

# Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes

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**BACKGROUND:** Ovarian stimulation for IVF profoundly alters the early luteal phase endometrial development. It has been hypothesized that this process has already started in the late follicular phase, as the endometrium has already been exposed to high steroid concentrations since that phase. The aim of the present study was to prospectively investigate the effect of multi-follicular ovarian stimulation for IVF on the late follicular phase endometrium histology and the expression of estrogen receptor (ER) and progesterone receptor (PR). **METHODS:** In a cross-over study, 11 infertile women with normal ovulatory function, participating in an IVF programme and treated with GnRH antagonist/recombinant FSH ovarian stimulation, were enrolled in the study. Endometrial biopsies were taken in a natural cycle on the day of the onset of the surge of the LH, and in a subsequent stimulation cycle on the day of hCG administration for final oocyte maturation. Endometrial histological dating was carried out according to Noyes' criteria. Immunohistochemistry was performed, using commercially available antibodies for ER and PR endometrial expression. The immunohistochemical signal was recorded in 1000 epithelial cells in each compartment (glands and stroma). Endometrial expression for each of the two receptors was graded on a scale of 0–3, based on the intensity of nuclear staining. Then a score range between 0 and 3000 was recorded, and expressed as a mean score per 1000 stroma or glandular cells per sample (range: 0–3). **RESULTS:** Histological examination of biopsies both in natural and stimulated cycles showed no secretory changes. However, in stimulated cycles, PR expression was significantly up-regulated compared to natural cycles in both glands (1.67 versus 1.34,  $P < 0.05$ ) and stroma (1.98 versus 1.62,  $P < 0.05$ ), whereas ER was down-regulated in glands (1.15 versus 1.43,  $P < 0.05$ ). In IVF cycles, the progesterone measurements, although within normal values (range 0.8–1.4 µg/l), were significantly higher than in natural cycles (0.99 vs 0.63 µg/l, respectively,  $P = 0.008$ ). An ongoing pregnancy rate of 37.5% was achieved in the stimulated cycles. **DISCUSSION:** Although the current study found no early secretory transformation in stimulated endometria before hCG administration, the ER and PR expression in these endometria is similar to the one described during the first days of the luteal phase in natural cycles. Supraphysiological concentrations of estradiol and subtle progesterone rises in the late follicular phase might be responsible for this modulated steroid receptor profile. This phenomenon indicates accentuated maturation of the endometrium in IVF cycles from the pre-ovulatory phase onwards.

**Key words:** endometrial receptivity/estrogen receptor/GnRH antagonist/pre-ovulatory endometrium/progesterone receptor

## Introduction

Despite remarkable advances in assisted reproductive techniques, the implantation rate following IVF still remains low at 13% (Nygren and Andersen, 2002). This has led to the hypothesis of decreased endometrial receptivity being responsible for this phenomenon (Paulson *et al.*, 1990; Fauser and Devroey, 2003).

Histological observations and the expression of markers of the implantation window have shown that ovarian stimulation for IVF alters the luteal phase endometrial development (Bourgain and Devroey, 2003), and possibly results in an incorrect timing of the interaction between a viable embryo and a receptive or sub-receptive endometrium. More specifically, previous observations have demonstrated that advanced

endometrial maturation is present on the day of oocyte retrieval in IVF cycles, using either a GnRH agonist or antagonist (Ubaldi *et al.*, 1997; Kolibianakis *et al.*, 2002). In contrast, in natural cycles, such an advancement of the endometrium is not present (Bourgain *et al.*, 2002). In these studies, we demonstrated that endometrial precocious secretory transformation was compatible with pregnancy achievement. However, the probability of ongoing pregnancy was significantly decreased in the presence of extreme endometrial advancement (Ubaldi *et al.*, 1997; Kolibianakis *et al.*, 2002).

Most studies in multi-follicular ovarian stimulation (multi-FOS) cycles focus on luteal phase inadequacy as the reason for low implantation rates in assisted reproductive treatments (Devroey *et al.*, 2004). Nevertheless, it should not be overlooked that in stimulated cycles the endometrium is also strongly exposed to supraphysiological steroid hormone elevation during the follicular phase (Fauser and Devroey, 2003). This might be responsible for the uniform presence of endometrial advancement on the day of oocyte retrieval and these inappropriate secretory changes could be present even before hCG administration for ovulatory triggering (Marchini *et al.*, 1991). As a possible mechanism for this early secretory transformation of pre-ovulatory endometrium, it was postulated that high estradiol mediates earlier expression of progesterone receptors in the early follicular phase, which can induce advancement of the endometrium in the late follicular phase even with normal progesterone values (Marchini *et al.*, 1991; Kolibianakis and Devroey, 2002). In addition, there is currently no information available about the status of endometrial steroid receptors in the follicular phase of patients undergoing ovarian stimulation for IVF and ICSI.

