Hormonal influence on the uterine contractility during ovarian stimulation

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High-frequency uterine contractions (UC) at the time of embryo transfer have been shown to hamper the outcome of in-vitro fertilization (IVF). As UC are postulated to be hormone-regulated, we aimed to investigate the role of plasma oestradiol and progesterone concentrations on UC during ovarian stimulation for IVF. A total of 59 women were studied on the day of administration of human chorionic gonadotrophin (HCG) and embryo transfer. Plasma oestradiol and progesterone concentrations were measured, and 5 min ultrasound scans of the uterus were digitized with an image analysis system to assess UC frequency and direction. Cycles were sorted according to whether progesterone concentrations on the day of embryo transfer were ≤100 (n = 34) or >100 (n = 25) ng/ml. On the day of HCG, UC frequency was similar in both groups at 4.5 ± 0.2 and 4.6 ± 0.3 UC/min (mean ± SE) respectively. On the day of embryo transfer, UC frequency remained steady in the low progesterone group, whereas it decreased (3.5 ± 0.2 UC/min) in the high progesterone group (P < 0.001), and was negatively correlated with progesterone concentrations (r = -0.56; P < 0.001). No influence of oestradiol on UC was noticed. These observations confirm the utero-relaxing effects of progesterone in the non-pregnant uterus and support the administration of progesterone before embryo transfer to increase tissue concentrations and improve the outcome of IVF.

Key words: embryo transfer in IVF/oestradiol/ovarian stimulation/progesterone/uterine contractions

Introduction

The contractility of the non-pregnant human uterus undergoes periodic variations according to the phase of the menstrual cycle. The follicular phase is characterized by a progressive increase in uterine contractile activity, which culminates at the pre-ovulatory period, as detected by either traditional intra-uterine pressure (Wilson and Kurzrok, 1938; Bickers, 1941; Henry and Browne, 1943; Garrett,
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1956; Csapo and Pinto-Dantas, 1966; Hendricks, 1966; Martinez-Gaudio et al., 1973) or transvaginal ultrasound (Oike et al., 1990; Lyons et al., 1991; IJland et al., 1996) measurements. These progressive changes in uterine contractile patterns throughout the proliferative phase of the cycle are probably a response to oestrogen stimulation. In support of this contention, early reports have indicated that the administration of oestrogens to women deprived of ovarian function as a result of surgical menopause (Krohn et al., 1937) or primary amenorrhoea (Wilson and Kurzrok, 1938; Henry et al., 1950) induces a normal proliferative pattern of uterine contractions (UC). However, the possibility that the supra-physiological concentrations of oestradiol used in ovarian stimulation could further enhance uterine contractility beyond the values observed in normal menstrual cycles remains to be investigated.

After ovulation, uterine contractility is characterized by a relative quiescence with small, slow, and superimposed UC, presumably as a response to the production of progesterone by the corpus luteum (Cibils, 1967). Indeed, a reorganization of UC is observed during the luteal phase with infrequent and long-lasting contractions, as detected by intra-uterine pressure (Bickers, 1941; Henry and Browne, 1943; Henry et al., 1950; Garrett, 1956; Eskes et al., 1970) and ultrasound (Abramowicz and Archer, 1990; Lyons et al., 1991) recordings. In addition, a predominance of converging contractions (from the fundus to the cervix and from the cervix to the fundus, concomitantly) has been also identified during the first 5 days after ovulation (IJland et al., 1996).

The physiological role of UC has not been fully elucidated. However, it is plausible that UC assist sperm ascension through the genital tract at midcycle (Lyons et al., 1991; Kunz et al., 1996) and take part in the embryo implantation process during the mid-luteal phase by helping its proper positioning, not only in animals (Pusey et al., 1980; Rogers et al., 1983) but also in humans. Conversely, aberrant patterns of UC may be detrimental to embryo implantation. Indeed, intense contractile patterns assessed by ultrasonography have been recently associated with lower pregnancy rates in spontaneous cycles (IJland et al., 1996) and during ovarian stimulation for in-vitro fertilization (IVF), at the time of embryo transfer (Fanchin et al., 1998).

Therefore, our understanding of hormonal influences on uterine contractility may provide the basis for corrective measures, such as the administration of oestradiol to improve fertilization by stimulating sperm ascension (enhancement of uterine contractility), or progesterone to improve embryo implantation rates by fostering the permanence of embryos in the uterine cavity (reduction of uterine contractility), in spontaneous or stimulated cycles. Hence, we investigated the possible role of plasma oestradiol and progesterone concentrations on the uterine contractility during ovarian stimulation for IVF.

