Human endometrial morphology around the time of implantation in natural and artificial cycles

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Introduction

Grosser (1910) stated that the most important physiological function of the endometrial cycle is the preparation for the reception of a fertilized ovum (implantation), while menstruation is only a secondary process, a degeneration of the 'mucus membrane' which has not been able to fulfil its purpose. From the earlier work of Hertig *et al.* (1956), it is thought that implantation starts ~6 days after ovulation. Croxatto *et al.* (1978) found that, in the natural cycle, the human embryo usually arrives in the endometrial cavity 96 h or more after the luteinizing hormone (LH) surge. In most in-vitro fertilization programmes, fertilized embryos are transferred to the endometrial cavity a day or two earlier, which is still compatible with successful implantation. In artificial cycles, the transfer of embryos to the endometrial cavity between Days 17 and 19 has been reported to result in successful pregnancy.

In this paper, the morphology of the human endometrium in the periimplantation period of the natural and artificial cycles will be discussed, with emphasis on the steroidal requirement for normal endometrial development.

Strategies for sampling endometrial tissue for morphological study

We obtained biopsy specimens of the endometrium by an outpatient procedure without the need for anaesthesia. The techniques and complications of outpatient endometrial biopsy have been reviewed (Li *et al.*, 1990a; Li & Cooke, 1990a; Li & Cooke, 1990b). We used the Sharman's biopsy curette (Down's Surgical Ltd, Sheffield, UK) to obtain the specimen because it is essential that the sample is of adequate size and intact. We have tried several other techniques of endometrial tissue sampling but the quality of sample obtained was not as good as with the Sharman's biopsy curette.

The sample should always be obtained from the upper or fundal part of the uterus, as the lower or isthmic part is structurally different from the upper part.

The collection of the endometrial sample should be precisely timed according to the LH surge, as it has been shown (Li *et al.*, 1987) that timing by this method is better than that based on the onset of the next menstrual period.

To reduce undue shrinkage of the tissue during fixation, the sample should be supported by pinning onto a piece of wax as soon as it is collected; a more gentle fixative, e.g. glutaraldehyde (which is in any case essential for ultrastructural studies), is preferred to formalin.

To optimize the results of any morphometric analysis, the section should be cut as thinly as possible. For this reason, we embedded our specimens in a plastic medium, JB-4 (Polysciences, Inc., USA) which enabled sections of 1–2 µm to be cut; in contrast, the thickness of paraffin-embedded sections is ≥ 5 µm.

Two methods may be used to study the morphology of the endometrium. The traditional method involves a set of subjective criteria proposed by Noyes *et al.* (1950) and measures only the overall maturity (histological dating); those with experience take only a few minutes. Another method, morphometry, involves quantitative measurements of individual histological features; this method is more objective and the results are more informative, but it takes longer.

Natural cycle

By the time the human embryo enters the endometrial cavity, ~ 4 days after the LH surge (Day LH + 4), the glandular epithelial cells have accumulated large amounts of intracellular secretory materials (Fig. 1a). By Day LH + 6, most of these secretory products have been discharged into the gland lumen. Teleologically, these secretory materials should have functions related to implantation, although their precise roles are unknown. They may be involved in the nourishment of the blastocyst, i.e. in histiotrophic nutrition (Finn, 1977; Johnson & Everitt, 1984). There is little information on the morphological changes of the luminal epithelium of the human endometrium around the time of implantation, or how the implanting human blastocyst goes through the luminal epithelium to reach the stroma. Presumably, the tight junctions at the apices of luminal epithelial cells have to be broken down to make a passage for the blastocyst. It is still a matter of debate as to the extent to which apoptosis, or programmed cell death, is involved in this process (Finn, 1977). We have observed apoptotic cells in the luminal epithelium around the time of implantation.

