Ratio of oestradiol concentration on the day of human chorionic gonadotrophin administration to mid-luteal oestradiol concentration is predictive of in-vitro fertilization outcome

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The role of luteal oestradiol for successful implantation in humans seems to be permissive rather than obligatory. Few studies have attempted to clarify the role of early luteal oestradiol in in-vitro fertilization (IVF) outcome, whether peri-implantation oestradiol is predictive of successful IVF outcome. We retrospectively analysed 106 women undergoing 106 IVF/embryo transfer cycles. Only the first treatment cycle per patient was analysed. Peak oestradiol denoted the concentration on the day of human chorionic gonadotrophin (HCG) administration. Mid-luteal oestradiol was obtained 3 days after embryo transfer (8 days after HCG administration). A total of 44 pregnancies were noted (41.51%). There were no differences in age, cycle day 3 follicle stimulating hormone (FSH), peak oestradiol, number of retrieved oocytes, number of embryo transfers, and mid-luteal oestradiol between pregnant and non-pregnant women. However, the ratio of day of HCG oestradiol to mid-luteal oestradiol was highly predictive of successful outcome: the ongoing pregnancy rate and implantation rate (sacs with fetal heart beat/embryo transfer) were 15.8 and 5.7% respectively if the above ratio exceeded 5.0 (n = 19), compared to 42.1 and 16.3%, and 53.3 and 26.5% if the ratio was between 0.4 and 2.5 (n = 57), and between 2.5 and 5.0 (n = 30) respectively. Our study suggests that the magnitude of decline in oestradiol concentrations after oocyte retrieval may be important in predicting IVF success. We postulate that endometrial integrity may become compromised when a dramatic drop in oestradiol occurs by the mid-luteal period. Whether these women benefit from oestradiol supplementation after oocyte retrieval remains to be investigated.

Keywords: implantation/IVF/luteal phase/oestradiol/pregnancy

Introduction

The role of oestradiol in endometrial priming during the follicular phase, including vascular, epithelial, glandular, and stromal proliferation, is well established. In addition, oestradiol induces the synthesis of specific proteins and growth factors, including oestradiol and progesterone receptors. While luteal progesterone is critical in allowing implantation and early pregnancy in most species, the role of luteal oestradiol is less clear. In rodents and rabbits, luteal oestradiol seems essential for implantation (Finn, 1977; Bill and Keys, 1983), while the role of luteal oestradiol in the hamster and subhuman primate is controversial (Morris et al., 1967; Ravindranath and Moudgal, 1987; Ghosh and Sengupta, 1994; Shetty et al., 1997).

The role of luteal oestradiol in humans is also controversial. There were no differences in early (4 days after ovulation) and mid-luteal oestradiol (8 days after ovulation) between conception and non-conception in spontaneously ovulating cycles, and no such differences were noted in ovulation induction cycles (Laufer et al., 1981). Only by the late luteal phase (12 days after ovulation), significant differences in oestradiol concentrations seen between conception and non-conception cycles both during spontaneous ovulation and ovulation induction (Laufer et al., 1981). In contrast, in a more recent study using daily oestradiol measurements, Stewart et al. found a significant difference in oestradiol concentrations as early as day 6 after the luteinizing hormone (LH) surge between conception and non-conception cycles in fertile women undergoing donor insemination (Stewart et al., 1993). The day 6 rise in luteal oestradiol in conception cycles compared to non-conception cycles was also noted in a group of 32 women attempting spontaneous pregnancy (Baird et al., 1997). These findings provide evidence that a trophoblastic stimulus is present before implantation and that the ovary is capable of responding appropriately by enhancing steroid production (Stewart et al., 1993; Baird et al., 1997).

Similarly, no clear consensus has emerged on the role of luteal oestradiol in stimulated cycles (Edgar, 1995). Based on the high rate of out of phase endometrial biopsies in the luteal phase of stimulated cycles (Garcia et al., 1984), and the worry about the integrity of the ovarian follicles after oocyte aspiration (Edwards et al., 1980; Garcia et al., 1981), routine progesterone supplementation became standard in assisted reproduction, especially since gonadotrophin-releasing hormone analogues (GnRHa) were introduced (Smitz et al., 1988; Smitz et al., 1993). Garcia et al. showed a disruption in luteal function with oocyte aspiration, occurring most severely in those cycles in which vigorous, repetitive aspirations, and multiple flushings of the follicles were performed which resulted in mechanical removal of a larger granulosa cell mass (Garcia et al., 1981). Muasher et al. investigated luteal phase oestradiol in 175 in-vitro fertilization (IVF) cycles. The mean serum oestradiol did not reveal any significant differences between conception and non-conception cycles until 10 days after oocyte retrieval (Muasher et al., 1984). These findings were later confirmed in GnRHa/human menopausal gonadotrophin (HMG) cycles (Smitz et al., 1988). This decline in late luteal oestradiol in

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unsuccessful cycles raised speculations that peri-implantation endometrial development may be compromised (Smitz et al., 1988).