Materials and methods

Patient characteristics

We studied 59 consecutive stimulated cycles for IVF, undertaken in 59 infertile women (aged 23–38 years). Only women aged ≤38 years, whose uteri were
morphologically normal as confirmed by hysteroscopy and ultrasound scans, and who had at least three good quality embryos (defined as uniform sized and shaped blastomeres, ooplasm having no granularity and a maximum fragmentation of 10%) available for embryo transfer were included. Clinical indications for IVF/embryo transfer were tubal abnormalities (39%), sperm abnormalities (54%), unexplained infertility (5%), and endometriosis (2%). Informed consent was obtained from all patients and this investigation received the approval of our internal Institutional Review Board.

**Stimulation protocol**

A single injection of a time-release gonadotrophin-releasing-hormone (GnRH) agonist, leuprolide acetate. (Enantone 3.75 mg i.m.; Takeda Pharmaceuticals, Paris, France) was administered on cycle day 2. After 18 days, complete pituitary desensitization was confirmed by documenting low plasma concentrations of oestradiol (<40 pg/ml) and luteinizing hormone (LH; ≤2 mIU/ml). Patients also had an ultrasound examination to exclude ovarian cysts and to verify that the endometrial thickness was <5 mm. Human menopausal gonadotrophin (HMG) therapy (Humegon; Organon Pharmaceuticals, Saint-Denis, France) was then initiated at a dosage of 225 IU/day for the first 5 days of ovarian stimulation. Further doses of HMG and the timing of HCG (Gonadotrophine Chorionique ‘Endo’, 10 000 IU i.m.; Organon Pharmaceuticals, Saint-Denis, France) administration were decided according to the usual criteria of follicular maturation determined by ultrasound and oestradiol findings. Administration of HCG was performed when at least three follicles were >17 mm in diameter and oestradiol concentrations per mature follicle (≥17 mm in diameter) were >300 pg/ml. Oocytes were retrieved 36 h after HCG administration by transvaginal ultrasound-guided aspiration. Follicles measuring <12 mm in diameter were not aspirated. All embryo transfers were performed 2 days after oocyte retrieval using a Frydman catheter (CCD Laboratories, Paris, France). The luteal phase was supported with 300 mg of micronized progesterone (Utrogestan; Besins-Iscovesco Pharmaceuticals, Paris, France) administered daily (100 mg in the morning, 200 mg in the evening) by the vaginal route, starting on the evening of the embryo transfer day.

**Uterine contractility assessment and definition of UC frequency groups**

Both on the day of HCG administration and just before embryo transfer, all patients underwent 5 min ultrasound scans of a sagittal plane of the uterus using a 7.5 MHz transvaginal probe (Siemens Elegra; Siemens S.A.S., Saint-Denis, France) at ~11:00 by one single operator. Environmental conditions were standardized throughout ultrasound examination. The present study respected similar methodological characteristics as previously described (Fanchin et al., 1998). Briefly, images were digitized on-line using a two images/s rate with a
Computerized assessment of uterine contractions (UC) frequency. After having determined the uterine section to be analysed (left panel), time-dependent changes in myometrial-endometrial interfaces that correspond to UC were assessed (right panel). Previously published in Fanchin et al., 1998. Reproduced by permission of Oxford University Press.

A computer-assisted image analysis system (IöTEC 3.1.2, IöDP, Paris, France) that allows objective quantification of frequency of the myometrial contractile activity.

As shown in Figure 1, frequency assessment was based on the analysis of time-dependent variation at the myometrial–endometrium interfaces. Direction of UC was assessed visually according to 20 images/s rate (10 times the normal speed) and classified arbitrarily into four types: (i) cervix-to-fundus or retrograde; (ii) fundus-to-cervix or antegrade; (iii) antagonistic (UC starting simultaneously on the cervix and on the fundus and meeting on the middle of the uterus); and (iv) non-propagated UC (local myometrial activity). Sequences were rated by two independent observers and the assessment of inter-rate reliability showed an adequate agreement ($\kappa = 0.71; P < 0.001$).

**Blood samples and hormone measurement**

In addition to routine hormonal monitoring of follicular development during ovarian stimulation, further blood samples were drawn on the day of HCG and embryo transfer for oestradiol and progesterone measurement.