The aim of the present study was to investigate the effect of multi-FOS with an antagonist protocol for IVF on the histology and the steroid receptor expression of estrogen receptor (ER) and progesterone receptor (PR) in pre-ovulatory endometrium.

## Materials and methods

### Patient population and study design

Between April 2003 and March 2004, 12 infertile patients were enrolled in this prospective cross-over study at the Centre for Reproductive Medicine of the Dutch-speaking Brussels Free University. The inclusion criteria were: (i) age <39 years; (ii) normal cycle length; (iii) male or tubal infertility; (iv) basal FSH <10 IU/l; and (v) more than three previous IVF failed attempts. Patients were monitored in a natural cycle and subsequently underwent an IVF stimulation cycle. Eleven ( $n = 11$ ) patients completed the study (one refused a biopsy to be taken in the stimulation cycle). The day of the onset of LH surge is crucial and correlates with the day of hCG administration in the stimulated cycles, thus allowing for correct dating comparison of the endometria. Endometrial biopsies were therefore taken during the natural cycle on the day of the initiation of the LH surge (Li *et al.*, 1987), and before the hCG administration (Acosta, 2000) during a stimulated cycle for IVF, in which an embryo transfer was performed (IVF cycle). All the patients gave their informed consent and the study protocol was approved by the Institutional Review Board of the Vrije Universiteit Brussel.

### Outcome measures

Two parameters were examined: (i) endometrial histology assessed by the criteria of Noyes *et al.* (1950); and (ii) expression of estrogen and progesterone receptors assessed by immunohistochemistry.

### Monitoring of the natural cycle

The monitoring of a natural cycle in all women ( $n = 11$ ) started from day 9 of the cycle with consecutive daily blood tests and ultrasound evaluation of the dominant follicle. The endometrium biopsy was taken on the day of the onset of the LH surge. Daily morning blood samples were taken until the detection of the onset of the LH surge. This was defined as a serum value of LH >14 IU/l (Bourgain *et al.*, 2002). To confirm the initiation of LH surge, we further monitored LH, estradiol ( $E_2$ ) and progesterone values during the next 3 days. An LH peak, a plateau or decrease of  $E_2$  and an increase in serum progesterone were considered as confirmatory variables. If the hormone values indicated incorrect evaluation of the day of the biopsy, we repeated the biopsy in a following natural cycle (only in one patient).

### Monitoring of ovarian stimulation cycle

The same 11 patients subsequently underwent multi-FOS using a GnRH antagonist/recombinant (r)FSH. According to the GnRH antagonist protocol, a blood test was performed on the first day of menstruation and, once estradiol level was <80 ng/l and progesterone <1.6  $\mu$ g/l, then on day 2 of the cycle, rFSH injections were initiated (Puregon; NV Organon, The Netherlands). The initial FSH dose was fixed for the rest of the treatment. On day 6 of the stimulation the s.c. administration of the GnRH antagonist was started (Orgalutran; Organon) with 0.25 mg daily up to and including the day of ovulation triggering by hCG. Monitoring of both follicular growth (by transvaginal ultrasound) and hormone concentrations (estradiol, FSH, LH and progesterone) were performed starting on day 6 of the stimulation and repeated as appropriate. The triggering dose of hCG was 10 000 IU (Pregnyl; NV Organon) administered i.m., when at least three follicles of 17 mm were present. An endometrial biopsy was performed before the administration of the hCG injection.

The luteal phase of the stimulated cycles was supported by vaginally administered progesterone (Utrogestan; Besins International, Belgium) at a dosage of 600 mg daily in three equal doses beginning immediately the day after oocyte retrieval. Oocytes were inseminated within 4 h after retrieval either by IVF or by ICSI. Embryo transfer was performed either on day 3 ( $n = 4$ ) or day 5 ( $n = 4$ ) of *in vitro* culture. A maximum of two embryos was transferred. To assess treatment outcome, serum  $\beta$ -hCG was measured 14 days after oocyte retrieval and repeated 3 days later. A rise in serum  $\beta$ -hCG on two consecutive blood tests indicated pregnancy. A clinical pregnancy was defined as the ultrasound observation of fetal cardiac activity after 7 weeks of gestation. Pregnancy losses before this period were assigned as preclinical miscarriages. An ongoing pregnancy was defined as a pregnancy with positive heart beat at ultrasound after 12 weeks of gestation.

Serum LH, FSH, hCG,  $E_2$  and progesterone were measured with the automated Elecsys immunoanalyser (Roche Diagnostics, Germany). Intra- and inter-assay coefficients of variation were: <3 and <4% for LH; <3 and <6% for FSH; <5 and <10% for  $E_2$ ; and <3 and <5% for progesterone, respectively.

