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The endometrium in stimulated cycles for IVF

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Ovarian stimulation for IVF is known to affect luteal phase function. The endometrium in IVF cycles is thus subject to an altered endocrinological environment and to a possible direct effect of the ovarian stimulation therapy. Factors influencing the endometrial receptivity in such cycles are poorly understood. Studies comparing the endometrium in IVF cycles with natural cycles as controls have shown premature secretory changes in the post-ovulatory and early luteal phase of IVF cycles, followed by a large proportion of dyssynchronous glandular and stromal differentiation in the mid-luteal phase. These findings suggest a profound modification of luteal endometrial development in stimulated cycles. This hypothesis is further supported by the demonstration of a modified endometrial steroid receptor regulation and a profound antiproliferative effect in IVF cycles. The time of maximal endometrial receptivity is defined as the implantation window and is characterized by the expression of various endometrial products, among which pinopodes, integrins and leukaemia inhibitory factor are best described. Premature expression of pinopodes and integrins are in line with the observation of precocious luteal transformation following ovarian stimulation, although the clinical relevance with respect to the establishment of a clinical pregnancy awaits further validation. Studies exploring the endometrium within the cycle of embryo transfer have shown a deleterious effect of severe peri-ovulatory maturation advancement exceeding 3 days, as no clinical pregnancies were obtained in this condition. Further unravelling of molecules involved in the implantation mechanism is needed for a better comprehension of the link between altered endometrial development and receptivity in IVF cycles.

Key words: endometrial receptivity/endometrium/IVF/luteal phase/ovarian stimulation

Introduction

Medical treatment for infertility has increased in recent years, and it is estimated that 1.33% of live births issue from assisted reproductive technologies including IVF and ICSI (Nygren and Anderson, 2001).

Arguments in favour of an affected endometrial environment are supported by a reduced implantation rate observed in IVF cycles as compared to natural cycles (review by Macklon and Fauser, 2000). This hypothesis is strengthened by the finding of a lower pregnancy outcome from a shared pool of oocytes in oocyte donors as compared to recipients in human (Check *et al*., 1992) and animal experiments (Ertzeid and Storeng, 2001). For human data, however, controversy regarding this issue persists, as, in a large retrospective analysis, implantation rates were similar in donor and recipient IVF patients (Levi *et al*., 2001).

In a model to calculate the probability of implantation in IVF cycles (Rogers *et al*., 1986), it has been assumed that a receptive endometrial environment accounted between 0.31 and 0.64 for the probability of successful implantation.

The assessment of endometrial function in terms of receptivity in IVF cycles is, however, a highly controversial area, as to date no unequivocal marker of receptivity has been defined even in natural cycle endometrium. IVF treatment is generally achieved through high-dose gonadotrophin ovarian stimulation and is thus associated with supraphysiological serum concentrations of estradiol (E_2) and progesterone. It is obvious that these high steroid concentrations may have an influence on endometrial development. Furthermore, a direct effect of the ovulation stimulation drugs and luteal phase support therapies can be responsible for an altered endometrial environment.

Endometrial histological maturation in IVF cycles

The histological changes that an endometrium undergoes during a natural menstrual cycle were described more than 50 years ago (Noyes *et al*., 1950). Dating yields several methodological flaws (only infertile patients were included in Noyes' criteria), is subject to intra- and inter-observer variability (Smith *et al*., 1995) and shows questionable relationship to endometrial receptivity (Murray *et al*., 2002). Interpretation is rendered even more difficult in the case of glandular–stromal dyssynchrony, where glandular and stromal maturation do not match the same cycle day

Table I. Endometrial morphology and morphometry in GnRH agonist and gonadotrophin stimulated cycles, classified according to biopsy timing