Plasma oestradiol was determined by an immunometric technique using an Estradiol-60 Amerlite kit (Ortho Clinical Diagnostics, Strasbourg, France), with a sensitivity of 14 pg/ml. Intra-assay and inter-assay coefficients of variation (CV) were 8 and 9% respectively for oestradiol. Plasma progesterone was measured by radioimmunoassay using a $^{125}$I Progesterone Coatria kit (Bio-Mérieux, Paris, France), with a sensitivity of 0.05 ng/ml. Intra-assay and inter-assay CV were 8 and 11% respectively for progesterone. Plasma follicle stimulating hormone (FSH) was measured by an immunometric technique using an Amerlite kit (Ortho Clinical Diagnostics, Strasbourg, France). Intra-assay and inter-assay CV were 5 and 7% respectively, and the sensitivity was 0.1 mIU/ml for FSH.
To assess the possible utero-relaxing properties of progesterone, we sorted our 59 cycles according to whether plasma progesterone concentrations on the day of embryo transfer were ≤100 ng/ml (low progesterone group, n = 34) or >100 ng/ml (high progesterone group, n = 25). The choice of this cut-off mark was arbitrary and corresponded to the mean progesterone concentrations at the time of embryo transfer in the present series.

**Statistical analysis**

Measures of central tendency used were means and measures of variability were standard errors. When data distribution was non-parametric, medians and ranges were used. Statistical assessment of our results was performed using factorial analysis of variance (ANOVA) and χ² tests when data distribution was normal. The Wilcoxon Signed Rank test was used for paired comparisons when normality of data could not be confirmed. Hormonal influences on uterine contractility were assessed using simple regression. Agreement between the two independent observers was measured by the κ statistic; R < 0.05 was considered to be statistically significant.

**Results**

**Overall characteristics of uterine contractility**

We failed to observe any significant variation either in the overall UC frequency (4.6 ± 0.2 and 4.2 ± 0.2 UC/min respectively), or in the overall prevalence of each UC type (cervix-to-fundus or retrograde, 62 and 61%; fundus-to-cervix or antegrade, 27 and 24%; antagonistic, 9 and 14%; and non-propagated, 2 and 1% respectively) on the day of HCG administration in comparison with the day of embryo transfer.

**Hormonal influence on uterine contractility**

On the day of HCG administration, plasma oestradiol (2 150 ± 124 pg/ml versus 3 043 ± 240 pg/ml, R <0.001), and progesterone (0.74 ± 0.08 ng/ml versus 1.06 ± 0.2 ng/ml, R <0.03) concentrations were higher in the high than in the low progesterone group. On the day of embryo transfer, plasma oestradiol concentrations in the low and in the high progesterone groups were comparable at 1065 ± 76 and 1189 ± 86 pg/ml respectively. Median progesterone concentrations were 61.9 ng/ml (range 7.4–98.0) in the low progesterone group and 128.8 ng/ml (range 102.9–232.0) in the high progesterone group.

The relation between ovarian hormones and UC frequency is illustrated in Figure 2. On the day of HCG administration, no significant association between either plasma oestradiol or plasma progesterone concentrations and UC frequency was observed. On the day of embryo transfer, although plasma oestradiol
concentrations still were not correlated with UC frequency, a significant negative correlation between plasma progesterone concentrations and UC frequency was identified ($r = -0.56; P < 0.001$).

Changes in UC frequency from the day of HCG administration to embryo transfer in both progesterone groups are depicted in Figure 3. In line with the negative correlation between plasma progesterone and UC frequency on the day of embryo transfer, we only observed a significant drop in the UC frequency from the day of HCG to embryo transfer in patients of the high progesterone group ($4.6 \pm 0.3$ to $3.5 \pm 0.2$ UC/min, $P < 0.001$). Conversely, in the low progesterone group, UC frequency remained unchanged during the same period ($4.5 \pm 0.2$ to $4.8 \pm 0.2$ UC/min).

In addition, on the day of HCG administration, UC patterns (relative prevalence of retrograde, antegrade, antagonistic and non-propagated UC) were comparable in the low and in the high progesterone groups (58, 32, 8, and 2%, and 68, 21, 10, and 1% respectively). On the day of embryo transfer, we observed a noticeable, yet not significant, increase in the prevalence of antagonistic UC in the high progesterone group (20%). This trend was not observed in the low progesterone group (8%). The prevalence of the remaining UC patterns on the
day of embryo transfer did not show conspicuous variation in comparison with the day of HCG administration (65, 26, and 0% in the low progesterone group, and 58, 20, and 2% in the high progesterone group, for retrograde, antegrade, and non-propagated UC respectively).

