Uterine glandular area during the menstrual cycle and the effects of different in-vitro fertilization related hormonal treatments

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Introduction
The uterine glandular epithelium undergoes a sequence of well characterized changes during the menstrual cycle (Noyes et al., 1950). Based on what is known from comparative studies in animal models, it can be assumed that these changes play an important role in preparation for blastocyst implantation (Rogers, 1993). In most species for which reliable data exist, the timing of embryo implantation is tightly controlled by the endometrium (e.g. rat: De Feo, 1963), resulting in a well defined implantation window. The defining of precise implantation receptive windows in different species has enabled the identification of numerous morphological and biochemical criteria that are involved in successful implantation, or rejection, of the blastocyst (Psychoyos and Casimiri, 1980). Despite the inherent value that implantation markers would have in the treatment of infertility patients and considerable recent research interest in this subject (Nikas et al., 1995; Saleh et al., 1995), no obligatory markers of human uterine receptivity have yet been identified.

By comparing the relative timing of different hormone replacement therapies (HRT) used by different in-vitro fertilization (IVF) groups to prepare the endometrium of women prior to receiving donated oocytes or embryos, it has been possible to establish that the implantation window in humans is relatively broad (Rogers, 1993). In addition, there is evidence that implantation in the human can occur when endometrial morphology is atypical or out of phase (Wentz et al., 1986; Rogers et al., 1989). These findings are problematic for those attempting to establish morphological criteria for receptive endometrium in humans. Most such studies to date have relied on relatively small groups of subjects with different infertility aetiologies and treatment regimens (e.g. Rogers et al., 1991; Serle et al., 1994). This approach can often fail to recognize the wide variability that exists in the normal population, as well as the fact that successful implantation may occur despite the endometrium having morphological criteria that are significantly different from normal.

In previous work, we have studied the effects of different IVF-related superovulation protocols and hormone replacement regimes on endometrial glandular development, and shown that down-regulation with the gonadotrophin releasing hormone analogue (GnRHa) buserelin acetate, followed by stimulation with human menopausal gonadotrophin (HMG), produces glandular development which is most consistent with that seen in normal peri-implantation endometrium. By comparison, clomiphene citrate (CC) was shown to have a suppressive effect on gland development that was only partly improved by the use of progesterone supplementation post-embryo transfer (Hosie et al., 1991). We have also shown that glandular development as determined morphometrically can be correlated with standard ultrasound evaluations of the endometrium (Rogers et al., 1991).
Table I. Details of subject groups used in the study

<table>
<thead>
<tr>
<th>Group n</th>
<th>Menstrual cycle stage(s)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All</td>
<td>Normal menstrual cycle controls</td>
</tr>
<tr>
<td>2</td>
<td>Late proliferative</td>
<td>IVF patients receiving CC/HMG</td>
</tr>
<tr>
<td>3</td>
<td>Early secretory</td>
<td>IVF patients receiving CC/HMG/P4</td>
</tr>
<tr>
<td>4</td>
<td>Early secretory</td>
<td>IVF patients receiving CC/HMG/P4</td>
</tr>
<tr>
<td>5</td>
<td>Early secretory</td>
<td>IVF patients receiving Buserelin down-regulation/HMG</td>
</tr>
<tr>
<td>6</td>
<td>Early secretory</td>
<td>IVFS patients receiving GnrH 'Flare'?HMG</td>
</tr>
<tr>
<td>7</td>
<td>Early, mid and late secretory</td>
<td>Donor oocyte patients receiving fixed E2/P4 HRT</td>
</tr>
<tr>
<td>8</td>
<td>Late proliferative and early secretory</td>
<td>Donor oocyte patients receiving variable E2/P4 HRT</td>
</tr>
<tr>
<td>9</td>
<td>Late proliferative and early secretory</td>
<td>Turner’s syndrome patients receiving E2/P4 HRT</td>
</tr>
</tbody>
</table>

HRT = hormone replacement therapy; IVF = in-vitro fertilization; CC = clomiphene citrate; HMG = human menopausal gonadotrophin; GnRH = gonadotrophin releasing hormone; E2 = oestrogen; P4 = progesterone.

Menstrual cycle and IVF endometrial gland volume

What has been lacking from our and other studies on glandular changes to date, however, has been an objective correlation of glandular changes caused by various IVF superovulation protocols and HRT, with the normal development of glandular epithelium during the entire menstrual cycle. The aim of the present work was to measure objectively glandular volume over the entire menstrual cycle and compare the results with eight different clinical superovulation or HRT subject groups.

