection for evidence of fertilization. The oocytes were considered to be normally fertilized when two pronuclei were visible. Fertilized oocytes were kept in culture for an additional 24 h (HTF + 15% serum) and checked for cleavage. The embryos were evaluated according to the number of blastomas and the degree of fragmentation. Up to four embryos were transferred, and any remaining high grade embryos were cryopreserved.

Embryo transfer and luteal support

Two-day-old embryos were transferred using the Frydman catheter (CCD Paris, France). The number of embryos transferred was typically two, and occasionally up to four in patients >39 years old or when embryo quality was poor. Luteal support was administered in the form of vaginal progesterone (Utrogestan; Besins International, Paris, France) 800 mg per day for 15 days, and hCG 1500 IU was injected 48 h after the transfer in patients whose peak E_2 levels were above the 90th percentile (>P90).

End points and outcome measures

The main outcome for plotting rFSH E_2 patterns for the three different age groups was E_2 levels from day 5 to d-hCG. IVF outcomes were expressed as the number of oocytes, number of embryos obtained, number of high grade embryos, E_2 peak levels and pregnancy rates (PRs) at day 5 and d-hCG. High, normal and poor responders were defined as those women with E_2 levels at day 5 and d-hCG >P90, between 10th and 90th percentile (P10–P90) and below the 10th percentile (<P10) respectively. The P10 and P90 cut-offs were chosen based on previous reports for other fields of gynaecology–obstetrics (Lubchenco *et al*., 1963; Leroy and Lefort, 1971).

Laboratory analysis

E_2 determination was performed using an immunoenzyme assay by sequential competitive technique on an Immuno-1 multiparameter analyser (Bayer reference ES2). The capture antibody used was a rabbit polyclonal antibody against E_2 conjugated to fluorescein. The competitive marker was constituted by E_2 conjugated to alkaline phosphatase. Separation was performed using a dispersed solid phase that is achieved by magnetic particles linked to monoclonal antibody anti-fluorescein. The signal was read on the spectrophotometer at 405 and 450 nm. E_2 standard USP in a saline buffer bovine albumin with sodium azoture was used for calibration. The molar conversion factor was 3.67 (pmol/l = pg/ml×3.67). The analytical range was from 0 to 3600 pg/ml (0 to 13 200 pmol/l). Samples with concentration values exceeding the maximal calibrator can be diluted in the zero calibrator or in an albumin buffer. Analytical sensitivity was 5 pg/ml, corresponding to twice the SD of the zero calibrator. The functional limit of detection at the 20% of inter-assay coefficient of variation (CV) was near 10 pg/ml (36.7 pmol/l). Inter-assays imprecision show CV <20% for a high concentration of E_2 (>300 pg/ml), and near 5% for a low concentration of E_2 (<30 pg/ml). Blood samples were collected in tubes without anticoagulant and separated by centrifugation. Assays were run on the day of sampling with 50 µl of serum. The method remained unchanged during the study period.

Statistical analysis

Results are expressed as means ± SD. Statistical comparisons were made by using the χ^2-test and analysis of variances when appropriate. Statistical significance was set at $P < 0.05$. Statview software (Abacus, F-4.5, 1999; Berkeley, CA, USA) was used for statistical analysis.

Results

E_2 thresholds (P10/P90) at day 5 and d-hCG were as follows: group A, 51/466 and 1007/3444 pg/ml; group B, 44/463 and 851/3414 pg/ml; and group C, 50/486 and 868/3155 pg/ml. Relatively high P10 and P90 levels for group C compared with group B may be explained by the higher starting doses of rFSH used for patients >38 years old. The percentile curves of E_2 levels from day 5 to d-hCG are presented in Figure 1. The general IVF outcomes of the three groups are presented