### Endometrial biopsy and histology dating

Aspiration biopsy of the endometrium was performed as an outpatient procedure using the Pipelle de Cornier (Laboratoire CCD, France). The biopsy was fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into 3 µm thick sections for light microscopical evaluation. The endometria were examined prospectively by a single observer blinded for the type of cycle. Endometrial histological dating was performed according to the criteria of Noyes *et al.* (1950).

### Immunohistochemistry

Immunohistochemistry was performed using commercially available antibodies for ERα and for both PR isoforms (Clone 6F11 and Clone 16 respectively at a dilution of 1:100; Novocastra, UK), with the labelled streptavidin–biotin method. Deparaffinated, rehydrated sections were incubated with 3% H<sub>2</sub>O<sub>2</sub> in methanol for 30 min to block endogenous peroxidase activity. Antigen retrieval was by microwave heating three times for 3 min in citrate buffer at pH 7.6. The sections were rinsed in phosphate-buffered saline (PBS), blocked with 10% normal goat serum for 30 min, and then incubated with the primary antibodies diluted in PBS overnight in a wet chamber at 4°C. A second biotinylated goat anti-mouse antibody at a dilution of 1:300 and streptavidin–peroxidase conjugate were used in accordance with the manufacturer's instructions of the (Amersham Pharmacia Biotech, UK). A chromogenic precipitate was obtained by immersion in diaminobenzidine solution for 7 min and in 0.5% CuSO<sub>4</sub> for 5 min. Sections were counterstained with haematoxylin before mounting. Positive controls were tissue sections containing the relevant antigens. Negative controls were obtained from known negative tissue sections and by substituting isotypic mouse serum for the primary antibody.

The immunohistochemical signal was recorded in epithelial cells from the endometrial glands and in stroma cells (both only in the functional endometrial layer and excluding surface epithelium and vessel wall). In each sample, and for each of the two monoclonal antibodies, 10 arbitrarily chosen microscopic fields were analysed and in each of them 100 stromal and 100 glandular cells (1000 cells per each type of cells per sample) were observed. Endometrial expression for each of the two receptors was graded on a scale of 0–3, based on the intensity of nuclear staining (0 = none; 1 = mild positive; 2 = moderate positive; and 3 = strong positive). Then a score range between 0 and 3000 was recorded, and expressed as a mean score per 1000 stroma or glandular cells (range: 0–3) per sample (Bourgain *et al.*, 2002). All observations of receptor staining was performed by one of the investigators blinded to the treatment group.

### Statistical analysis

Statistical analysis was performed using SPSS version 11.5 software. Fisher's exact test was used to analyse nominal variables in the form of frequency tables. Sign rank test was used for non-normally distributed paired metric variables. All tests were two-tailed with a confidence level of 95% ( $P < 0.05$ ). Values are expressed as mean  $\pm$  SEM.

## Results

### Cycle characteristics and pregnancy outcome

In total, 11 women (aged 30.7 years) underwent an endometrial biopsy first in a natural cycle, and subsequently in a stimulated cycle with embryo transfer. The majority of the patients had primary infertility and the main indication for

**Table I.** Patient characteristics ( $n = 11$ )

Age (years) (mean $\pm$ SEM)	30.7 $\pm$ 0.9
Infertility aetiology, $n$ (%)	
Male	6 (55)
Tubal	4 (36)
Combined	1 (9)
Type of infertility, $n$ (%)	
Primary	10 (91)
Secondary	1 (9)
Mean duration of infertility (years) (mean $\pm$ SEM)	4.2 $\pm$ 0.8

IVF treatment was a male factor (Table I). Cycle characteristics and hormone values are shown in Table II. As was expected, all the hormone parameters were lower in natural cycles compared to the IVF cycles. The progesterone levels in IVF cycles, although within normal values, were significantly higher than in natural cycles ( $P = 0.008$ ). In three patients, no embryo transfer was carried out, because of no available embryos on the day of the transfer ( $n = 2$ ), or because of ovarian hyperstimulation syndrome ( $n = 1$ ). A maximum of two embryos was transferred and an ongoing pregnancy rate of 37.5% was achieved.

### Endometrium histology

None of the endometria, either in the natural cycles or in the stimulated cycles, showed early secretory changes (Figure 1). All of them were dated as late proliferative phase (Table II).

### Endometrial steroid receptor expression

Staining for ER and PR was limited to nuclei. Figures 2 and 3 show the immunohistochemistry image from an endometrial biopsy taken from the same patient in a stimulated cycle for IVF. In both stimulated and natural cycles, PR immunoreactivity was stronger in the stroma compared to the glands (Figure 4). However, in stimulated cycles (on the day of hCG triggering), there was a statistically significant up-regulation of the expression of the PR in both compartments (Table III). On the contrary, on the same day, the signal for the estrogen receptor (ER) in epithelial gland cells was significantly less intense in stimulated cycles (down-regulation) compared to natural cycles ( $P < 0.01$ ) (Table III). Moreover, up-regulation of PR in the stimulated cycle was shown to be present with a wide range of estradiol concentration on the day of hCG (Figure 5).