Materials and methods

All subjects were recruited to the study on the basis of fully informed consent. Institutional ethical approval was obtained for these studies. Endometrial biopsies were taken from control normal menstrual cycle subjects, and eight other smaller groups of women who had received different IVF-related treatments (see Table I). All endometrial biopsies were taken on an outpatient basis using a Pipelle suction curette (Prodimed, 60530: Neuilly-en-Thelle, France). Control biopsies (group 1) were predominantly collected from women undergoing investigation for infertility, where the investigation subsequently showed the cause of the infertility to be clearly unrelated to uterine or endocrine factors (i.e. tubal damage and/or male infertility). Tissues were not collected from women who had received any form of exogenous hormones or had used an intrauterine contraceptive device (IUD) in the previous 3 months, or who had any known or demonstrable uterine pathology.

Details of the superovulation and HRT protocols for each subject group have been published elsewhere (groups 2, 3 and 4: Rogers et al., 1986a,b; group 5: MacLachlan et al., 1989; group 6: Norman et al., 1991; groups 7, 8 and 9: Leeton et al., 1991) and will not be repeated here. Subject groups 3, 4 and 5 have already been analysed morphometrically and the results published previously (Rogers et al., 1991; Hosie et al., 1991). The results from these groups are included in the present study to allow comparison with normal menstrual cycle data that was not previously available.

For normal menstrual cycle control patients (group 1) biopsies were taken across the full range of the menstrual cycle and haematoxylin and eosin stained sections were dated according to the general classifications of Noyes et al. (1950). The control biopsies were then divided into nine discrete menstrual cycle stages that could be clearly defined, and easily and repeatedly identified from the appearance of the endometrium (Rogers et al., 1992a). These nine stages and their identifying criteria were as follows: (i) menstrual: fragmented tissue with fibrin thrombi, condensed stroma, collapsed glands surrounded by nuclear debris; (ii) early proliferative: small, short tubular glands lined by cuboidal to columnar epithelium with ovoid nuclei. Mitoses seen in glands and stroma; (iii) early-mid proliferative: glands elongated but not tortuous. Increasing number of mitoses. No stromal oedema; (iv) mid proliferative: increasing tortuosity of glands but less coiling than at later stages; (vi) late proliferative: coiled and very elongated glands lined by tall columnar cells with pseudostratification of nuclei. High number of mitoses. Mild stromal oedema; (v) mid-late proliferative: increasing tortuosity of glands but less coiling than at later stages; (vi) late proliferative: coiled and very elongated glands lined by tall columnar cells with large pseudostratified nuclei. Stromal density with oval nuclei and small amount of cytoplasm; (vii) early secretory: post-ovulation days (POD) 3–4. POD 3: >50% glands with subnuclear vacuoles. POD 4: supranuclear vacuoles; (vii) mid secretory: POD 5–10. POD 5–6: tortuous glands with intraluminal secretions. No mitoses and no stromal changes. POD 7–8: marked stromal oedema, commencement appearance spiral arterioles. POD 9–10: increasing pseudodecidualization of stroma commencing around spiral arterioles; (ix) late secretory: POD 11–14: POD 11: pseudodecidualization of stroma under surface epithelium. POD 12–13: infiltration of neutrophil polymorphs and granulocytes. POD 14: early ‘peri-glandular’ nuclear debris.

Biopsies were processed for light microscopy as already detailed (Hosie et al., 1991). One slide with at least two sections was chosen from each patient, and at least two fields of view from each section were measured. For this study to evaluate the effects of the different treatments/conditions, we chose to measure the total area of glandular epithelium as a percentage of the area of endometrial tissue as this has previously been shown to be a reliable indicator of changes in uterine glands (Hosie et al., 1991; Rogg et al., 1991). The measurements on each section of each slide were made using the ×10 objective of a Zeiss microscope with a colour video camera attached and connected to a Tracor Northern Image Analysis System (Tracor Northern, USA) programmed to collect the glandular area information as specified above and from a completed binary image, formed from the original microscopic image. Particles of <50 μm² were automatically excluded from the final binary image as these were considered artefacts. Stromal cells not excluded by the system were manually removed from each image before any data were collected. Where gland profiles had no lumen either because they were sectioned on the periphery of the gland or the lumen was not enclosed, no luminal measurements were made; it was estimated that this type of profile occurred equally in all the sections measured.