We therefore investigated the role, if any, of mid-luteal oestradiol in implantation and early pregnancy in an IVF population. We also tested the hypothesis that the magnitude of decline in luteal oestradiol, and not just the oestradiol concentration in the mid-luteal phase, is important in predicting IVF success, as measured by the ratio of peak oestradiol to mid-luteal oestradiol.

Materials and methods

Population

We retrospectively evaluated 106 patients undergoing IVF and embryo transfer between April 1997 and August 1998. Only the first treatment cycle per patient was analysed. All patients had been on an oral contraceptive for at least 3 weeks (range 3–6 weeks) prior to initiating ovarian stimulation to ensure ovarian quiescence.

Forty patients had tubal disease (37.7%), 22 had male factor infertility (20.8%), 22 had diminished ovarian reserve (20.8%), 12 had endometriosis (11.3%), and 10 had ovulatory dysfunction (9.4%). Some patients had more than one infertility factor.

Stimulation protocols

Women with basal follicle stimulating hormone (FSH) <8.5 IU/l, or younger than 38 years of age underwent a standard long luteal leuprolide acetate (LA) protocol (Lupron; TAP Pharmaceuticals, North Chicago, IL, USA) using a step-down regimen. After pituitary desensitization, FSH and/or HMG (Metrodin and Gonal, F; Serono, Randolph, MA, USA; and Humegon and Follistim; Organon, West Orange, NJ, USA) were started at 150–300 IU/day. The starting dose was not modified for the first 4 days, the number of ampoules was then adjusted depending on oestradiol concentrations and ultrasound findings. For women older than 38 years and those with FSH ≥8.5 IU/l, a modified microdose LA flare protocol was used. This consists of 50 μg twice daily of LA along with 450 IU daily of FSH and/or HMG starting on day 3 of a withdrawal bleed (Scott et al., 1993; Scott and Navot, 1994; Surrey et al., 1998). Using these criteria, 64 women were stimulated using a long luteal LA protocol, and 42 women were stimulated using a microdose LA flare protocol. When at least three follicles were ≥18 mm, 10 000 i.u. human chorionic gonadotrophin (HCG) (Profasi; Serono, Randolph, MA, USA) were administered. Oocyte retrieval was performed 34–36 h later. None of the follicles was flushed with media during the oocyte retrieval, and only one puncture per follicle was performed. Embryo transfer was performed 72–78 h later under ultrasound guidance using a Wallace catheter (Edwards-Wallace Catheter, Marlow Technologies, Willoughby, OH, USA). Luteal phase supplementation using 50–100 mg of intramuscular progesterone in oil was given to all patients, and none received HCG supplementation. Serum concentrations of oestradiol and progesterone were also obtained 8 days after HCG administration (day 8 oestradiol), corresponding to the peri-implantation period. If a viable pregnancy was confirmed by ultrasound, progesterone supplementation was continued until 8–9 weeks of gestation.

Laboratory analysis

For serum FSH, a microparticle enzyme immunoassay was used (Abbott Axsym system, Abbott Pharmaceuticals, Abbott Park, IL, USA). The interassay and intra-assay coefficients of variation were 3.48 and 4.52% respectively. The upper limit of normal for FSH in our laboratory is 10 IU/l (conversion factor to SI units, 1.0), which is equivalent to 18 IU/l by radioimmunoassay (RIA) (Leeco Diagnostics, Southfield, MI, USA). For oestradiol and progesterone, an RIA was used (Coat-a-count, Diagnostic Products Corporation, Los Angeles, CA, USA). The interassay and intra-assay coefficients of variation for oestradiol were 7.8 and 5.8% respectively. The interassay and intra-assay coefficients of variation for progesterone were both <10%.

Statistical analysis

Data are expressed as mean ± SD. Student's t-test, Wilcoxon signed rank test, Mann-Whitney rank sum test, Fisher's exact test, and χ² analysis were used as appropriate. P was significant at < 0.05.

Results

The mean age of the 106 patients was 33.0 ± 4.0 years (range 24–43). Mean basal (cycle day 3) FSH was 6.62 ± 2.0 IU/l (range 1.4–10.4). The mean peak oestradiol was 2543 ± 993 pg/ml (range 797–5292, conversion to SI units, 3.671), and the mean number of retrieved oocytes was 14.96 ± 5.85 (range 5–34). The mean number of pre-embryos replaced was 3.7 ± 1.0 (range 1–6). The mean day 8 oestradiol was 1037 ± 669 pg/ml (range 57–3362). Progesterone concentrations were >20 ng/dl in all patients on day 8. There was a significant decrease in oestradiol concentrations between the day of HCG administration and day 8 oestradiol (P < 0.0001). There were 44 clinical pregnancies noted (41.5%): three missed abortions, and 41 ongoing or delivered pregnancies.