## Discussion

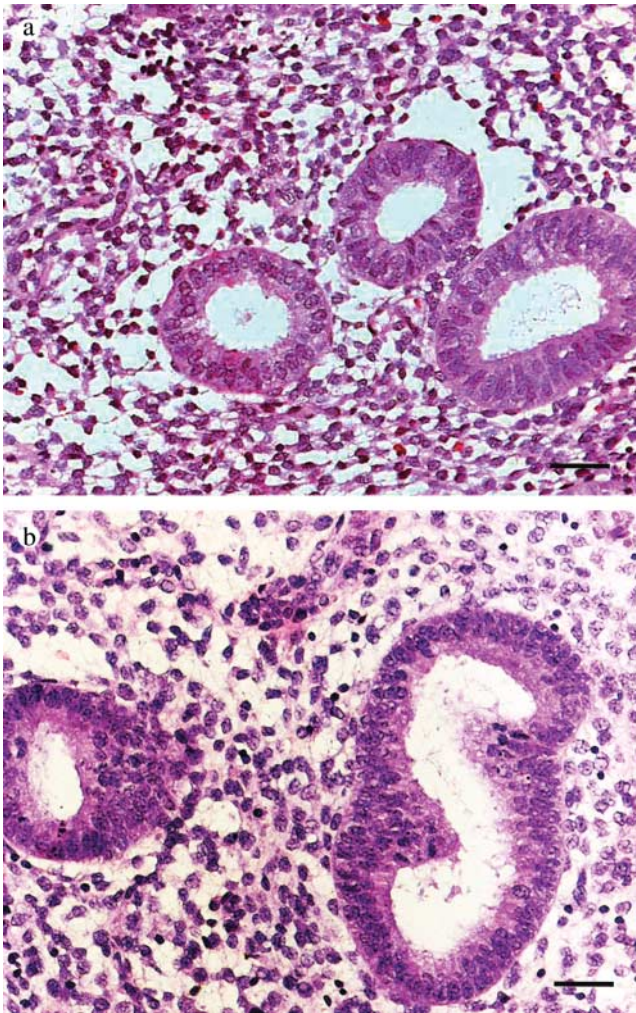
In the present study, endometrial biopsies were taken in natural and stimulated cycles on the day of LH rise and hCG administration respectively. In our study design, patients served as their own controls, thereby eliminating inter-patient variability, which frequently causes interpretation bias in this type of study. To the best of our knowledge, this is also the first study that explores steroid receptors in the follicular phase of stimulated cycles. Our results indicate that before the ovulatory administration of hCG, multi-FOS for IVF treatment with a GnRH antagonist/rFSH protocol does not induce visible secretory changes in the endometrium. It appears, however, that supraphysiological hormone



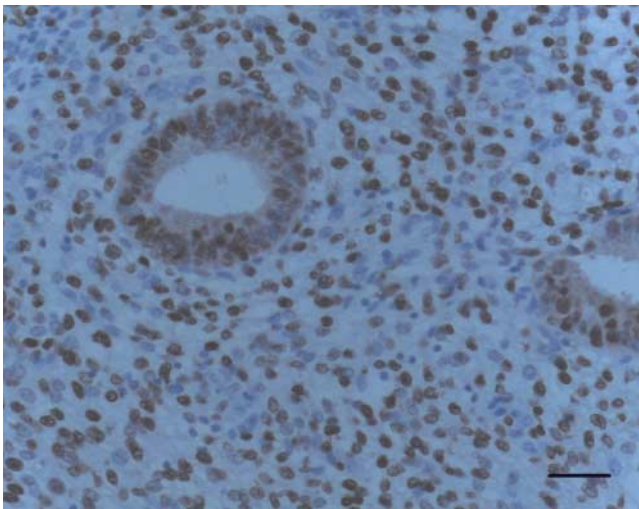
**Table II.** Cycle characteristics, hormone values and endometrial histology

	Natural cycles (n = 11)	Stimulation cycles (n = 11)	P
Mean day of biopsy <sup>a,b</sup>	13 (10–18)	11 (8–15)	0.09
LH (U/l) <sup>c</sup>	19.5 ± 3.0	1.1 ± 0.2	<0.001
FSH (U/l) <sup>c</sup>	6.6 ± 0.8	13.7 ± 1.8	0.005
Estradiol (ng/l) <sup>b</sup>	274 (193–454)	1863 (436–4591)	0.004
Progesterone (µg/l) <sup>b</sup>	0.63 (0.3–1.0)	0.99 (0.8–1.4)	0.008
Endometrium thickness (mm) <sup>c</sup>	8.2 ± 0.6	8.9 ± 0.7	NS
Mean units of rFSH used <sup>c</sup>		2815 ± 202	NA
No. of follicles > 11 mm <sup>c</sup>	1	11.7 ± 1.8	NA
Mean follicular diameter in NC <sup>a,c</sup>	18.8 ± 0.6		NA
No. of cumulus–oocyte complexes <sup>a</sup>		13 ± 2.6	NA
Cycles with embryo transfer, n (%)		8 (73)	NA
No. of embryos transferred <sup>c</sup>		1.3 ± 0.2	NA
Pregnancy outcome per embryo transfer, n (%)			
Positive hCG		4/8 (50.0)	NA
Clinical pregnancy		3/8 (37.5)	
Ongoing pregnancy		3/8 (37.5)	
Histological dating of endometrium, n (%)			
Early secretory changes	0	0	NS
Late proliferative phase	11 (100)	11 (100)	