Author	Stimulation protocol	Luteal support	Biopsies	Timing	Dating results
Marchini *et al*. (1991)	Buserelin/hMG	None	21 stimulated	Pre-ovulatory: E_2 >250 pg/ml	21 early secretory
	Natural cycle controls		20 controls	Follicle >17 mm	18 proliferative 2 secretory
Ubaldi *et al*. (1997)	Buserelin/hMG	Vaginal progesterone	41 stimulated	Oocyte retrieval	39 advanced 2 in phase
Lass *et al*. (1998)	Buserelin or naferelin/ FSH or recombinant FSH	Not mentioned	33 stimulated	Oocyte retrieval	15 in phase 15 advanced 3 delayed
Noci *et al*. (1997)	Buserelin s.c./FSH	None	12 stimulated	Oocyte retrieval +2	11 stroma advanced
Barash *et al*. (1992)	Buserelin/hMG hMG Contraceptive/gonadotrophin	Not mentioned	20 stimulated 5 stimulated 2 controls	Oocyte retrieval +7 2 h	25 normal day 16–17 aspect 2 atrophy
Macrow *et al*. (1994)	Goserelin/hMG	None	11 stimulated	Oocyte retrieval +4	No difference compared to controls
	Natural cycle controls		11 controls	Ovulation +4	
Ragni *et al*. (1999)	Decapeptyl/FSH	15 vaginal progesterone 15 i.m. progesterone	30 stimulated	hCG +6	30 in phase
Seif *et al*. (1992)	Buserelin s.c./hMG	15 None 15 hCG 1×	30 stimulated	hCG +7	5 inadequate samples 3 gland delay 14 advanced stroma
Bourgain *et al*. (1994)	Buserelin s.c./hMG	10 None 41 hCG 28 vaginal progesterone 12 i.m. progesterone	51 stimulated	hCG +7	Non-supplemented cycles: 70% out of phase Supplemented cycles: 80% out of phase
Kolb and Paulson (1997)	Leuprolide/FSH	None	7 stimulated	hCG +7	1.8 days advanced compared to controls
	HRT cycle controls		20 controls	7 days progesterone	
Basir *et al*. (2001)	Buserelin/hMG	Not mentioned	26 stimulated	hCG +7	15 gland–stroma dyssynchrony
	Natural cycle controls		12 controls	LH +7	11 gland–stroma synchrony 12 in phaseglands
Meyer *et al*. (1999)	Leuprolide/FSH	9 none	20 stimulated	hCG +8	1 advanced, 12 dyssynchrony, 7 normal
	Natural cycle controls	11 i.m. progesterone	20 controls	LH+8	3 advanced, 5 dyssynchrony, 12 normal
Balasch *et al*. (1991)	Buserelin/hMG	21 hCG	21 stimulated	hCG+11–13	19 normal 2 deficient

(Deligdisch, 2000). Despite these limitations, no other method has proven yet to be more efficient than dating to estimate endometrial development. Dating accuracy can be improved by applying strict criteria to determine the chronological cycle day by taking the day of LH surge as reference point (Acosta, 2000) and biopsy timing (Castelbaum *et al*., 1994).

In IVF cycles, the day of oocyte retrieval is generally designated as equivalent to day 14 in a natural cycle (Develioglu *et al*., 1999; Creus *et al*., 2003).

Early studies using IVF protocols with clomiphene citrate or gonadotrophins already indicated an adverse effect of ovarian stimulation on endometrial development (Garcia *et al*., 1984; Sterzik *et al*., 1988; Rogers *et al*., 1991). Nowadays, most ovarian stimulation protocols include co-treatment with GnRH analogues adjunct to gonadotrophins for prevention of a premature LH rise. Most data on the endometrial histology have been reported in GnRH agonist and gonadotrophin stimulation protocols (Ben-Nun *et al*., 1992; Seppala and Tiitinen, 1995; Deligdisch, 2000; Tavaniotou *et al*., 2001). In those studies, the morphological aspect of the endometrium was related to that expected from the chronological equivalent in a natural cycle and dated according to Noyes' criteria or by morphometric assessment. The day of oocyte retrieval was either considered to be equivalent to day 14 in a natural 28 day cycle, or the ovulatory stimulus was considered equivalent to the natural cycle LH surge. Results from these studies varied according to the timing of the endometrial biopsy (Table I).

The only study performing biopsies in the pre-ovulatory phase showed accentuated proliferative aspects and early secretory changes, even before any serum progesterone rise was observed (Marchini *et al*., 1991).

In the peri-ovulatory phase, a generally advanced endometrial maturation was observed. On the day of oocyte retrieval, an advancement of 2–4 days was reported in 100% (Ubaldi *et al*., 1997) and 45.5% (Lass *et al*., 1998) of cycles. The different percentages can partially be explained by patient selection. In the

first study, only women without known endometrial pathology attending ICSI cycles were included, while the latter study concerned observations in women with endometrial polyps.