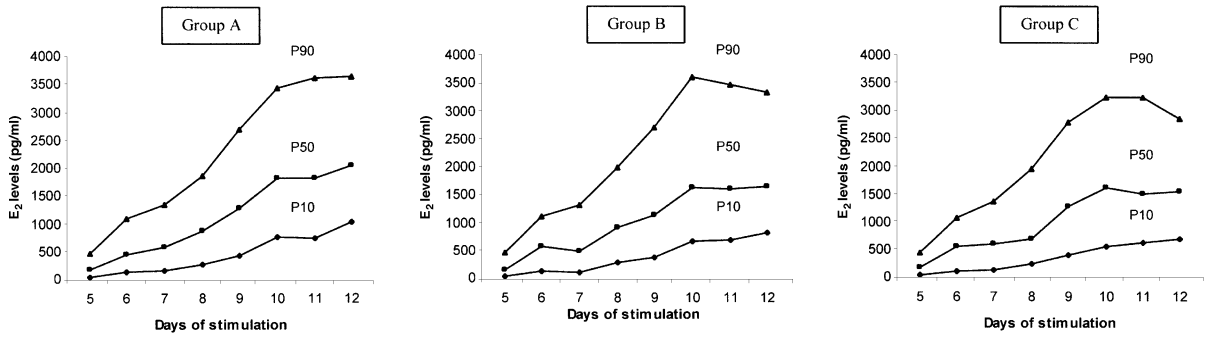

Figure 1. Estradiol percentile curves for group A (<35 years), group B (35–38 years) and group C (>38 years).

Table I. Reproductive outcome of three ages groups stimulated with rFSH in GnRH agonist short protocol

Variable	Group A (<35 years)	Group B (35–38 years)	Group C (>38 years)
Cycles	461	234	210
Age	31.2 ± 2.6	36.5 ± 0.8	39.8 ± 1.4
Ampoules rFSH (75 IU/ml)	25.2 ± 16.5	29.7 ± 18.0	32.1 ± 18.7
Peak E_2 (pg/ml)	2204.5 ± 1024.2	2031.0 ± 954.9	1974.0 ± 949.8
Oocytes	8.2 ± 5.3	6.7 ± 4.0	6.1 ± 3.7
Metaphase II	6.5 ± 4.2	5.4 ± 3.1	4.9 ± 3.2
Total embryos	4.2 ± 3.1	3.4 ± 2.6	3.2 ± 2.7
High grade embryos	3.4 ± 2.7	2.7 ± 2.1	2.6 ± 2.3

Values are means ± SD.

in Table I. As expected, lower doses of rFSH were used in younger patients (group A), resulting in higher peak E_2 values, a higher number of total and metaphase II oocytes retrieved and a greater number of total and high grade (grade I and II) embryos obtained.

IVF outcomes for high, normal and poor responders in groups A, B and C are presented in Table II. There was no difference between the ages of poor, high and normal responders for all groups. This eliminates the possibility of bias for comparisons between different responders in the same group of patients. The number of oocytes, total embryos and high grade embryos was higher in >P90 and lower in <P10 responders in all age groups. Differences were statistically significant ($P < 0.05$) only in younger patients (group A) at day 5. For d-hCG results, statistical significance was reached for all groups. A possible interpretation for this is that between day 5 and d-hCG, the dose of rFSH is adjusted according to the ovarian response. Pregnancy rates were also higher in high responders and lower in poor responders, but differences did not reach statistical significance in any group. Three patients (0.3%) (two from group A and one from group B) were hospitalized for severe OHSS. Interestingly, the three patients belonged to the P10–P90 group. Seven patients (0.8%), four from the P10–P90 group and three from the >P90 group, were treated as out-patients for moderate OHSS.

Discussion

The challenge of COS is to achieve a reasonable balance between two conflicting outcomes, namely obtaining the maximum number of mature oocytes and embryos without exposing patients to the risk of severe OHSS. It is useful to identify high responders early in order to try to prevent OHSS, and to identify poor responders to prevent cancellation. The difficulty is to properly define high, normal and poor responders to COS. This was the subject of a recent discussion (McDonough, 1999) which pointed out that most studies suffered from small sample sizes and nearly all used different definitions based upon E_2 cut-off levels. In addition, determination of E_2 values depends on many factors, including hormonal assay methodology. Therefore, cut-off levels expressed as absolute values of E_2 in pg or pmole/l from one centre may not be used universally.

For poor responders identified at day 5, P10 E_2 were ~50 pg/ml, which are similar to those previously described in IVF cycles stimulated with gonadotrophins, either with or without GnRH agonist. In the study by Phelps *et al*. E_2 levels <75 pg/ml at day 4 yielded poor IVF outcomes (Phelps *et al*. 1998), and for Khalaf *et al*. E_2 levels <50 pg/ml at day 6 had higher cancellation rates and poorer IVF results (Khalaf *et al*., 2000). Sharara and McClamrock defined poor responders as patients who had <500 pg/ml E_2 at d-hCG (Sharara and McClamrock, 1999). In the present study, it is of interest that poor responders at day 5 had better IVF outcomes than poor responders identified later at d-hCG for all groups of patients. This can be explained by the fact that doses of rFSH were increased in cases of poor response at day 5, and this increase obviously improved the ovarian response. This difference was not observed for normal responders, probably because of the unchanged initial dose of rFSH in these patients.