<sup>a</sup>On the day of LH rise (in natural cycles, NC) or hCG administration (in stimulation cycles) respectively.  
<sup>b</sup>Mean (range).  
<sup>c</sup>Mean ± SEM.  
NS = non-significant; NA = non-applicable.



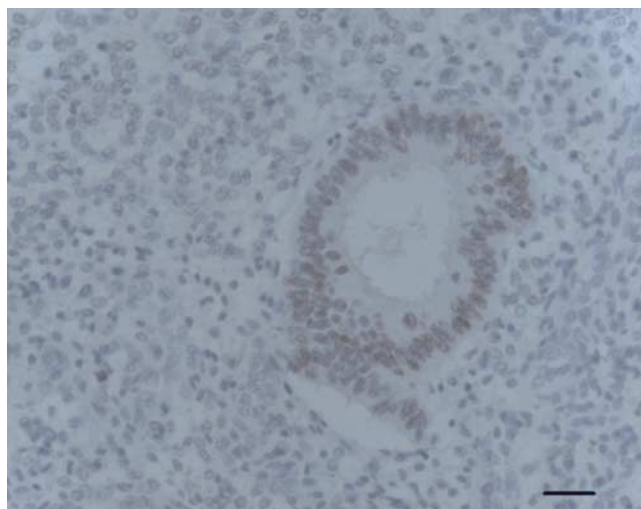
**Figure 1.** Light microscopy of the same patient’s endometrium on the day of the LH surge onset in a natural cycle (a) and on the day of hCG injection in a stimulated cycle (b). Scale bar = 50 µm.



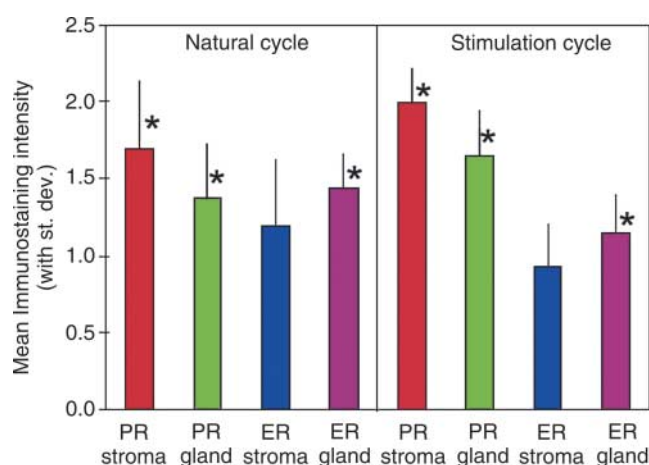
**Figure 2.** Immunohistochemistry image of progesterone receptor expression on the day of hCG administration during a stimulated cycle for IVF. Scale bar = 50 µm.

concentrations deviate the steroid receptor expression in the glandular and stromal compartment of the pre-ovulatory endometrium, indicating that accentuated maturation of endometrium takes place.

Only one study (Marchini *et al.*, 1991) has reported on late follicular phase endometria in IVF cycles. The authors of this study have shown that ovarian stimulation treatment with GnRH agonist and hMG could induce early secretory changes in all stimulated patients. The study, however, has its limitations. First, natural and stimulated cycle biopsies were not performed in the same patient and the two groups had different infertility aetiology. Second, there was no precise timing of the biopsies between the two groups, as in the control group the biopsy was taken once a follicle of 17 mm



**Figure 3.** Immunohistochemistry image of estrogen receptor expression on the day of hCG administration during a stimulated cycle for IVF (same patient as in Figure 2). Scale bar = 50  $\mu$ m.



**Figure 4.** Endometrial steroid receptor expression in the natural cycle (on the day of the LH surge onset) and in the stimulation cycle (on the day of hCG injection) (\* $P < 0.05$  comparing natural cycles to stimulated cycles).

was present and/or  $E_2$  was  $> 250$  ng/l, and in the stimulation group the biopsy was taken the day before hCG administration. Furthermore, in only half the patients, progesterone measurement had been performed on the day of the biopsy, and secretory changes were found in the late follicular phase in the control group (natural cycles) as well. The authors suggested that, although secretory changes were not related to higher pre-ovulatory progesterone in stimulated patients as compared to controls, these changes might be caused by an endometrium more sensitive to physiological levels of pre-ovulatory serum progesterone; the reason could be an increase of PR because of the elevated circulating  $E_2$  levels (Marchini *et al.*, 1991).