On day 2 following oocyte retrieval, discordant stromal maturation with precocious edema and vascular hypertrophy was reported in 91% of biopsies (Noci *et al*., 1997). On this cycle day, other studies found in-phase maturation compared to the chronological cycle day and no statistical difference between stimulated and natural cycles (Bourgain *et al*., 2002; Tavaniotou *et al*., 2003).

In the early to mid-luteal phase, 72 h and 4 days after oocyte retrieval respectively, glandular development was also similar in stimulated cycles compared to natural controls (Barash *et al*., 1992; Macrow *et al*., 1994).

Mid-luteal biopsies frequently showed a glandular–stromal dyssynchrony with a glandular delay (Seif *et al*., 1992; Meyer *et al*., 1999; Basir *et al*., 2001). In cycles where a luteal support with either hCG or i.m. or vaginal progesterone was used, normal 'in phase' histology was reported and no differences were seen related to the luteal support administration route (Bourgain *et al*., 1994; Ragni *et al*., 1999). One report mentioned advanced endometrial maturation in the absence of luteal support on day hCG +7 (Kolb and Paulson, 1997). All the patients from that study, however, presented premature elevation of serum progesterone on the day of hCG injection.

In the late luteal phase, on days 11–13 after hCG injection, normal endometrial development was found (Balasch *et al*., 1991).

Data on the endometrial morphology in cycles using GnRH antagonists adjunct to gonadotrophins are much scarcer. Comparing biopsies in agonist and antagonist cycles on the day of oocyte retrieval showed a similar endometrial advancement of 2–4 days (Kolibianakis *et al*., 2002). In the mid-luteal phase, in comparison to agonist cycles, preliminary data show less endometrial delay in antagonist cycles without luteal phase support (Kolibianakis *et al*., 2003b).

The results of the different published studies are difficult to compare. Stimulation regimens in terms of the type of agonist and administration route were different. A luteal support therapy was not always present and not similar in the different studies. The methods of endometrial biopsy analysis varied from simple dating methods to complex morphometrical analysis, with a large inter-study variation both for the different endometrial parameters assessed as for the application of dating criteria. Patient selection criteria and endocrinological parameters were also highly variable.

Despite the aforementioned considerations, a general trend emerges from these studies. In the peri- and post-ovulatory period, an advanced maturation of the endometrium is present, followed by a 'normal' aspect of the endometrium in the early luteal phase and resulting in frequent glandular–stromal dyssynchrony in the mid- and late luteal phase (Figure 1). The observations in GnRH agonist cycles lend support to the clinical need for luteal supplementation in these cycles (Pritts and Atwood, 2002), as all types of luteal support corrected mid-luteal glandular delay.

These findings are supported by results from studies evaluating the endometrial proliferation index in stimulated cycles. Early luteal severe antiproliferative effects of the stimulation protocol were observed in both glandular and stromal cells when compared to natural cycle controls (Bourgain *et al*., 2002). This difference was no longer present on later cycle days (Bebington *et al*., 2000).

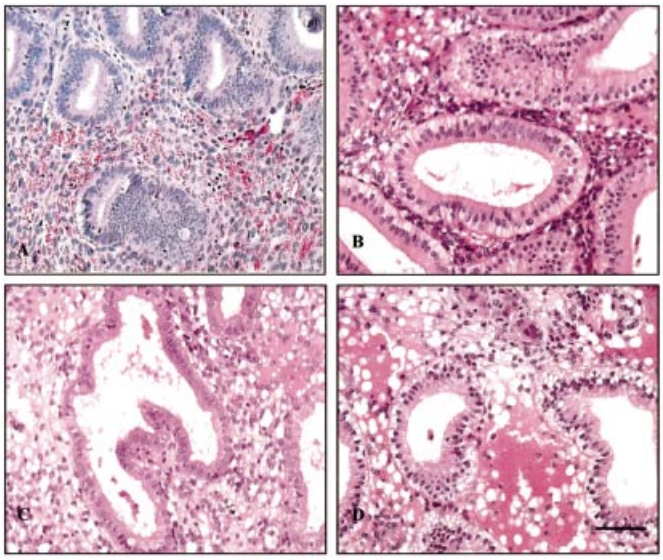

Figure 1. Histological maturation in natural and stimulated cycles. Light microscopy of the endometrium on luteal phase day 0 (**A**, **B**) and day 7 (**C**, **D**) of the luteal phase in natural (**A**, **C**) and stimulated (**B**, **D**) cycles. On the day of natural ovulation, a pseudostratified epithelium without vacuoles is seen (**A**). On the day of oocyte retrieval, the glandular cells show subnuclear vacuolization and very few mitotic figures (**B**). On day 7, stimulated endometria show glandular–stromal dyssynchrony with persistent vacuoles in the glands (**D**). Scale bar = 100 μm.