In the stroma, typical changes include the appearance of stromal oedema (Fig. 1b), proliferation of blood vessels and the decidual reaction (Fig. 1c). In the human endometrium, these changes begin around Day LH + 8, regardless of whether a blastocyst is present or not. In mice, decidual transformation only takes place in the presence of an adequately primed endometrium and a stimulus to the surface of the uterine epithelium by the trophoblast, although this can be replaced under experimental conditions by an artificial stimulus, such as a drop of oil (Finn & Keen, 1962). From their morphological appearance, the decidual cells appear to be actively engaged in the synthesis of certain substances. They have been shown to produce a number of endometrial and placental proteins (for review, see Bell, 1986) and synthesize enzymes such as alkaline phosphatase (Wilson, 1969). The decidual tissue has been considered to play a role in restraining the invasiveness of the conceptus (Johnson & Everitt, 1984).

The mechanisms of implantation and the relative roles of the blastocyst and the endometrium in implantation differ considerably in species. We do not know much about the mechanism of implantation in man, e.g. whether the closure reaction, the first stage of implantation observed in mice (Pollard & Finn, 1972), exists in man. We know that the trophoblast appears to burrow actively between the luminal epithelial cells and that the luminal epithelium appears undisturbed (Finn, 1985; Edwards & Fishel, 1986). Once the blastocyst is inside the endometrium, the uterine glandular tissue and the stromal tissue most adjacent to the invading blastocyst is destroyed. This releases large quantities of primary metabolic substrates which are then taken up by the blastocyst for growth and development. Later, the maternal blood vessels are also eroded, with the result that

Fig. 1. Morphological features of the endometrium in the periimplantation period of the natural cycle: (a) glandular epithelium from a normal woman at Day LH + 4 showing prominent basal vacuoles of secretory material ($\times 960$); (b) view of stroma between glands in a normal woman at Day LH + 8. The stromal cells appear well-dispersed, probably due to oedema, which is maximal at this time ($\times 960$); (c) electron micrograph taken from the stroma of a normal woman at Day LH + 12. A vessel, containing two red blood cells, is becoming surrounded by stromal cells. The 'decidualization' process is typical of this stage in the normal cycle ($\times 4100$).

Table 1. Age, duration of infertility and the prevalence of retarded endometrial development among four groups of infertile women and a group of normal fertile women

<i>n</i> =	Type of infertility				
	Group I (tubal) 34	Group II (male) 39	Group III (endometriosis) 21	Group IV (unexplained) 48	Group V (normal) 68
Age (years)	3.25 ± 4.0 NS	30.8 ± 4.0 NS	34.0 ± 2.9 NS	32.7 ± 4.4 NS	33.4 ± 4.0
Duration of infertility (years)	6.1 ± 3.3 NS	6.8 ± 2.7 NS	6.9 ± 2.9 NS	6.0 ± 3.3 NS	—
Prevalence of retarded endometrium (histological dating by traditional criteria)	1/34 = 2.9% NS	3/39 = 7.7% NS	6/21 = 29% <i>P</i> < 0.01	10/48 = 21% <i>P</i> < 0.01	3/68 = 4.4%

Data (except prevalence of retarded endometrium) are means ± s.d.

The results in the four groups of infertile subjects are compared individually to the normal fertile subjects by two sample *t* tests or 2 × 2 contingency table analysis.

NS = not significant.

the trophoblastic cells are bathed in maternal blood, and finally haemotrophic support of the conceptus is established.

Recently, more refined, quantitative data on endometrial morphology in the periimplantation period have been obtained on precisely timed specimens from fertile subjects (Johannisson *et al.*, 1982; Johannisson *et al.*, 1987; Li *et al.*, 1988a; Dockery *et al.*, 1990). Dockery *et al.* (1988) found a small intersubject variation in the morphology of endometria obtained between Days LH + 2 and LH + 5 from normal, fertile subjects; the results suggest that cellular events during this period are tightly controlled.