Table I shows the outcome of the pregnant compared to non-pregnant women with respect to various parameters. There were no significant differences in age, basal FSH, peak oestradiol, number of gonadotrophin units, duration of stimulation, fertilization rate, number of oocytes, number of replaced embryos, and day 8 oestradiol between pregnant and non-pregnant women.

Table II and Figure 1 show the pregnancy rate (PR) and implantation rate (IR) according to five arbitrary groups of day 8 oestradiol values as follows: group 1, day 8 oestradiol concentration <200 pg/ml; group 2, 200–600 pg/ml; group 3, 601–1000 pg/ml; group 4, 1001–2000 pg/ml; and group 5, oestradiol >2000 pg/ml. Evaluation of different ratios showed that a threshold for a negative effect was noted only when the ratio was >5.0. Working with different ratios between 0.4 and 5.0 did not lead to any differences in IR and PR. Again, there were no differences in either PR or IR between the five groups with the exception of a significantly decreased IR between <200 pg/ml and the 200–600 pg/ml groups (P = 0.012).

We found no difference in the mid-luteal oestradiol concentrations between conception and non-conception cycles. The median and interquartile ranges for the peak oestradiol to day 8 oestradiol ratio in conception and non-conception cycles were 2.3 (1.7–4.5) and 2.5 (1.7–3.2) (P = 0.967). We then analysed those results in relation to the magnitude of decrease in oestradiol concentrations between the day of HCG administration and the mid-luteal phase. Table III and Figure 2 show the ongoing PR and IR (sacs with fetal heart beat/embryo transfer) according to the peak oestradiol/day 8 oestradiol ratios between conception and non-conception cycles. The ratios between conception and non-conception cycles. The ratios between conception and non-conception cycles.
concentrations between the days of HCG administration and 8 days later, measured by the ratio of peak oestradiol to mid-luteal oestradiol, did predict IVF outcome. A sharp decline in the mid-luteal oestradiol, defined in our study as an elevated peak oestradiol to mid-luteal oestradiol ratio >5, resulted in a significantly lower ongoing IR and PR.

Our results indicate that such an occurrence is not uncommon, occurring in 17.9% of our patients. The decline in late luteal oestradiol in unsuccessful cycles raised speculations that peri-implantation endometrial development may be compromised. This decline may be due to either an embryonic problem (insufficient HCG production with insufficient oestradiol production from the ovaries as a result), or an ovarian problem (sufficient embryonic HCG production but insufficient oestradiol production from the ovaries due to disruption of granulosa cells during follicular aspiration), or a combination of both. To our knowledge, this is the first study specifically evaluating the magnitude of the decrease in oestradiol concentrations between the day of HCG administration and the peri-implantation phase in relation to IVF outcome, rather than the actual values on either days. Larger studies are needed to confirm our findings.

While the use of progesterone for luteal support is not in debate in assisted reproduction, the role of luteal oestradiol in the peri-implantation period seems to be permissive rather than obligatory (Kapetanakis and Pantos, 1990; Stewart et al., 1993; Ghosh and Sengupta, 1994; De Ziegler, 1995; Edgar, 1995; Ghosh and Sengupta, 1995; Stassart et al., 1995). No clear evidence exists that a certain amount of luteal oestradiol is required for implantation and pregnancy maintenance. The donor oocyte model in women with no ovarian function provides an optimal opportunity to investigate further the role of luteal oestradiol. While most donor oocyte programmes routinely use luteal oestradiol supplementation in addition to progesterone during and beyond the artificial luteal phase, several case reports of successful outcomes when supplemental luteal oestradiol was inadvertently omitted raise doubts about the need for oestradiol in the luteal phase (Kapetanakis and Pantos, 1990, Stassart et al., 1995). This is consistent with early studies which suggest that luteal oestradiol may have only a facilitating role in early pregnancy maintenance, and that it is no longer necessary once progesterone is introduced (Csapo et al., 1973a,b). In addition, histological data suggest that there are no adverse effects of omitting luteal oestradiol supplementation on endometrial integrity and dating when evaluated by endometrial biopsies on days 21 and 25 of a programmed cycle, suggesting that supraphysiological concentrations of progesterone may compensate for the absence of oestradiol (De Ziegler et al., 1992; Younis et al., 1994; De Ziegler et al., 1995).