The particular endometrial development in IVF cycles is most likely due to several factors. An early and increased exposure to progesterone of the endometrium in stimulated cycles may explain both early secretory transformation (Fanchin *et al*., 1995) and subsequent mid-luteal glandular maturation arrest (Ezra *et al*., 1994). Elevated serum E_2 concentrations in stimulated cycles have also been associated with more frequent glandular–stromal dyssynchrony (Basir *et al*., 2001). HCG injection to achieve final oocyte maturation is a further possible cause for disrupted endometrial luteal phase morphology. Indeed, a direct effect of hCG in terms of advanced endometrial maturation and acquisition of a luteal phase phenotype has been well documented in both *in vitro* experiments (Tang and Gurpide, 1993; Han *et al*., 1999) and hormone replacement cycles (Fanchin *et al*., 2001). Finally, it has been demonstrated that GnRH and its agonists have antiproliferative effects on the endometrium (Kim *et al*., 1999; Meresman *et al*., 2002). The hypothesis of multiple factors regulating endometrial development in IVF cycles is sustained by the finding of a large variability of endometrial patterns for similar hormone values (Bourgain *et al*., 1994; Seppala and Tiitinen, 1995) and the absence of a clear correlation between individual serum hormone measurements and endometrial dating (Ubaldi *et al*., 1997).

Endometrial steroid receptors in stimulated cycles

Early reports evaluating the endometrial steroid receptor content have used homogenized endometrial samples not permitting differentiation of the receptors in glandular and stromal cells. Compared to natural cycles, luteal cytosolic receptors were reduced in stimulated cycles when assessed with a dextran

charcoal assay (Forman *et al*., 1989; Molina *et al*., 1989). Other studies found cytosolic reduction of estrogen receptors (ER) but not of progesterone receptors (PR) in correlation with high ovarian response (Toner *et al*., 1991). Using enzyme immunoassays, no receptor difference between natural and stimulated cycles was seen (Balasch *et al*., 1992).

More recent studies evaluated the receptor status by immunohistochemical techniques. These techniques allow for differentiation between glandular and stromal cell receptor expression. Newly available monoclonal antibodies against the different receptor isoforms have further permitted more detailed insight in the endometrial hormonal regulation (Lecce *et al*., 2001; Mote *et al*., 2001). The comparison of literature data should, however, be made with caution. The different monoclonal antibodies and tissue processing methods are known to result in variable immunohistochemical staining patterns (Mote *et al*., 2001). Moreover, wide variation exists throughout the studies regarding the methods reporting the staining intensity.

In stimulated cycles, both glandular and stromal PR are found to be reduced in the peri-ovulatory and luteal phase. Data on endometrial estrogen receptors in stimulated cycles are less clear since both overall decrease and glandular ER increase has been described. On day 2 after oocyte retrieval, low overall PR associated with either high or reduced glandular ER were found (Noci *et al*., 1997; Bourgain *et al*., 2002). Hadi *et al*. (1994) found a reduction in PR in the endometrium after ovulation induction compared to natural cycle controls on day 4 of the luteal phase, which was not associated with detectable morphological changes. A decreased amount of both glandular and stromal ER and PR was seen throughout the luteal phase in stimulated cycles (Develioglu *et al*., 1999). Lower glandular and stromal mid-luteal PR expression was found in supplemented than in non-supplemented cycles (Bourgain *et al*., 1994). Other studies showed few or no difference in steroid receptors between natural and stimulated cycles on various luteal phase cycle days, but observed a differential regulation of progesterone-related molecules such as growth factors and ubiquitin in natural and stimulated cycles (Salat-Baroux *et al*., 1994; Bebington *et al*., 2000).

The results of these studies further support the notion of a substantially modified endometrial environment in stimulated as compared to natural cycles.

Markers of the implantation window in stimulated cycles

The implantation window is defined as the limited period during which the uterus is receptive for implantation of the free-lying blastocyst. Clinical evidence for an endometrial 'implantation window' has been demonstrated (Navot *et al*., 1991; Wilcox *et al*., 1999). It is suggested that in the human natural cycle, blastocyst apposition begins about day LH +6 and is completed by day LH +10 (Lessey, 2000).