Endometrial morphology of infertile subjects

We compared endometrial development in the luteal phase of fertile and infertile subjects and found that the prevalence of retarded endometrial development (histological dating based on the criteria of Noyes *et al.*, 1950, more than 2 days behind chronological dating based on the LH surge) in the infertile population (20/142 = 14%) was significantly higher than in the fertile population (3/68 = 4.4%). However, when the infertile subjects were subdivided into groups of different aetiology, women with endometriosis and unexplained infertility had significantly higher prevalence of retarded endometrium than women with normal fertility; the prevalence in women of infertility associated with tubal or male infertility was not significantly different from that of the fertile subjects (Table 1).

We examined the endometrial morphology in those with unexplained infertility by morphometric techniques (Li *et al.*, 1990b) and found that the abnormalities were all related to the glandular, but not the stromal, component of the endometrium (Fig. 2). Immunohistochemical study of these specimens using monoclonal antibody D9B1 confirmed that the secretory activity of endometrial glands in women with unexplained infertility was abnormal (Graham *et al.*, 1990). Most of the women with abnormal endometrial development had normal progesterone concentrations, suggesting that abnormal response of the endometrium, rather than subnormal progesterone production, is a common cause of abnormalities in such cases. (Li *et al.*, in 1990c).

Endometrial response to antiprogesterone and progesterone

We previously reported that the administration of a single dose of progesterone receptor blocker (mifepristone) in the periimplantation period (Days LH + 2 to LH + 6) resulted in

Fig. 2. Endometrial morphology in women with unexplained infertility ($n = 30$); the results of each individual morphometric measurement are shown on a background of the normal range (shaded area) established from a fertile population ($n = 70$); (●) overall endometrial development (histological dating) is normal; (○) overall endometrial development is retarded, vv = volume fraction of, wbc = white blood cell. First published in *British Journal of Obstetrics and Gynaecology*, 1990.

profound changes in endometrial morphology, including the inhibition of glandular secretory activity, acceleration of degenerative changes, increased stromal mitosis, dilatation of venules and high stromal extravasation (Li *et al.*, 1988b). More recently, we examined the morphological response of normally developing endometrium ($n = 8$) to a single intramuscular dose of 100 mg of progesterone administered between Days LH + 1 and LH + 6, by the use of morphometric techniques and transmission electron-microscopy (T. C. Li, unpublished data). We were unable to detect any significant effect of the administered progesterone on endometrial morphology. It is possible that a normally developing endometrium at this stage is already maximally stimulated by progesterone, so that the administration of a large dose of exogenous progesterone does not result in any further change. Perhaps, this is not surprising, considering the rapid changes of endometrial morphology in the first half of the luteal phase.

Artificial cycle

The artificial cycle refers to one produced in women with nonfunctioning or no ovaries, by the administration of steroid hormones, oestrogen and progesterone, in a sequential manner so as to mimic the natural cycle of normal, regularly cycling women. The artificial cycle provides a unique human model for studying the relationship between peripheral steroid hormones and the

morphology and function of the endometrium, and to address critical physiological aspects of implantation, such as the implantation window and morphological markers of implantation.

Intersubject variability of endometrial response in the artificial cycle

In a recent study, we examined the variability of the endometrial response to a standard hormone replacement therapy (HRT) (Navot *et al.*, 1986) in 18 women with premature ovarian failure, by serial ultrasonographic measurement of endometrial thickness and histological study of endometrial biopsy taken on Day 19 of the artificial cycle. Women with idiopathic premature ovarian failure ($n = 10$) had a significantly thicker endometrium and more-advanced histological development than women with Turner's syndrome ($n = 4$); whereas women with premature ovarian failure associated with previous chemotherapy had intermediate responses (Table 2). A previous study by Fraser *et al.* (1989) on the endometrial response of post-menopausal women to combined oestrogen and progestogen HRT also suggested wide intersubject variation. These observations suggest that women with (premature) ovarian failure should not be taken to represent a homogeneous population. A satisfactory response in some women to a HRT regimen may not necessarily imply the same in others. For this reason, in planning clinical trials involving the comparison of two or more HRT regimens, a cross-over design may be more appropriate than a cohort one, as the former reduces the variance of results due to individual difference in the response of the endometrium. While successful pregnancy in women with Turner's syndrome treated with HRT and donor oocyte has been reported (Salat-Baroux *et al.*, 1988), it will be of interest to compare the pregnancy rate of women with Turner's syndrome participating in a donor oocyte programme, with that of women with other causes of premature ovarian failure, to determine whether the observed difference in endometrial development is of functional and prognostic significance.

