Histological evaluation of endometrium on the day of oocyte retrieval after gonadotrophin-releasing hormone agonist/follicle stimulating hormone ovulation induction for in-vitro fertilization*

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Introducing

The disappointingly low implantation rate after replacement of morphologically normal embryos is still a major enigma in in-vitro fertilization (IVF) treatment. The development of an adequate receptive endometrium is a crucial factor in success for implantation. A few authors have investigated the maturation of the endometrium in different stages of the luteal phase, from the day of embryo transfer onwards, and demonstrated conflicting results (Garcia et al., 1984; Abate et al., 1987; Sterzik et al., 1988; Forman et al., 1989; Sharma et al., 1990; Ben-Nun et al., 1992; Benadiva and Metzger, 1994). However, the maturation of the endometrium in the critical period during the transformation from proliferative to secretory phase is not clear. The objective of this study was to evaluate the histopathological characteristics of endometrial biopsies taken on the day of oocyte recovery in IVF cycles with a satisfactory response to ovulation induction, which could not proceed to embryo transfer.

Materials and methods

The data from 5736 oocyte recovery cycles for IVF performed at Bourn Hall Clinic during the period January 1991 to May 1997 were analysed retrospectively. In 83 (1.4%) cycles endometrial polyps were suspected, on transvaginal ultrasonography during the monitoring phase, were studied. Following oocyte recovery, hysteroscopy, polypectomy and endometrial curettage were performed. Dating of endometrial glands and stroma was carried out in the tissue not containing the polyps. The total dose of follicle stimulating hormone (FSH), duration of ovulation induction, peak oestradiol and luteinizing hormone (LH) concentrations, thickness of endometrium and number of oocytes were recorded and compared to the endometrial dating of the specimens. In 15 cycles (45.5%), the endometrium was classified as ‘in phase’ (group I), ‘advanced’ by 2–4 days in a further 15 (45.5%, group II), and in the remaining three cycles (9%) it was delayed in maturation (group III). Younger age was correlated with advanced staging of the endometrium (r = –0.42; P = 0.015). Women with ‘in phase’ and ‘advanced’ maturation were similar in their response to ovulation induction; however, there was a strong correlation between advanced dating of endometrium and number of oocytes retrieved (r = 0.49; P = 0.04). Endometrial staging on the day of oocyte retrieval varied widely in patients treated by the same gonadotrophin-releasing hormone agonist (GnRHa)/FSH protocol for ovulation induction. This difference was not predictable by parameters monitored through the cycles.

Key words: endometrial dating/endometrial maturation/IVF/oocyte retrieval

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were at least three follicles of ≥ 16 mm in average diameter. Transvaginal ultrasound directed oocyte recovery, mainly under general anaesthesia, was performed 32–36 h after the HCG injection. In-vitro culture and insemination were carried out according to our normal laboratory procedures (Elder and Avery, 1992).

Statistical analysis
Statistical analysis was performed using Student’s unpaired t-test and Pearson’s correlation test when appropriate. A P value of < 0.05 was considered to be statistically significant. Because of the small number of patients in group III (n = 3), it was omitted from statistical analysis.

Results
In 32 cycles, the dating of the endometrium ranged from day 12 to 19, without any significant asynchrony between glands and stroma. In the 33rd patient, aged 40 years, the glands were dated as day 7 and the stroma day 10. Notably, in this patient, the peak oestradiol concentration on the day of HCG administration was low (330 pg/ml) and the only two oocytes that were retrieved failed to fertilize. Increasing age was negatively correlated with endometrial date (r = -0.42; P = 0.0135). In women younger than 35 years old, the endometrium was advanced by 1.5 days (15.9 versus 14.2, not significant) compared with women aged 35 and above. Women showing ‘advanced’ and ‘in phase’ endometrium were similar in their age and response to ovulation induction in terms of total dose of FSH, duration of stimulation, peak oestradiol and LH concentrations and the thickness of endometrium on the day of HCG administration (Table I). More oocytes were retrieved in group II (13.4 versus 9, P = 0.046) without significant difference in the fertilization rate. Progesterone concentrations on the HCG administration day appeared higher in group II (15.9 versus 14.2, not significant) without significant difference in the fertilization rate. Progesterone concentrations on the HCG administration day appeared higher in group II (15.9 versus 14.2, not significant).