Phelps *et al*. considered high responders to be patients with E_2 levels >200 pg/ml at day 4 (Phelps *et al*., 1998). Interestingly, none of our patients with E_2 >P90 at day 5 was hospitalized for OHSS. This may be explained by the reduction of rFSH dose for those patients after day 5. For our high responders identified at d-hCG, P90 E_2 levels were similar to those of high responders defined by others (Simon *et al*., 1998; Sharara and McClamrock, 1999). Generally, in our study, patients with E_2 levels >P90 had significantly better IVF outcomes (expressed as numbers of oocytes, total embryos and high-grade embryos) than normal responders (P10–P90) and, as expected, than poor responders. In the only study in which E_2 percentile curves were used to predict IVF outcomes, Forman *et al*. showed lower PRs for patients with E_2 >P90 (>2320 pg/ml) (Forman *et al*., 1988). Of note is that this

Table II. IVF outcomes for group A (<35 years), group B (35–38 years) and group C (>38 years)

Variable	<P10 day 5	P10–P90 5 day	>P90 day 5	*P*-value	<P10 day hCG	P10–P90 day hCG	>P90 day hCG	*P*-value
Group A								
Age	31.3 ± 2.6	31.2 ± 2.5	30.8 ± 3.0	NS[a]	31.5 ± 2.1	31.1 ± 2.5	30.6 ± 3.1	NS[a]
Peak E_2	1587 ± 630	2178 ± 975	3268 ± 1259	–	733 ± 180	2035 ± 638	4233 ± 928	–
No. oocytes	7.8 ± 4.5	8.0 ± 5.5	9.4 ± 5.0	NS[a]	4.3 ± 3.4	7.9 ± 4.6	13.6 ± 7.7	< 0.05[a]
No. total emb.	3.8 ± 2.9	4.1 ± 3.1	5.5 ± 3.9	< 0.05[a]	2.2 ± 1.5	4.1 ± 2.9	6.4 ± 3.8	< 0.05[a]
No. high grade emb.	2.8 ± 2.6	3.3 ± 2.7	4.4 ± 3.2	< 0.05[a]	1.8 ± 1.4	3.3 ± 2.6	5.3 ± 3.4	< 0.05[a]
PR (%)	29.7	21.2	33.3	NS[b]	15.5	22.2	30.4	NS[b]
Group B								
Age	36.6 ± 0.8	36.4 ± 0.8	36.7 ± 0.6	NS[a]	36.5 ± 0.8	36.4 ± 0.8	36.5 ± 0.8	NS[a]
Peak E_2	1311 ± 747	1985 ± 903	2972 ± 661	–	698 ± 130	1963 ± 661	3904 ± 520	–
No. oocytes	5.4 ± 3.7	6.8 ± 4.1	6.6 ± 2.6	NS[a]	2.5 ± 1.5	6.8 ± 3.8	9.3 ± 4.5	< 0.05[a]
No. total emb.	2.3 ± 2.0	3.4 ± 2.6	4.2 ± 2.4	NS[a]	1.3 ± 1.0	3.4 ± 2.4	5.3 ± 3.1	< 0.05[a]
No. high grade emb.	1.8 ± 2.1	2.7 ± 2.1	3.3 ± 1.9	NS[a]	1.0 ± 0.9	2.6 ± 2.0	4.2 ± 2.3	< 0.05[a]
PR (%)	17.6	19.8	21.0	NS[b]	8.6	20.1	21.7	NS[b]
Group C								
Age	40.0 ± 1.4	39.7 ± 1.2	39.4 ± 1.2	NS[a]	39.9 ± 1.4	39.8 ± 1.3	39.6 ± 1.4	NS[a]
Peak E_2	1188 ± 546	1891 ± 841	3026 ± 962	–	610 ± 175	1887 ± 595	3897 ± 781	–
No. oocytes	5.7 ± 3.3	5.8 ± 3.4	7.5 ± 4.4	NS[a]	2.8 ± 2.2	5.9 ± 3.1	10.5 ± 4.8	< 0.05[a]
No. total emb.	3.2 ± 3.4	2.9 ± 2.3	4.1 ± 3.1	NS[a]	1.9 ± 1.5	3.1 ± 2.3	5.5 ± 3.5	< 0.05[a]
No. high grade emb.	2.4 ± 2.0	2.3 ± 2.0	3.4 ± 2.6	NS[a]	1.0 ± 1.2	2.5 ± 2.2	4.8 ± 2.8	< 0.05[a]
PR (%)	29	13	29	NS[b]	5.2	15.5	14.2	NS[b]

Values are means ± SD.
[a]Analysis of variance.
[b]χ^2-test.
NS = not significant.

study was published before the GnRH agonist era. Our data are consistent with those previously published by Sharara and McClamrock (Sharara and McClamrock, 1999).