Oestrogen priming: is an incremental regimen necessary?

The earlier HRT regimens for artificial cycles were designed to mimic the natural cycle and so the oestrogen was administered in a variable, incremental manner to reproduce an oestrogen surge in the late proliferative phase. However, whether such a surge is required for normal endometrial maturation is unclear. Serhal & Craft (1987) suggested that the variable, incremental regimens could be simplified to a fixed oestrogen regimen throughout the artificial cycle; they subsequently reported successful pregnancies in women who received this type of HRT regimen (Serhal & Craft, 1989). Cameron *et al.* (1989) reported that normal endometrial morphology was achieved in artificial cycles produced by a fixed, 2 mg daily dose of oestradiol valerate.

These observations suggest that normal endometrial development is not dependent on the oestrogen surge in the late proliferative phase. This is in keeping with our previous observation in the natural cycle that endometrial development in the luteal phase was not related to the magnitude of the oestrogen surge (Li *et al.*, 1990c).

In comparison with the variable, incremental regimen, the fixed dose regimen is simple and is likely to increase acceptability by patients and compliance. In a consecutive series of 20 women with premature ovarian failure prescribed the variable, incremental regimen in our clinic, two (10%) reported mistakes in taking the correct dose of drugs; whereas none of 14 consecutive women who were prescribed the fixed dose regimen recorded any error.

Optimum doses of oestrogen

The serum oestradiol concentrations in artificial cycles produced by a number of HRT regimens, such as those of Navot *et al.* (1986), Lutjen *et al.* (1984) and Serhal & Craft (1987), were higher than those of the natural cycle. In our studies, the serum concentrations of oestradiol in artificial cycles produced by the regimen of Navot *et al.* (1986) were 2–5 times those of the natural cycle.

Preliminary data from our laboratory suggest that, although such high concentrations of oestradiol do not appear to produce any obvious abnormality under the light microscopy, features in keeping with excessive oestrogenic influence were detected by transmission electron microscopy (P. Dockery, unpublished data).

Analysis of results reported by other investigators revealed similar evidence of excessive oestrogen stimulation in the glands, such as persistence of subnuclear vacuoles (Borrero *et al.*, 1989) and relative glandular retardation (Droesch *et al.*, 1988). Perhaps further studies are required to define the optimal oestrogen doses for priming of the endometrium in artificial cycles.

Duration of oestrogen priming

Navot *et al.* (1989) studied the effect of variable duration of oestrogen priming and reported that endometrial development in the artificial cycle was similar in those with a short duration of priming (6 days), a standard duration of priming (2 weeks), or a long duration of priming (3–5 weeks). Their results suggested that the duration of oestrogen priming is likely to be flexible.

Effect of varying doses of progesterone

In a prospective, cross-over study, we examined the effect of various doses of progesterone on endometrial development in the artificial cycles (T. C. Li, unpublished data). The HRT of Navot *et al.* (1986) was used as a standard regimen, which involved oral administration of a variable daily dose (1–6 mg) of oestradiol valerate and intramuscular administration of 25–50 mg of progesterone in oil from Days 15 to 28 of the cycle. In the modified regimens, the doses of oestradiol valerate were unaltered but the doses of progesterone were increased to five times of the standard regimen, i.e. 125–250 mg (high progesterone regime), or reduced to only one-fifth of the standard regimen, i.e. 5–10 mg (low progesterone regime). In all cycles studied, an endometrial specimen was obtained on Day 19, i.e. on the 5th day of progesterone therapy. While suboptimal doses of progesterone result in retarded endometrial development, large or supranormal doses of progesterone do not advance endometrial development. The minimal doses of progesterone required to produce normal endometrial development should lie somewhere between the standard and low progesterone regimens. These results are in agreement with the observations of Devroey *et al.* (1989), who reported that normal endometrial maturity in the midluteal phase (Day 21) of artificial cycles could be achieved with a rather wide range of serum progesterone concentrations (from < 10 ng/ml to > 30 ng/ml). However, progesterone concentrations ≤ 5 ng/ml appeared to result in abnormal development.