Discussion
In spite of much progress since the development of IVF, including improvements in ovulation induction, oocyte retrieval and culture medium, the maximum conception rate, even in the most successful units, still rarely exceeds 30% per embryo transfer, and up to 90% of apparently normal embryos fail to implant (HEFEA, 1997). Attention has therefore been focused on endometrial receptivity around the actual time of embryo transfer (day 16–17). Garcia et al. (1984), Forman et al. (1989) and Sharma et al. (1990) demonstrated, using different ovulation induction protocols, advanced maturation of the endometrium in up to 85% of cases; this was related to significantly increased progesterone concentrations in these patients. Others, in contrast, showed that in up to 50% of biopsies taken on the day of planned embryo transfer, the endometrium was delayed in maturation (Abate et al., 1987; Sterzik et al., 1988). The degree of delay was enhanced with the use of clomiphene citrate compared with human menopausal gonadotrophin (HMG) (Sterzik et al., 1988). Moreover, there was no consistency in these studies regarding the definition of ‘out of phase’ between 1 or 2 and more days of discrepancy. In these studies, similar to ours, the method used for endometrial maturation was histopathological dating according to Noyes criteria (Noyes et al., 1950) in spite of its limitations (Li et al., 1988). While it offers unique information concerning the secretory component and the stroma, other techniques (i.e. histochemical) may be superior for luminal epithelium evaluation.

Advanced endometrium might be explained by supraphysiologic concentrations of progesterone in stimulated cycles causing accelerated transformation to secretory endometrium at the time of embryo transfer. The elevated progesterone concentration suppresses the production of oestrogen and progesterone receptors. Forman et al. (1989) demonstrated that advanced endometrial maturity tended to be associated with low concentration of progesterone receptors.

Miyara et al. (1996) performed endometrial biopsy in the late follicular phase of natural menstrual cycles and demonstrated a positive correlation between ultrasonographic patterns of the endometrium and the size of glands and number of stromal cells. However, the idea of performing an endometrial biopsy on the day of oocyte recovery in cycles with the intention of proceeding to embryo transfer was rejected because of anticipated damage to the endometrium and a consequent decrease in implantation rate. Ubaldi et al. (1997) recently suggested that it is a safe procedure by achieving similar pregnancy rates in 40 women who underwent endometrial biopsy on the day of oocyte recovery after GnRHa/HMG follicular stimulation and in 20 women without biopsy. They found advanced endometrial maturity with secretory activity in all 40 cases. The maturation was significantly more advanced in women with increased progesterone concentrations than in women with normal progesterone concentrations on the day of HCG injection. However, the dating was correlated neither with progesterone exposure nor with the number of days of increased follicular phase serum progesterone concentrations. They explained that in women with subtle
raised progesterone concentrations, the advanced maturation, although statistically significant, was less than should be expected. Moreover, because of the relatively wide window of implantation, the pregnancy and implantation rates were similar in both groups. Their findings support other studies claiming that raised progesterone concentrations on the day of HCG probably have no detrimental effect on pregnancy rates (Bustillo et al., 1995; Ubaldi et al., 1996).

Our study is the first to evaluate endometrial maturity following GnRH-a/FSH ovulation induction for IVF on the day of oocyte recovery. We found much lower rates of advanced endometrium (45.5%) than Ubaldi et al. (1997) and, in agreement with Sharma et al. (1990), the likelihood of advanced maturity seems to be associated with the progesterone concentration on the HCG day. The reason for the difference in the proportion of endometrium showing advanced maturity between our study and that of Ubaldi et al. (1997) is not clear, but may be a result of using different ovulation induction protocols. In the current study, of the six women with progesterone concentrations of <0.9 ng/ml on the day of HCG injection, only one had advanced endometrium while Ubaldi et al. (1997) identified 100% advanced maturation in women with raised progesterone. While asynchrony between glands and stroma is a frequent finding in the late luteal phase in stimulated cycles (Benadiva and Metzger, 1994), it is uncommon at the time of oocyte retrieval, probably due to the short exposure to progesterone.

The dating of the endometrium could not be predicted by any other parameters of the ovulation induction cycle, although increased female age was correlated with delayed maturation. Sterzik et al. (1988) is in agreement with our study, that in women older than 35 years, the incidence of delayed maturation is significantly higher. There were no other differences in response to ovulation induction between the two age groups. It appears, therefore, that increased age can independently delay the maturation of the endometrium and that factor might contribute to the lower pregnancy rate and increased miscarriage rate in women towards the end of their reproductive life. Interestingly, in women with advanced maturation of the endometrium, significantly more oocytes were retrieved, although we have no explanation for this phenomenon and it might be a result of relatively small numbers of patients in each group.

In IVF treatment, the embryo is transferred to the uterus ~48–52 h after oocyte recovery, compared with 72–96 h post-ovulation in natural conception cycles (Croxatto et al., 1978). Therefore Garcia et al. (1984) suggested that advanced endometrium may have some benefit for embryo implantation. However, Ben-Nun et al. (1992) summarized the literature and calculated that in endometrial biopsies performed around the time of embryo transfer in 198 women on different ovulation induction protocols, 50% of them showed delayed maturation. It might be that in some patients with an apparently normal response to ovulation induction and morphologically normal-looking embryos, inadequate endometrial maturation is the cause of implantation failure. Thus, it seems possible to detect this group of patients as early as the day of oocyte recovery by endometrial biopsy. If these findings are confirmed in larger studies, and the safety of the procedure is guaranteed, then by understanding the optimal development of receptive endometrium and as a consequence, regulating its maturation, we would be able to improve implantation rates.

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References


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