The current study confirmed that an iatrogenic effect induced by ovarian stimulation is already present in the late follicular phase, though in the absence of secretory changes. Indeed, we found higher expression of the PR in glands and

**Table III.** Estrogen and progesterone receptors endometrial immunostaining<sup>a</sup> in natural and IVF cycles before ovulation

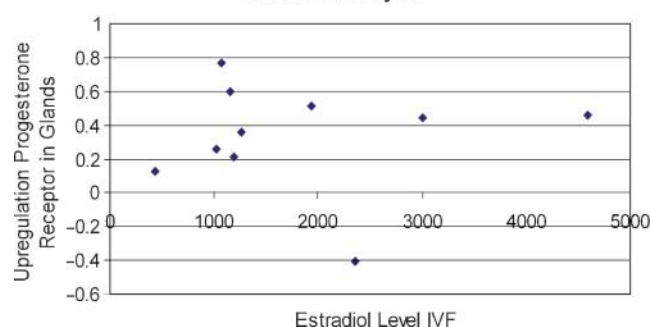
	Natural cycles <sup>b</sup>	Stimulation cycles <sup>b</sup>	<i>P</i>
Estrogen receptor			
Glands	1.43 ± 0.07	1.15 ± 0.08	0.01
Stroma	1.18 ± 0.14	0.91 ± 0.09	NS
Progesterone receptor			
Glands	1.34 ± 0.10	1.67 ± 0.09	0.02
Stroma	1.62 ± 0.12	1.98 ± 0.07	0.01

<sup>a</sup>Mean ± SEM

<sup>b</sup>Receptors were analysed in only 10 patients due to the lack of tissue in one biopsy specimen of a natural cycle in the 11th patient.

NS = non-significant.

**Upregulation Progesterone Receptor in Glands vs. Estradiol Level in IVF Cycle**



**Figure 5.** Up-regulation of progesterone receptor expression in relation to different serum estradiol concentrations in stimulated cycles.

stroma and down-regulation of the expression of ER in glands of the stimulated endometria (Table III). The significantly increased expression of PR in glands and stroma can be explained by the increased  $E_2$  concentration during the follicular phase in stimulated cycles. It is well known that a pre-ovulatory surge of estrogen or exogenous estrogen administration up-regulates the expression of the PR in endometrium. In parallel, progesterone has no effect on the endometrium in the absence of estrogen priming, due to the lack of PR expression (Kreitmann *et al.*, 1979). The same up-regulation would be expected also for ER as, in general, ER $\alpha$  isoform expression appears to be up-regulated by  $E_2$ . This autologous up-regulation of ER is attenuated by progesterone during the menstrual cycle (Taylor, 2001). However, this was not the case in our patients during the stimulation cycle, as we found significant ( $P < 0.01$ ) down-regulation of the ER in the glands (Figure 4).

A possible explanation is that, in stimulated cycles, the significant (Table II) progesterone elevation observed before hCG administration, although remaining within arbitrarily defined 'normal' limits, has a biologically significant activity in the presence of PR up-regulation (lower threshold). This might cause acceleration of the maturation process of endometrium, being first recognized as down-regulation of the ER. Indirect evidence supporting this hypothesis can be retrieved from studies in natural cycles. Premature exposure to small



amounts of exogenous administered progesterone in the early part of the pre-ovulatory phase (day 2 to day 6) did not exert any significant effect on the histological structure of the endometrium, whereas the same dose from day 7 to day 11 significantly diminished the number of glandular mitoses and the height of the glandular epithelium (Shuminet *et al.*, 1983). In addition, our findings support the proposed facilitating/activating mode of hormonal control of endometrial receptivity (de Ziegler, 1995). According to this theory, once endometrium is primed by estradiol—independently of the duration and serum concentrations (Serhal and Craft, 1987; Steingold *et al.*, 1991)—the duration of exposure to progesterone is the crucial point leading to a receptive endometrium.