During the receptive phase, the endometrium secretes proteins in a temporary fashion that will be recognized by the embryo and facilitate its growth and differentiation (Lessey, 2000). The most cited factors involved in implantation include the formation of luminal epithelial 'pinopodes', expression of adhesion molecules and of cytokines.

Pinopodes were described originally in rats and mice as epithelial projections with pinocytic activity (Enders and Nelson, 1973). The structures that have been currently described by scanning electron microscopy as 'pinopodes' in human endometrium, however, show important morphological and functional differences compared to their rodent counterpart (Murphy, 2000; Adams *et al*., 2001). In normally fertile women, pinopode formation and regression is closely related to serum progesterone concentrations as well as to the down-regulation of the progesterone receptor B in glandular and luminal cells (Stavreus-Evers *et al*., 2001). Pinopodes were demonstrated at the apical surface of the luminal epithelial cell during the implantation window (day 20–22) (Nikas *et al*., 1999), therefore claimed strongly as a possible receptivity marker. Recent studies have questioned this assumption, as pinopode appearance varied up to 5 days between women and a direct involvement of these structures in embryo attachment was not found (Bentin-Ley, 2000). Their synchrony with other presumed markers of implantation has also been debated (Acosta, 2000; Creus *et al*., 2003).

In early reports on CC and hMG/hCG schemes for ovarian stimulation, endometrial pinopodes were found to be diminished or absent (Martel *et al*., 1987). In GnRH agonist and gonadotrophin stimulation, an early appearance of 1–2 days prior to the expected cycle day and a wider range of cycle days displaying endometrial pinopodes has been reported as compared to natural cycles (Develioglu *et al*., 1999; Nikas *et al*., 1999; Novotny *et al*., 1999). These observations led to the hypothesis of a possible shift in the implantation window in IVF cycles. However, a recent study assessing natural and stimulated cycles within the same patient found no difference in pinopode expression (Creus *et al*., 2003).

Integrins are cell surface adhesion molecules involved in a wide variety of cellular processes (Hii and Rogers, 1998). Three integrins ($\alpha_1\beta_1$, $\alpha_4\beta_1$ and $\alpha_v\beta_3$) are thought to be important for endometrial receptivity, as they are expressed in the implantation window (Lessey *et al*., 1996). Their exact role remains controversial (Creus *et al*., 1998). In IVF cycles (Table II), premature expression of α_1 and α_4 integrin subunits has been found on day 2 following oocyte retrieval, consistent with advanced secretory transformation (Tavaniotou *et al*., 2003). In the mid-luteal phase, variable results were reported. Ovarian stimulation induced either lower (Meyer *et al*., 1999; Thomas *et al*., 2002), similar (Wang *et al*., 2000; C.Bourgain *et al.*, unpublished data) or higher integrin expression (Creus *et al*., 2003).

Overall, $\alpha_v\beta_3$ integrin expression correlated well with endometrial maturation (Figure 2). In the studies reporting a lower expression, stimulated cycles from oocyte donors were assessed, where luteal support was not systematically provided. In those cycles, the lowered glandular integrin expression correlated with a morphological delay in glandular maturation. In endometria with more advanced glandular development, integrin expression was also found at a higher level.

Leukaemia inhibitory factor

Leukaemia inhibitory factor (LIF) is a pleiotrophic cytokine from the gp130 family. LIF is the first cytokine that appeared to be critically involved in embryonic development and implantation, as female mice without functional LIF gene fail to implant, although their blastocysts can be successfully transplanted into wild-type recipient females (Stewart *et al*., 1992). In the human, cyclic endometrial LIF expression patterns (Laird *et al*., 1997; Tsai *et al*.,

Table II. Integrin expression in stimulated cycles classified according to biopsy timing