The issue of deleterious effects of high E_2 levels on uterine receptivity has been under debate for many years. High levels of steroid hormones induce morphological alterations of the endometrium (Garcia *et al*., 1984; Bladford *et al*., 1997) and alter endometrial E_2/progesterone ratios, which is associated with impairment of endometrial receptivity (Gidley-Baird *et al*., 1986). In COS, a significant reduction of the nuclear receptors for progesterone and E_2 has been demonstrated in both glands and stroma (Hadi *et al*., 1994). Several studies have shown a negative effect of high E_2 levels on pregnancy and implantation rates (Testart *et al*., 1986; Pellicer *et al*., 1996; Simon *et al*., 1998). Moreover, Valbueña *et al*. found that a high E_2 concentration affects embryonic adhesion (Valbueña *et al*., 2001). However, most of these studies used small sample sizes and different definitions of high responders, or dealt with in-vitro models. Our results are from a large cohort of human subjects are consistent with previously reported data (Chenette *et al*., 1990; Tarin *et al*., 1992; Loumaye *et al*., 1997; Sharara and McClamrock, 1999; Levi *et al*., 2001). According to our study, we did not find any deleterious effect of high E_2 levels, and patients with E_2 >P90 had better IVF outcomes in all group of patients. Our three cases of severe OHSS belonged to group A (<35 years) and were normal responders (d-hCG E_2 = P10–P90). No cases of severe OHSS were detected in patients with E_2 >P90 at d-hCG, in contrast to previously published data which reported incidences of 0.94 and 2.9% of severe OHSS (Asch *et al*., 1991; Jaffe *et al*., 1993).

An important difference between our study and prior reports (Simon *et al*., 1998; Sharara and McClamrock, 1999; Khalaf *et al*., 2000) is the stimulation regimen. We cannot exclude the possibility that the type of gonadotrophin influences oocyte and embryo quality. Moreover, high responders with rFSH may behave differently. We used both types of rFSH (follitropin-α and -β) and we do believe that this has affected our results, as previous reports have shown that there are no differences in the biological and clinical activity of the two rFSH hormone preparations (Brinsden *et al*., 2000; Harlin *et al*., 2002).

In conclusion, we believe that percentile curves can help practitioners to follow stimulation cycles and to adjust the dose of the stimulation regimen according to the ovarian response. Percentile curves have been used successfully in other fields of gynaecology–obstetrics, such as fetal ultrasonography, and can be useful in COS monitoring. Our high responders (E_2 >P90) had better IVF outcomes than normal and poor responders in all age groups. More studies with larger sample sizes using the same protocols have to be performed for further confirmation of our results.

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References

Asch, R.H., Li, H.P., Balmaceda, J.P., Weckestein, L.N. and Stone, S.C. (1991) Severe ovarian hyperstimulation syndrome in assisted reproductive technologies: definition of high risk groups. *Hum. Reprod*., **6**, 1395–1399.

Bladford, A.J., Najmabadi, S. and Paulson, R.J. (1997) Ultrastructural characteristics of the luteal phase endometrium in donors undergoing controlled ovarian hyperstimulation. *Fertil. Steril*., **67**, 625–630.

Brinsden, P., Akagbosu, F., Gibbons, L.M., Lancaster, S., Gourdon, D., Pharm, D., Engrand, P. and Loumaye, E. (2000) A comparison of the efficacy and tolerability of two recombinant human follicle-stimulating

hormone preparations in patients undergoing *in vitro* fertilization–embryo transfer. *Fertil. Steril*., **73**, 114–116.

Chenette, P.E., Sauer, M.V. and Paulson, R.J. (1990) Very high serum E_2 levels are not detrimental to clinical outcome if *in vitro* fertilization. *Fertil. Steril*., **54**, 858–863.

Forman, R., Fries, N., Testart, J., Belaisch-Allart, J., Hazout, A. and Frydman, R. (1988) Evidence for an adverse effect of elevated serum E_2 concentration on embryo implantation. *Fertil. Steril*., **49**, 118–122.

Garcia, J.E., Acosta, A.A., Hsiu, J.G. and Jones, H.W. (1984) Advanced endometrial maturation after ovulation induction with human menopausal gonadotrophin/human chorionic gonadotrophin for *in vitro* fertilization. *Fertil. Steril*., **41**, 31–37.