The down-regulation of estradiol receptors is typical of the early luteal phase and marks the initiation of the secretory changes in endometrium (Lessey *et al.*, 1988). Nevertheless, in natural cycles, the first post-ovulatory 2 day period of the luteal phase is called the 'mute' phase of the endometrium, as obvious histologically secretory changes appear only after 36–48 h post-ovulation. Hence, although we did not notice secretory changes in any of the specimens taken in a stimulated cycle, the ER and PR expression of these endometria resembles that being observed during the first days of the natural cycle luteal phase. In order to observe early secretory phenomena immediately prior to hCG administration in stimulated cycle endometria (as Marchini *et al.*, found),  $\geq 3$  days of maturation advancement are needed (36 h from hCG injection to oocyte retrieval added to the mute 36 h phase). This scenario seems unlikely, because 3 days endometrial advancement was found only in a minority of cycles at the day of oocyte retrieval (Ubaldi *et al.*, 1997; Kolibianakis *et al.*, 2002). In addition, it has been recently shown that histological endometrial dating by Noyes' criteria is less accurate than originally described, due to considerable inter-subject, intra-subject and inter-observer variability (Murray *et al.*, 2004) especially during the mid-luteal phase (Myers *et al.*, 2004).

On the other hand, we cannot preclude the observations by Marchini *et al.*, in as much as the exact threshold concentration of progesterone necessary for the secretory transformation of the endometrium is not known. In their study, progesterone in the late follicular phase might have exceeded threshold levels for a certain period of time (data not provided by these authors). Moreover, it cannot be excluded that in GnRH agonist-stimulated cycles, the longer follicular phase compared to antagonist treatment (Al-Inany and Aboulghar, 2002) provides more time for progesterone (once a lowered threshold is reached) to transform the endometrium into a secretory phenotype.

The patients included in our study had a median of four previous unsuccessful IVF trials. Thus the probability of becoming pregnant was decreased as these patients represent a negatively selected population. Nevertheless, the ongoing pregnancy rate (37.5%) that we observed is in accordance with our previously published data (Ubaldi *et al.*, 1997; Kolibianakis *et al.*, 2002), which indicates that endometrial biopsy has no detrimental effect on the probability of pregnancy in IVF cycles. Moreover, in parallel with our obser-

vations on the day of oocyte retrieval (Bourgain *et al.*, 2002), the subtle changes observed in the endometrial steroid receptor expression on the day of hCG injection appear not to be related to a decreased endometrium receptivity. It would be interesting to investigate the kinetics of the receptors along with other endometrium receptivity markers during the implantation window. However, these patients were studied during IVF cycles with an embryo transfer and it could be hazardous for a patient to undergo repeated endometrial biopsy. This limitation, in addition to the limited number of patients recruited in this study, restrict our ability to evaluate the association between steroid expression and pregnancy rate.

What mediates between the follicular phase and the luteal phase of a stimulation cycle is the hCG administration in the presence of supernumerary follicles. Around the ovulatory dose of hCG, two events might be crucial for the endometrial receptivity status within the implantation window. The first is the premature pre-ovulatory rise of progesterone observed in stimulated cycles in contrast with natural ovulation. It has been postulated that elevation of plasma progesterone  $>0.9$  ng/ml on the day of hCG administration carries a poor prognosis, particularly if the overall response of the ovary to hMG/FSH is weak (Fanchin *et al.*, 1997). In addition, findings from oocyte donation programmes suggest that the deleterious effect of premature progesterone elevation is exerted on the endometrium but not on the oocyte quality (Younis *et al.*, 1996). However, in the presence of an adequate response to ovarian stimulation, high progesterone levels in the late follicular phase are not associated with lower pregnancy rates, indicating that good quality embryos may compensate for the endometrial alteration (Fanchin *et al.*, 1997). Furthermore, it appears that it is the duration of exposure to progesterone rather than actual concentrations or the duration of estradiol priming that is crucial for the proper triggering of endometrial receptivity (de Ziegler, 1995). The second event that has implications for endometrium receptivity is the resulting progesterone profile in the early luteal phase. Develioglou *et al.* (1999) have found that a progesterone value  $>6$  ng/ml on the day after hCG administration was correlated with an earlier down-regulation of PR expression, and with accelerated glandular development and pinopode expression. Considering that the timing of steroid receptor down-regulation in the epithelium coincides with the establishment of endometrial receptivity (Lessey *et al.*, 1996), it is possible that the advanced endometrial maturation relative to embryonic development might result in early closure of the implantation window and decreased pregnancy rates in stimulation cycles.

We conclude that endometrium development in stimulated cycles for IVF treatment is already altered on the day of hCG ovulation triggering, despite the absence of early secretory transformation. Supraphysiological concentrations of estradiol and subtle progesterone rises in the late follicular phase lead to a modulated steroid receptor profile resembling that of the early luteal phase. However, steroid fluctuations early in the luteal phase, the establishment of embryonic–endometrium dialogue and inpatient constitutional endometrial

phenotypes may account for the ability of endometrium to compensate with a wide range of variations, without affecting later luteal phase receptivity.