**Patients, ovarian stimulation, embryology, and implantation data in the low and the high progesterone groups**

The low and high progesterone groups were not statistically different in regard to the age of patients (median 31 years, range 26–38, versus median 32 years, range 26–38 respectively), indications for IVF (tubal abnormalities, 42 versus 35%; sperm abnormalities, 52 versus 57%; unexplained infertility, 6 versus 4%; endometriosis, 0 versus 4%), ovarian reserve assessed by FSH (5.2 ± 0.3 mIU/ml versus 4.5 ± 0.3 mIU/ml) and oestradiol (30 ± 2 pg/ml versus 29 ± 3 pg/ml) concentrations on cycle day 3 performed during the 2 or 3 months prior to ovarian stimulation, number of 75 IU HMG ampoules administered (32.8 ± 2.2 versus 34.6 ± 2.1), duration of stimulation (11.2 ± 0.2 days versus 11.9 ± 0.3 days), number of mature oocytes retrieved (8.4 ± 0.8 versus 9.2 ± 0.9), and number of available embryos (4.2 ± 0.5 versus 4.9 ± 0.7) and transferred embryos (median 3, range 3–4, in both groups). Incidentally, endometrial thickness measured on the day of HCG (10.5 ± 0.3 mm versus 9.8 ± 0.4 mm respectively) and embryo transfer (9.7 ± 0.4 mm versus 9.5 ± 0.3 mm respectively) was comparable in the low and high progesterone groups, further supporting that the uterine exposure to oestrogen was similar in both groups.

The relatively small number of cases included in this analysis did not permit
meaningful comparison of pregnancy rates between groups. Therefore, the observed data are merely descriptive. Clinical pregnancy rates (defined as presence of intrauterine gestational sac with cardiac activity) were 27 and 46%, ongoing pregnancy rates (≥12 weeks of amenorrhea) were 18 and 35%, and implantation rates (number of intrauterine gestational sacs transferred embryos×100) were 13 and 19%, in the low and high progesterone groups respectively.

Discussion

The present study investigated the possible influence of circulating concentrations of ovarian hormones (oestradiol and progesterone), measured on the day of HCG administration and at the time of embryo transfer, on UC characteristics. Myometrial contractile activity was visualized by ultrasound and assessed objectively by a computerized module specially fitted for such analysis (Fanchin et al., 1998). Three major points summarize the results observed. Firstly, overall UC frequency and patterns did not change significantly from the end of the follicular phase of ovarian stimulation (day of HCG administration) to the day of embryo transfer. Secondly, we failed to observe any relationship between plasma oestradiol concentrations and UC characteristics either on the day of HCG or on the day of embryo transfer. Finally, plasma concentrations of progesterone were negatively correlated with UC frequency on the day of embryo transfer, and women whose progesterone concentrations exceeded the arbitrary mark of 100 ng/ml displayed significantly lower UC rates than those included in the low progesterone group, with a trend for a reorganization of UC patterns (increased prevalence of antagonistic UC).

Given the remarkable changes observed in ovarian hormone concentrations, with halved plasma oestradiol and, especially, a massive increase in plasma progesterone from the day of HCG administration to embryo transfer, the putative utero-stimulating action of oestrogens (Wilson and Kurzrok, 1938; Bickers, 1941; Henry and Browne, 1943; Garrett, 1956; Csapo and Pinto-Dantas, 1966; Hendricks, 1966; Martinez-Gaudio et al., 1973; Oike et al., 1990; Lyons et al., 1991; IJland et al., 1996) and the utero-relaxing properties of progesterone (Bickers, 1941; Henry and Browne, 1943; Henry et al., 1950; Garrett, 1956; Cibilis, 1967; Eskes et al., 1970; Abramowicz and Archer, 1990; Lyons et al., 1991), it was reasonable to anticipate a significant reduction in the uterine contractile activity from the day of HCG to embryo transfer. We failed, however, to observe any significant modification in the overall UC frequency and patterns during this period. This observation is consistent with results from other investigators who failed to note a decrease in UC rate after HCG administration (Lesny et al., 1998). However, women whose progesterone concentrations were markedly high on the day of embryo transfer (high progesterone group) experienced a significant reduction of UC frequency and a trend for reorganization
of contractility patterns. Further, plasma oestradiol concentrations did not influence UC characteristics.