Author	Cycles	Biopsies	Integrins	Timing	Results
Tavaniotou *et al*. (2003)	Buserelin/recombinant FSH	7 stimulated	α_1	Oocyte retrieval +2	Higher and more frequent integrin expression in stimulated cycles
	Natural cycle controls	23 controls	α_4, $\alpha_v\beta_3$	Ovulation +2	
Thomas *et al.* (2002)	Synarel/recombinant FSH	15 stimulated	$\alpha_v\beta_3$, α_1, α_4, β_1, β_3	hCG +7	Decrease of $\alpha_v\beta_3$, $\alpha_4\beta_1$ and $\alpha_1\beta_1$ in glandular epithelium of stimulated cycles
	Natural cycle controls	15 controls		LH surge +7	Decrease of $\alpha_v\beta_3$ in luminal epithelium of stimulated cycles
Meyer *et al*. (1999)	Leuprolide/FSH	20 stimulated	$\alpha_v\beta_3$	hCG +8	Decreased integrin expression in stimulated cycles
	Natural cycle controls	20 controls		LH surge +8	
Creus *et al*. (2003)	Triptorelin/FSH	8 stimulated	$\alpha_v\beta_3$	Oocyte retrieval +7–8/ oocyte retrieval +11–12	Increased integrin expression in stimulated cycles
	Natural cycle controls	8 controls		Ovulation +7–8/ ovulation +11–12	

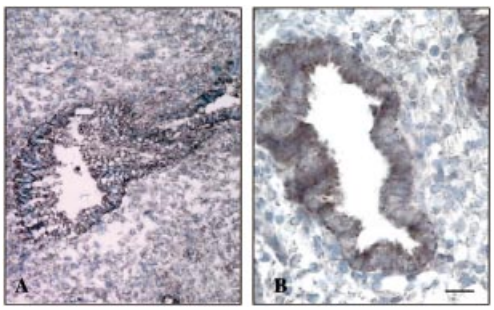

Figure 2. Immunohistochemical staining for $\alpha_v\beta_3$ integrin (**A**) and leukaemia inhibitory factor (LIF) (**B**) on day 7 of the luteal phase in a stimulated cycle. The glandular epithelium shows membranous staining for integrin, and intense basal and apical cytoplasmic staining for LIF. Scale bar = 100 μm.

2000) and clinical association between LIF deficiency and infertility (Hambartsoumian, 1998; Giess *et al*., 1999) also suggest an important function in implantation.

In IVF cycles, there are few data on LIF regulation. One study reported higher LIF expression on cycle day 10 compared to day 20 in endometrial explants from patients in a simulated menstrual cycle (Hambartsoumian *et al*., 1998) but the effect of the *in vitro* culture system on LIF expression cannot be excluded in this type of study. Higher LIF expression was found on day 7 of the luteal phase in HRT and stimulated cycles compared to natural cycle controls (Figure 2) (Ledee-Bataille *et al*., 2002; C.Bourgain *et al.*, unpublished data). The exact importance of LIF in IVF cycles awaits further investigation.

Endometrial development in IVF cycles with embryo transfer

In an attempt to correlate endometrial development and the establishment of an ongoing pregnancy, an endometrial biopsy was performed on the day of oocyte retrieval within the actual embryo transfer cycle (Ubaldi *et al*., 1997; Kolibianakis *et al*., 2002). In IVF cycles with either GnRH agonists or antagonists, no deleterious effect of the endometrial biopsy on clinical pregnancy was recorded.

As illustrated in the aforementioned studies, virtually all endometria on the day of oocyte retrieval in both stimulation regimens showed advancement of ⩾2 days as compared to a natural cycle endometrium on the day of ovulation.

This advancement was more pronounced in cycles with premature serum progesterone rise on or before the day of hCG injection, but for an individual patient, no correlation could be found between endometrial secretory development and absolute progesterone values or number of days of premature progesterone elevation (Ubaldi *et al*., 1997).

Using multiple regression analysis in GnRH antagonist cycles, the degree of endometrial advancement could be predicted by high LH concentration at initiation of recombinant (r)FSH stimulation and long duration of rFSH stimulation before antagonist inhibition (Kolibianakis *et al*., 2002). This correlation was not present in GnRH agonist cycles, where low serum LH concentrations are observed as a result of pituitary desensitization.

Endometrial morphological features of precocious secretory transformation in both stimulation regimens included appearance of uniform glandular subnucleolar vacuoles displacing the nucleus (Figure 1). These histological parameters were associated with a decreased proliferation index and PR content in both glands and stroma (Bourgain *et al*., 2002).

Only a minority of endometria (7/39 in GnRH-agonist and 6/55 in GnRH antagonist cycles) presented extreme endometrial advancement of >3 days as compared to a natural cycle ovulation day (Ubaldi *et al*., 1997; Kolibianakis *et al*, 2002) (Figure 3). In these biopsies, glandular vacuoles were present also at the luminal cell pole, mitosis were absent from glands and stroma and a variable stromal edema was present. In a natural cycle, these features are not expected prior to days 4–5 of the luteal phase. Although no cross-over studies are available with natural cycle endometria on luteal cycle days 4 and 5, glands in these IVF cycles appeared less tortuous and contained less intraluminal secretion as described in Noyes' criteria for these cycle days.