Navot *et al.* (1989) attempted to address the question of whether secretory transformation in the endometrium of artificial cycles could be accelerated by high doses of progesterone. At a daily dose of 150 mg, progesterone did not advance endometrial development in the early luteal phase, but the authors claimed that endometrial maturation in the late luteal phase was accelerated. However, one criticism of their study is that endometrial samples were obtained 2 days earlier in the cycles treated with high doses of progesterone than those treated with standard doses of progesterone, 'because of the anticipated hastened secretory transformation'.

Effect of different preparations of progesterone

Oral progesterone. Dehou *et al.* (1987) reported that delayed endometrial maturation appeared typical of artificial cycles treated with oral progesterone at a daily dose of 300–600 mg: subnuclear vacuoles, nuclear channel systems and giant mitochondria were absent on Days 16 to 19, whereas pseudostratification and glandular mitoses were prominent. In contrast, cycles treated with intramuscular progesterone (50–100 mg/day) revealed normal glandular maturation, both at light-microscopic and electron-microscopic levels. Later, Devroey *et al.* (1989) compared the effects of 300 mg of oral progesterone with those of 100 mg of intramuscular progesterone and presented similar findings. In both of the above studies, serum progesterone concentrations were significantly

lower in cycles treated with oral progesterone than in those treated with intramuscular progesterone, suggesting that a probable explanation of the difference was the poor absorption of progesterone when administered orally. Increasing the oral dose of progesterone may not be appropriate because of side effects, especially sleepiness (Devroey *et al.*, 1989).

Vaginal progesterone. In artificial cycles treated with vaginal progesterone at 300–600 mg, Devroey *et al.* (1989) found that the histological and electron-microscopic pictures of the endometrium on Day 21 of the cycle were similar to cycles treated with intramuscular progesterone at a daily dose of 100 mg, although the serum concentrations of progesterone in cycles treated with intramuscular progesterone were five times higher than those treated with vaginal progesterone. (The serum concentrations of progesterone in the latter were, in turn, higher than those cycles treated with oral progesterone at the same dose.) Progesterone (micronized) administered vaginally has been reported to support implantation and pregnancy in artificial cycles (Devroey *et al.*, 1989).

Dydrogesterone. Dydrogesterone (Duphaston) has been claimed to be useful in the treatment of luteal-phase defect (Balasch *et al.*, 1982) and successful in inducing endometrial secretory changes in post-menopausal women (King & Whitehead, 1986; Lane *et al.*, 1986). However, a recent cross-over study by Pellicer *et al.* (1989) comparing the use of dydrogesterone (10–20 mg/day, given orally) and progesterone administered intramuscularly (25–50 mg/day) in artificial cycles revealed out-of-phase endometrium in 3 of 8 cases treated with dydrogesterone, but in none of 8 cases treated with intramuscular progesterone. In addition, (a) stromal haemorrhagic suffusion and increased oedema were noted in the endometrium of all those treated with dydrogesterone and (b) the total protein content of uterine lavages (10 ml normal saline via a Foley catheter) was significantly lower in those treated with dydrogesterone than in those treated with intramuscular progesterone. It was, therefore, concluded that endometrium supplemented with dydrogesterone failed to result in optimal endometrial development and should not replace intramuscular progesterone as the progestin in steroid replacement cycles.