Two hypotheses may be drawn from these results. Firstly, at the end of ovarian stimulation, patients are exposed to supra-physiological oestradiol concentrations as a result of the multiple follicular development. It is, therefore, possible that the utmost stimulating action of this hormone has been reached progressively during the follicular phase, and that additional variations in oestradiol concentrations, e.g. those occurring from the day of HCG to embryo transfer, cannot modify UC characteristics. This observation is in agreement with those of other investigators (Kunz et al., 1998). Furthermore, the relatively high overall frequency of UC observed on the day of HCG administration (4.6 ± 0.2 UC/min), which exceeds slightly that reported on previous ultrasonographic studies during the late follicular phase of spontaneous cycles (3–4 UC/min) (Abramowicz and Archer, 1990; Lyons et al., 1991; IJland et al., 1996), is consistent with this. Repeated measurements of uterine contractility throughout the follicular phase of ovarian stimulation are, however, needed to further investigate the potential stimulating role of oestrogens on UC during stimulation.

Another plausible explanation for the lack of decrease in the overall contractile activity of the myometrium after HCG is that, notwithstanding the remarkable 100-fold increase in mean plasma progesterone concentrations from the day of HCG to embryo transfer, the resultant exposure of the uterus to this hormone during the first 4 days of the luteal phase of ovarian stimulation may be insufficient to significantly alter UC characteristics. It is possible that the hyper-oestrogenized uterine milieu during ovarian stimulation may interfere with the myo-relaxing action of progesterone, particularly when plasma progesterone concentrations remain <100 ng/ml on the day of embryo transfer. The observation of a negative correlation between plasma progesterone concentrations and UC frequency, concurring with the trend for a reorganization of UC patterns in the high progesterone group on the day of embryo transfer, support the hypothesis that the bulk of progesterone necessary to rebut the utero-exciting oestrogen effects and reduce uterine contractility in stimulated cycles may be very high, probably >100 ng/ml on the day of embryo transfer.

Finally, by design, the present study failed to assess the tissue uterine concentrations of progesterone. It is possible that noticeable discrepancy exists between circulating and tissue progesterone concentrations, which challenges the mere appraisal of peripheral blood progesterone concentrations in the evaluation of the progesterone effects on the uterine contractility. Indeed, studies on the action of progesterone administered i.m. or vaginally have evidenced a conspicuous variability between plasma and endometrial progesterone concentrations (Miles et al., 1994; Fanchin et al., 1997). Other studies on the possible relation between UC characteristics and uterine progesterone concentrations, assessed directly by endometrial biopsies or indirectly by the evaluation of endometrial echogenicity patterns, may be momentous to further examine this question.

In addition, the latest UC measurements performed during the luteal phase of ovarian stimulation in the present analysis took place 4 days after HCG
administration (day of embryo transfer). It is likely, however, that additional reduction of myometrial contractile activity may take place subsequently during mid- or late luteal phase, when the exposure of the uterus to progesterone is longer and possibly cumulative. Investigation of a plausible relationship between UC and plasma progesterone concentrations at the time of blastocyst transfers, as well as the potential implication of the uterine contractility in the rates of blastocyst implantation, 5–7 days after fertilization, may extend our understanding of the hormonal control of the uterine contractility and role of UC in the embryo implantation process.

Incidentally, in the present study on 59 IVF cycles, pregnancy and implantation rates tended to be higher in the high progesterone group. Although the small number of cases studied precluded any definitive conclusion on IVF outcome, the trend observed is in keeping with our previous results that showed a deleterious effect of high-frequency uterine contractility at the time of embryo transfer on pregnancy and implantation rates in IVF (Fanchin et al., 1998). It is also noteworthy that, as previously (Fanchin et al., 1998), our methodological approach for UC frequency benefited from software specially tailored for UC assessment. The possibility of fast play image sequences (10-fold normal speed), without detriment to image resolution and quality, permits the reliable appraisal of UC direction.

In conclusion, the present results indicate that UC characteristics are influenced by plasma progesterone concentrations on the day of embryo transfer, and are refractory to the changes in plasma oestradiol concentrations occurring on the day of HCG and embryo transfer. Moreover, on the day of embryo transfer in comparison with the day of HCG administration, only patients displaying high progesterone concentrations (>100 ng/ml) experienced a reduction in UC frequency with a trend for a reorganization of UC patterns. This suggests that high tissue concentrations of progesterone are needed to alter uterine contractility during the early luteal phase of ovarian stimulation. As high-frequency UC at the time of conventional embryo transfer has been reported to adversely affect pregnancy and implantation rates in IVF (Fanchin et al., 1998), exogenous administration of progesterone before embryo transfer may constitute an attractive and safe measure to increase tissue concentrations, reduce UC frequency, and improve the outcome of IVF. Further investigation is, however, required to confirm this hypothesis.

References


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