The accuracy and inter-observer reproducibility of endometrial dating has been subject to debate, but were reported to be very high for overall dating (proliferative versus secretory) and reasonable to high for individual post-ovulatory days providing a dating error allowance of 1 day (Duggan *et al*., 2001). In our hands, including both Noyes' criteria and a semiquantitative method taking serum

LH surge as reference day (Li *et al*., 1988), inter- and intra-observer variability was <5%.

Implantation correlated negatively with important endometrial maturation advancement of >3 days on the day of oocyte retrieval, as no pregnancies were observed in such cycles. Taking into account that severe endometrial advancement was found to be associated with high follicular LH concentrations (Kolibianakis *et al*., 2002), further support for a deleterious effect of extreme advancement was provided from a recent clinical study in 111 patients stimulated with GnRH agonists and recombinant FSH. Indeed, in these patients, high early follicular phase LH and E_2 was also associated with reduced pregnancy rate (Kolibianakis *et al*., 2003a).

On the day of oocyte retrieval, no other endometrial marker was related to clinical pregnancy outcome (Table III) (Bourgain *et al*., 2002). These findings are in line with recent observations on LIF secretion on the day of oocyte retrieval in IVF cycles with embryo transfer, where LIF expression as assessed by endometrial flushing was also not different in pregnant and non-pregnant women (Olivennes *et al*., 2003).

The observations in stimulated cycles with embryo transfer suggest that altered endometrial development as a result of IVF therapy has probably less impact than initially thought on the actual endometrial receptivity. Alternatively, good embryo quality may to a certain extend compensate for less optimal endometrial development. These findings lend support to the presumed multiple and redundant pathways regulating implantation events.

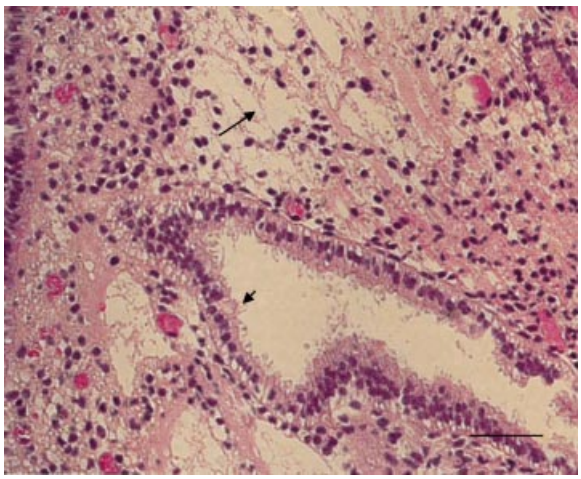

Figure 3. Histological aspect of extremely advanced endometrial development on the day of oocyte retrieval. The glandular cells show apical secretion (short arrow) and absent mitotic figures. The stroma contains large amounts of edema (long arrow). Scale bar = 100 μm.

Conclusions

There is strong evidence from histological observations and expression of implantation window markers that ovarian stimulation for IVF profoundly alters the luteal phase endometrial development. From studies in IVF cycles with embryo transfer, only extremely deviant endometrial morphology seems to affect receptivity for implantation. Further unravelling of molecules involved in the implantation mechanism is needed for a better comprehension of the link between altered endometrial development and receptivity in IVF cycles.

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Table III. Endometrial morphology, proliferation and hormone receptors and clinical pregnancy

	Cell type	Pregnant	Not pregnant	*P*
Histological dating (days post oocyte retrieval)		2.18 (0.22)	2.86 (0.15)	< 0.039
Proliferation index (positive cells/1000 cells)	Glandular	28.27 (6.9)	34.88 (8.5)	NS
	Stromal	33.72 (8.32)	26.86 (5.96)	NS
Estrogen receptor (mean H-score/1000 cells)	Glandular	0.84 (0.1)	0.85 (0.07)	NS
	Stromal	0.16 (0.03)	0.13 (0.02)	NS
Progesterone receptor (mean H-score/1000 cells)	Glandular	2.34 (0.04)	2.26 (0.05)	NS
	Stromal	1.78 (0.05)	1.68 (0.05)	NS

Data are represented as mean (SEM).
NS = non-significant.

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