Comparative studies of the natural and artificial cycles

Comparative morphological studies of endometria obtained from various cycles known to be capable (or not capable) of supporting implantation may provide important information on which of the morphological features are consistently associated with normal function, i.e. implantation, and so may be used as marker(s) of implantation. We compared the morphology of endometrium in the periimplantation period in (a) natural cycles of women with proven fertility, and (b) artificial cycles of women with idiopathic premature ovarian failure receiving the hormone replacement regimen of Navot *et al.* (1986). In the artificial cycles ($n = 12$), all endometrial specimens were taken on Day 19, i.e. the Day 5 of progesterone therapy and this corresponds to Day LH + 5 of the natural cycle. The endometrial specimens were dated and further analysed by established morphometric techniques (Li *et al.*, 1988a; Dockery *et al.*, 1988, 1990). The results are shown in Table 2 and Fig. 3.

The results indicate that, while the HRT regimen of Navot *et al.* (1986) produced endometrial morphology similar in many aspects to the corresponding Day (LH + 5) of the natural cycle, subtle differences in the stroma were detected by morphometry. The stromal cell dimensions (Fig. 3) are similar to those of Day LH + 2 from the natural cycles. However, the volume fraction of the total tissue (endometrium) occupied by the stromal cell nuclei is similar to the corresponding period (Days LH + 4 to LH + 6) of the natural cycle. When compared with the natural cycle, the smaller stromal cell nuclei but similar volume fraction (ratio) in the artificial cycle together suggest an increase in cell density, a finding which is supported by the earlier report of Dehou *et al.* (1987). Given that both these cycles are capable of supporting implantation, perhaps the stromal cell size and density are of little functional significance as far as implantation is concerned.

So far, many different HRT regimes have been reported to be capable of supporting implantation in the artificial cycle (Lutjen *et al.*, 1984; Navot *et al.*, 1986; Rosenwaks, 1987; Serhal & Craft, 1987;

Table 2. Endometrial morphology between Day 19 of artificial cycle, from 12 women with idiopathic ovarian failure receiving the hormone replacement therapy regimen of Navot *et al.* (1986) and Day LH + 5 of natural cycles from 8 previously fertile women

Measurements	Day 19 of artificial cycle (<i>n</i> = 12)	Day LH + 5 of natural cycle (<i>n</i> = 8)	<i>P</i> value
Histological dating (overall development)	4.9 (4.1–6.4)	5.3 (3.5–6.7)	NS
Mitotic activity /1000 gland cells	0.5 (0–2.0)	0.5 (0–3.0)	NS
/1000 stromal cells	1.0 (0–2.0)	1.0 (0–2.0)	NS
Volume fractions (%)			
Gland occupied by gland cells	86 (67–89)	85 (71–93)	NS
Endometrium occupied by gland	34 (24–47)	30 (17–34)	NS
Secretory vacuoles/1000 gland cells:			
Supranuclear	10 (5–20)	18 (6–47)	NS
Subnuclear	68 (30–92)	69 (22–81)	NS

Results are medians, with ranges in parentheses NS, not significant, according to Wilcoxon nonparametric test.

Fig. 3. Data on stromal cell nuclei (a, b and c) and endometrial stroma (d) in endometrial specimens obtained on Day (D) 19 of artificial cycles in 12 women with idiopathic premature ovarian failure receiving the hormone-replacement therapy regimen of Navot *et al.* (1986) (▨), and endometrial specimens collected from the early luteal phase of natural cycles from 12 normal, fertile women (□). LH = luteinizing hormone. The data on the natural cycles have been published (Dockery *et al.*, 1990). Each bar represents the result in arithmetic mean, each vertical line represents the s.e.