Earlier studies showing a decline in late luteal oestradiol in non-conception cycles compared to conception cycles raised speculations that peri-implantation endometrial development may be compromised (Lauffer et al., 1981; Muasher et al., 1984; Smits et al., 1988). To test this hypothesis, Smits et al. used 6 mg daily of oestradiol valerate beginning 6 days after HCG administration along with vaginal progesterone, compared to only vaginal progesterone for luteal support in a large prospective, randomized trial involving 378 patients undergoing IVF or zygote intra-Fallopian transfer. These investigators could not find any improvement in PR whether or not oestradiol valerate was added. Furthermore, an analysis of the singleton conceptions did not reveal any differences in oestradiol concentrations between the two groups until 12 days after HCG administration (Smits et al., 1993). These investigators also noted a higher preclinical pregnancy loss (16.9 versus 10.9%) in the group that did not receive oestradiol supplementation. In our study, despite the low pregnancy rate and the small number of women in the group with a ratio of peak oestradiol to mid-luteal oestradiol >5, it is worth noting that the incidence of early pregnancy loss was 50%, possibly suggesting that even if an implantation occurs, the endometrium may be significantly compromised leading to pregnancy loss. No cases of early pregnancy loss occurred in the other two groups, which may be related to the relatively small sample size. In contrast to our results, however, Smits et al. did not evaluate if differences in luteal oestradiol concentrations were found between conception and non-conception cycles in the two groups, and therefore we believe the issue of whether to give oestradiol supplementation in the luteal phase is still unresolved. Currently, the vast majority of IVF programmes, including ours, do not routinely use supplemental oestradiol in the luteal phase.

The ideal method to study the role of oestradiol in the luteal phase is to prevent its synthesis. Most recently, Shetty et al. used fadrozole (a new aromatase inhibitor) in five female bonnet monkeys mated with fertile males for three consecutive natural cycles (Shetty et al., 1997). The use of fadrozole from days 14 to 26 of each cycle resulted in the complete prevention of pregnancy in a total of 14 ovulatory cycles. Following the withdrawal of treatment, all five monkeys became pregnant within 10 ovulatory cycle exposures (Shetty et al., 1997). As expected, fadrozole had no effect on progesterone concentrations and totally abolished oestradiol production. The authors suggest that a total elimination of oestradiol, and possibly, oestradiol-derived growth factors or proteins, prevents the establishment of a pregnancy. Confirmation of this work is needed. Multiple studies have also shown an improvement in luteal oestradiol and progesterone production, and a higher PR when HCG is used for luteal support compared to progesterone (Hutchinson-Williams et al., 1990; Grazi et al., 1991; Soliman et al., 1994). The argument against the routine use of HCG has been the higher incidence of ovarian hyperstimulation syndrome. The higher PR in those women given HCG for luteal support may be due to the increase in oestradiol production, along with progesterone, by the corpora lutea.

Smits et al. also evaluated the endometrium in their above mentioned study. Endometrial biopsies were performed between days 8 and 10 after HCG administration in 10 women receiving oestradiol valerate and progesterone and 12 women receiving only progesterone, all of whom had no embryos to transfer (Smits et al., 1993). Endometrial maturation was similar in both groups with 25% of the biopsies being out of phase. No endometrial biopsies were performed in our study as all patients underwent embryo transfer. It is tempting to speculate that the incidence of out-of-phase biopsies in our
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study would be high in the group with a peak oestradiol to mid-luteal oestradiol ratio >5.

Prior results from artificially prepared cycles showing normal endometrial morphology in the mid- or late-luteal phase if oestradiol substitution therapy was withdrawn when progesterone was initiated may not be directly comparable to stimulated IVF cycles (De Ziegler et al., 1992; Younis et al., 1994). In IVF, wide supra-physiological serum oestradiol fluctuations are known to occur, especially after oocyte retrieval as noted both prospectively identified, we believe a prospective, randomized (1993). Since those women with HCG which can interact with the ovary to increase steroid expression remains to be investigated. Controlling for oocyte and embryo quality needs to be addressed in future studies since a linear increase in oestradiol concentrations during ovarian stimulation is predictive of good quality oocytes and pre-embryos (Scott and Smith 1998).

In addition, whether those women with an elevated ratio would benefit from oestradiol supplementation starting in the mid-luteal phase remains to be investigated, but we believe that a low mid-luteal oestradiol (<200 pg/ml), and more importantly an elevated peak oestradiol to mid-luteal oestradiol ratio (>5) is already an early sign of a failed conception (not absolute, however, since three ongoing pregnancies were noted in this group). Thus, any attempt at oestradiol supplementation should be started in the early, not mid, luteal phase. As reported previously, mRNA for HCG has been found in the human 6- to 8-cell embryo, and that blastocysts grown in culture secrete HCG which can interact with the ovary to increase steroid production (Stewart et al., 1993). Since those women with low mid-luteal oestradiol and/or an elevated ratio cannot be prospectively identified, we believe a prospective, randomized trial using oestradiol supplementation in addition to progesterone, versus only progesterone, beginning on the day of oocyte retrieval is warranted.

References