Once collected, the measurements of glandular area for different stages of the menstrual cycle or different treatment groups were analysed using either Student’s t-test or Kolmogorov–Smirnov tests.

Results

Results are presented graphically in Figures 1 and 2. The data from biopsies taken across the control menstrual cycle display a curve, with the lowest area of endometrium occupied by glands during the proliferative stages and highest proportion of glands during the secretory stages. There is a significantly greater gland area in the early secretory stage of the cycle than at any time between the early proliferative through to the mid-late proliferative stages (P < 0.05).

Results of biopsies from the five IVF superovulation groups are shown in Figure 1, superimposed on the control cycle data.
Patients in groups 2 and 3 receiving clomiphene citrate and human menopausal gonadotrophin (CC/HMG) alone had significantly less glandular area than those in the control groups at equivalent stages of the menstrual cycle. The use of progesterone supplementation in these patients removed this significant difference (group 4). Group 6 patients on the ‘Flare’ regime had the highest gland area, although this was not significantly different from controls. Buserelin down-regulation (group 5) gave a gland area that was closest to the normal cycle controls.

The three HRT groups are shown in Figure 2. A feature of these results is the high variability shown between patients. Thus, even though group 8 (variable oestradiol/progesterone HRT) has substantially reduced gland volume compared to controls, this is not significantly different.

Discussion

This study has undertaken an extensive evaluation of uterine glandular development during the normal menstrual cycle and has shown that it is possible to differentiate the effects of some superovulation treatments on uterine glandular epithelium using measurements of the proportion of endometrial area occupied by glandular epithelia.

Our findings on the changing volume of glandular epithelium during the menstrual cycle are consistent with descriptive examinations previously published (Noyes et al., 1950) in that we find glands occupy a greater proportion of endometrium during secretory phase than earlier, but our study places these findings on a quantitative basis which can serve as a baseline for determining the influence of various hormonal conditions. Our results for the secretory stages of the cycle agree with those of earlier workers (Li et al., 1988); however, these earlier studies did not include menstrual or proliferative stage endometrium. Our data also support the findings of Bonhoff et al. (1993), who found that secretory phase endometrial biopsies from women stimulated with CC had fewer and smaller glands than controls, although the difference in this study was not statistically significant.

Perhaps one of the most important observations to arise from this study is the apparent wide latitude in glandular volume that will support successful implantation. For many years CC/HMG (patient groups 2 and 3) was the most widely used IVF stimulation protocol, resulting in large numbers of pregnancies. From this it can be concluded that a significantly reduced gland volume does not preclude implantation. However, there have been suggestions based on statistical modelling of IVF multiple pregnancy data that CC/HMG results in reduced uterine receptivity compared to HMG alone (Rogers et al., 1986a). Thus it may be possible that reduced gland volume is an indicator of a sub-optimal endometrial environment, but that this alone will not preclude implantation in all cases. Such a finding would fit well with the concept of implantation as a complex process, the success of which is dependent on a large number of interacting variables.

The relative merits of the different superovulation protocols used in this study remain arguable. It is still the case that the ‘success’ of a superovulation protocol is assessed almost entirely on the number and quality of the oocytes obtained, and not the appearance of the endometrium. It is unlikely that this scenario will change until more specific markers of human endometrial receptivity are established.

Our data provide good evidence to support a central role for oestrogen in controlling gland growth during the proliferative stage of the menstrual cycle. In groups 2 and 3, where the anti-oestrogen CC is acting to block oestrogenic activity, and in group 8 on low dose oestrogen HRT, there is reduced glandular volume. By contrast, with the Flare protocol (group 6), where oestrogen concentrations are elevated above those in the normal cycle (Norman et al., 1991), gland volumes are raised.

The wide variability in gland volumes seen in groups 7, 8 and 9 may reflect the unusual reproductive status and histories of some of these women. In view of the fact that pregnancy rates are usually quite high in donor oocyte programmes, treating such cases provides further evidence to support the hypothesis that the endometrial prerequisites for implantation in the human may not be very strict at all.
Other data from this group of patients to support this statement include the reporting of successful implantation in Turner’s syndrome patients apparently lacking uterine epithelial tight junctions (Rogers et al. 1992b), and a single case of implantation in an endometrium with a primarily proliferative phase appearance (Rogers et al., 1989).

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References


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