Asch *et al.*, 1988; Droesch *et al.*, 1988; Devroey *et al.*, 1989; Steingold *et al.*, 1989; Ben-Nun *et al.*, 1989). Among these regimens, the oestrogen may be administered orally as oestradiol valerate (Lutjen *et al.*, 1984; Navot *et al.*, 1986; Serhal & Craft, 1987), micronized oestradiol (Rosenwaks, 1987; Asch *et al.*, 1988), conjugated oestrogen, e.g. Premarin (Ben-Nun *et al.*, 1989), or the oestrogen may be given via a vaginal ring (Rosenwaks, 1987; Steingold *et al.*, 1989), or a transdermal patch (Droesch *et al.*, 1988). The progesterone may be given intramuscularly (Lutjen *et al.*, 1984; Navot *et al.*, 1986; Asch *et al.*, 1988) or via a pessary (Rosenwaks, 1987; Lutjen *et al.*, 1986; Devroey *et al.*, 1989). It seems that endometrial receptivity to embryo implantation could be achieved by a rather wide range of HRT regimens with various preparations of oestrogen and progesterone.

The Norfolk group of investigators (Rosenwaks, 1987; Droesch *et al.*, 1988; Navot *et al.*, 1989; Steingold *et al.*, 1989) consistently reported on glandular stromal disparity (stroma more advanced than gland by 2–3 days) in artificial cycles produced by various types of HRT regimens. However, we do not have the same experience with endometrial samples obtained on Day 19 of the artificial cycle. If there is a glandular stromal disparity, our data suggest that the stroma is relatively retarded, not advanced. Glandular stromal disparity in artificial cycles has not been reported by other investigators.

Among the various HRT regimens known to be capable of supporting implantation and pregnancy in artificial cycles, the overall endometrial development was reported to be normal in some (Navot *et al.*, 1986; Lutjen *et al.*, 1986; Hung *et al.*, 1989), but retarded in others, e.g. by 2–3 days (Borrero *et al.*, 1989). None reported on the advancement of endometrial development. These observations suggest that some degree of retardation of endometrial development may be compatible with successful implantation in the artificial cycle.

Window of implantation and transfer

It is generally agreed that a certain degree of synchrony is required between the arrival of the blastocyst at the site of implantation and the stage of development of the endometrium in order to achieve successful implantation (McLaren, 1984; Edwards & Fishel, 1986). In rats and mice, there appears to be a narrow window during which implantation can be initiated and outside which implantation may not take place. In mice, for the first 2–3 days after fertilization, replaced embryos do not survive in the uterus. The ensuing 36–48 h is a period in which embryos could survive in the uterus, but implantation would not be possible. Then there is a narrow window of just a few hours during which implantation can occur. After this time, the uterine environment becomes hostile, and any unimplanted embryo degenerates (Psychoyos, 1961). Although the window of implantation is rather narrow, exact synchrony between endometrial and embryo development is not required, because during the 36–48 h prior to this window, the endometrium is not hostile to the developing embryo. It is possible for a more advanced embryo to remain dormant in a relatively retarded endometrium to allow the latter to catch up. The embryo needs to be at the same stage as, or slightly more advanced than, the endometrium; the embryo cannot afford to lag behind.

A similar window is thought to exist in man. From the study of Croxatto *et al.* (1978), in the natural cycle, the human embryo usually arrives in the endometrial cavity 96–120 h after the LH surge. In stimulated, in-vitro fertilization cycles, embryos often arrive in the endometrial cavity around Day LH + 3, i.e. 1–2 days earlier than the natural cycle, but implantation is still possible. In artificial cycles, experience from various centres with established donor oocyte programmes suggests that the embryos are best transferred to the endometrial cavity 2–4 days after the commencement of progesterone therapy (Navot *et al.*, 1986; Serhal & Craft, 1987; Rosenwaks, 1987). Investigators in the Monash Centre, however, suggest transferring the embryo slightly earlier, 1–3 days after the start of progesterone therapy (Chan *et al.*, 1987). On the whole, it seems likely that the window of transfer in man covers 3–4 days.

Conclusions

The study of artificial cycles in women with premature ovarian failure has provided a unique human model for understanding the steroidal requirement for normal endometrial development and implantation. Clinically, highly rewarding results have been obtained as artificial cycles are now capable of supporting implantation. Comparative study of the natural and artificial cycles has resulted in a better definition of the implantation window, and will undoubtedly help to answer other important questions in reproductive biology, e.g. morphological markers of implantation and embryo–endometrial interaction.

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