## References

- Acosta AA (2000) Endometrial dating and determination of the window of implantation in healthy fertile women. *Fertil Steril* 73,788–798.
- Al-Inany H and Aboulghar M (2002) GnRH antagonist in assisted reproduction: a Cochrane review. *Hum Reprod* 17,874–885.
- Bourgain C and Devroey P (2003) The endometrium in stimulated cycles for IVF. *Hum Reprod* 9,515–522.
- Bourgain C, Ubaldi F, Tavaniotou A, Smits J, Van Steirteghem AC and Devroey P (2002) Endometrial hormone receptors and proliferation index in the periovulatory phase of stimulated embryo transfer cycles in comparison with natural cycles and relation to clinical pregnancy outcome. *Fertil Steril* 78,237–244.
- de Ziegler D (1995) Hormonal control of endometrial receptivity. *Hum Reprod* 10,4–6.
- Develioglu OH, Hsiu JG, Nikas G, Toner JP, Oehninger S and Jones HW Jr (1999) Endometrial estrogen and progesterone receptor and pinopode expression in stimulated cycles of oocyte donors. *Fertil Steril* 71, 1040–1047.
- Devroey P, Bourgain C, Macklon NS and Fauser BC (2004) Reproductive biology and IVF: ovarian stimulation and endometrial receptivity. *Trends Endocrinol Metab* 15,84–90.
- Fanchin R, Righini C, Olivennes F, Ferreira AL, de Ziegler D and Frydman R (1997) Consequences of premature progesterone elevation on the outcome of in vitro fertilization: insights into a controversy. *Fertil Steril* 68,799–805.
- Fauser BCJM and Devroey P (2003) Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. *Trends Endocrinol Metab* 14,236–242.
- Kolibianakis E, Bourgain C, Albano C, Osmanagaoglu K, Smits J, Van Steirteghem A and Devroey P (2002) Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. *Fertil Steril* 78,1025–1029.
- Kolibianakis EM and Devroey P (2002) The luteal phase after ovarian stimulation. *Reprod Biomed Online* 5 (Suppl 1),26–35.
- Kreitmann B, Bugat R and Bayard F (1979) Estrogen and progestin regulation of the progesterone receptor concentration in human endometrium. *J Clin Endocrinol Metab* 49,926–929.
- Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL and McCarty KS Jr (1988) Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metab* 67,334–340.
- Lessey BA, Yeh I, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P et al. (1996) Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. *Fertil Steril* 65,477–483.
- Li TC, Rogers AW, Lenton EA, Dockery P and Cooke I (1987) A comparison between two methods of chronological dating of human endometrial biopsies during the luteal phase, and their correlation with histological dating. *Fertil Steril* 48,928–932.
- Marchini M, Fedele L, Bianchi S, Losa G, Ghisletta M and Candiani GB (1991) Secretory changes in preovulatory endometrium during controlled ovarian hyperstimulation with buserelin acetate and human gonadotropins. *Fertil Steril* 55,717–721.
- Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, Zeng D and Fritz MA (2004) A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. *Fertil Steril* 81,1333–1343.
- Myers ER, Silva S, Barnhart K, Groben PA, Richardson MS, Robboy SJ, Leppert P and Coutifaris C (2004) Interobserver and intraobserver variability in the histological dating of the endometrium in fertile and infertile women. *Fertil Steril* 82,1278–1282.
- Noyes RW, Hertig AJ and Rock J (1950) Dating the endometrial biopsy. *Fertil Steril* 1,3–25.
- Nygren KG and Andersen AN (2002) Assisted reproductive technology in Europe, 1999 Results generated from European registers by ESHRE. *Hum Reprod* 17,3260–3274.
- Paulson RJ, Sauer MV and Lobo RA (1990) Embryo implantation after human in vitro fertilization: importance of endometrial receptivity. *Fertil Steril* 53,870–874.
- Serhal PF and Craft IL (1987) Ovum donation—a simplified approach. *Fertil Steril* 48,265–269.
- Shumin X, Johannisson E, Landgren BM and Diczfalusy E (1983) Pituitary, ovarian and endometrial effects of progesterone released prematurely during the proliferative phase. *Contraception* 27,177–193.
- Steingold KA, Matt DW, de Ziegler D, Sealey JE, Fratkin M and Reznikov S (1991) Comparison of transdermal to oral estradiol administration on hormonal and hepatic parameters in women with premature ovarian failure. *J Clin Endocrinol Metab* 73,275–280.
- Taylor AH (2001) Action of sex steroid hormones. In Al-Azzawi and Wahab M (eds) *Hormone replacement therapy and the endometrium*. Parthenon, London, pp. 49–70.
- Ubaldi F, Bourgain C, Tournaye H, Smits J, Van Steirteghem A and Devroey P (1997) Endometrial evaluation by aspiration biopsy on the day of oocyte retrieval in the embryo transfer cycles in patients with serum progesterone rise during the follicular phase. *Fertil Steril* 67,521–526.
- Younis JS, Simon A and Laufer N (1996) Endometrial preparation: lessons from the oocyte donation. *Fertil Steril* 66,873–884.

*Submitted on September 27, 2004; resubmitted on December 3, 2004; accepted on January 14, 2005*