Gidley-Baird, A.A., O'Neil, C., Sinosich, M.J., Porter, R.N., Pike, I.L. and Saunders, D.M. (1986) Failure of implantation in human *in vitro* fertilization and embryo transfer patients: the effects of altered progesterone/estrogen rations in humans and mice. *Fertil. Steril*., **45**, 69–75.

Hadi, F.H., Chantler, E., Anderson, E., Nicholson, R., McClelland, R.A. and Seif, M.W. (1994) Ovulation induction and endometrial steroid receptors. *Hum. Reprod*., **9**, 2405–2410.

Harlin, J., Aanesen, A., Csemiczky, G., Wramsby, H. and Fried, G. (2002) Delivery rates following IVF treatment, using two recombinant FSH preparations for ovarian stimulation. *Hum. Reprod*., **17**, 304–309.

Jaffe, S.B., Jaffe, L.H. and Jewelewicz, R. (1993) Incidence of severe ovarian hyperstimulation syndrome with extremely elevated serum estrogen levels. *Gynecol. Obstet. Invest*., **35**, 222–227.

Khalaf, Y., Taylor, A. and Braude, P. (2000) Low serum E_2 concentrations after five days of controlled ovarian hyperstimulation for *in vitro* fertilization are associated with poor outcome. *Fertil. Steril*., **74**, 63–66.

Leroy, B. and Lefort, F. (1971) A propos du poids et de la taille des nouveaus-nés à la naissance. *Rev. Fr. Gynecol*., **66**, 391–395.

Levi, L.J., Drews, M.R., Bergh, P.A., Miller, B.T. and Scott, R.T. (2001) Controlled ovarian hyperstimulation does not adversely affect endometrial receptivity in in-vitro fertilization cycles. *Fertil. Steril*., **76**, 670–674.

Loumaye, E., Engrand, P., Howles, C.M. and O'Dea, L. (1997) Assessment of the role of serum luteinizing hormone and E_2 response to follicle-stimulating hormone on *in vitro* fertilization treatment outcome. *Fertil. Steril*., **67**, 889–899.

Lubchenco, L.O., Hansman, C., Dressler, M. and Boyd, E. (1963) Intrauterine growth as estimated from liveborn birthweight data at 24 to 42 weeks of gestation. *Pediatrics*, **32**, 793–800.

McDonough, P. (1999) low and high responders—at what level of serum E_2 do things start to get fuzzy? *Fertil. Steril*., **71**, 584–586.

Morris, R.S., Paulson, R.J., Sauer, M.V. and Lobo, R.A. (1995) Predictive value of serum E_2 concentrations and oocyte number in severe hyperstimulation syndrome. *Hum. Reprod*., **10**, 811–814.

Pellicer, A., Valbueña, D., Cano, F., Remohí, J. and Simón, C. (1996) Lower implantation rates in high responders: evidence for an altered endocrine milieu during the preimplantation period. *Fertil. Steril*., **65**, 1190–1195.

Phelps, J.Y., Levine, A.S., Hockman, T.N., Zacur, H.A., Wallach, E.E. and Hinton, E.L. (1998) Day 4 E_2 levels predict pregnancy success in women undergoing controlled ovarian hyperstimulation for IVF. *Fertil. Steril*., **69**, 1015–1019.

Sharara, F.I. and McClamrock, H.D. (1999) High E_2 levels and high oocyte yield are not detrimental to *in vitro* fertilization outcome. *Fertil. Steril*., **72**, 401–405.

Simón, C., Garcia Velasco, J.J., Valbueña, D., Peinado, J.A., Moreno, C., Remohí, J. and Pellicer, A. (1998) Increasing uterine receptivity by decreasing E_2 levels during the preimplantation period in high responders with the use of a follicle-stimulating hormone step-down regimen. *Fertil. Steril*., **70**, 234–239.

Tarín, J.J., Sampaio, M.C., Calatayud, C., Castellvi, R.M., Bonilla-Musoles, F. and Pellicer, A. (1992) Relativity of the concept 'high responder to gonadotrophins'. *Hum. Reprod*., **7**, 19–22.

Testart, J., Belaisch-Allart, J. and Frydman, R. (1986) Relationship between embryo transfer results and ovarian response and *in vitro* fertilization rates: analysis of 186 human pregnancies. *Fertil. Steril*., **45**, 237–243.

Valbueña., D., Martin, J., de Pablo, J.L., Remohí, J., Pellicer, A. and Simón, C. (2001) Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. *Fertil. Steril*., **76**, 962–968.

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