CHAPTER 20

Endometrial preparation for IVM

Anne-Maria Suikkari

INTRODUCTION

Implantation depends both on the quality of the embryo and the endometrium. Proliferation and secretory changes of the endometrium are directly and indirectly influenced by the steroid hormones estrogen and progesterone. An inadequate exposure of the endometrium to these hormones may lead to implantation failure[1]. In different assisted reproductive techniques (ART), such as in-vitro fertilization (IVF), oocyte donation, frozen-thawed embryo transfer, and in-vitro maturation (IVM), the aim is to achieve synchronization between the receptive endometrium and embryo development. In spontaneous cycles these events are related to ovulation and cyclic hormonal changes. This is not the case in IVM. Therefore artificial preparation of the endometrium is necessary to open the window of implantation.

Although Cha et al. were the first to achieve a live birth after IVM in a recipient of a donated oocyte[2], it was Trounson et al. who first successfully transferred embryos in the same cycle in which the immature oocytes were retrieved using ultrasound guided transvaginal aspiration of small follicles[3]. One of the challenges of this method is to prepare the uterus in only a few days between the oocyte retrieval and embryo transfer. Because immature oocytes are usually retrieved before the dominant follicle develops, the endometrium is exposed to relatively low levels of estradiol by the time of immature oocyte collection (IOC). As a result, there is a dyssynchrony between the phase of the endometrium and the cleaved embryo. This is thought to be one of the reasons for the low success of the early days of IVM development[4]. Since then the clinical outcome of IVM has greatly improved and protocols for endometrial preparation have been developed.

This chapter will discuss the quality of endometrium in different applications of ART, the rationale for endometrial preparation in IVM, and current protocols used for the preparation of endometrium.

WHAT IS A RECEPTIVE ENDOMETRIUM?

The development of a receptive endometrium is a complex process which involves the sex steroid hormones and various local factors and cell surface structures such as cell adhesion molecules, integrins, cytokines, extracellular matrix proteins, and pinopodes[5,6]. The only known undisputed marker for uterine receptivity in the human, however, is implantation[6]. Proliferation

of the endometrium is regulated by estradiol produced by the granulosa cells of the growing follicle/s. The transformation into secretory and subsequently receptive endometrium requires adequate progesterone exposure from the corpus luteum. A mathematic calculation by Rogers et al. estimated that the uterine receptivity accounts for about 31–64% of implantation[7].

The morphologic changes of the endometrium during a spontaneous menstrual cycle were described by Noyes et al. in 1950 and are still considered as the gold standard today[8]. However, normal morphology does not always imply adequate functional capacity of the endometrium[9]. In estrogen/progesterone-supplemented cycles, there is a frequently observed glandular–stromal asynchrony in endometrial biopsies taken in the mid luteal phase[10]. Furthermore, endometrial morphology differs according to the hormone replacement preparation and route of administration[11].

Specific genes and molecular markers for implantation and endometrial receptivity have been investigated[5,12,13]. The roles of the various adhesion molecules, cytokines, and other factors are being debated[5,6]. The expression of, e.g., $\alpha_v\beta_3$ integrin and leukemia inhibitory factor (LIF) coincides with the implantation window, but their exact biological action remains unclear[6]. Pinopodes are ultrastructural formations of the receptive endometrium. The appearance of pinopodes lasts less than 48 h and they coincide with the implantation window on cycle days 20–22. Pinopodes tend to form earlier in stimulated cycles and later in hormone replacement cycles compared with natural cycles[14]. However, the appearance of pinopodes varies individually and evidence for direct involvement in embryo attachment is still lacking[6].

ENDOMETRIUM IN STIMULATED CYCLES FOR IVF

Ovarian stimulation for IVF is known to cause an advancement of 2–5 days in the histology of the late follicular phase endometrium and a dyssynchronous glandular and stromal differentiation in the midluteal phase in 45–90% of stimulated IVF cycles[1,6,15–18]. When this endometrial advancement exceeded 3 days, no pregnancies occurred[1,19]. In the mid luteal phase, on the other hand, a delay in endometrial development has been observed[20]. This asynchronous endometrial development during GnRH-agonist and gonadotropin stimulated IVF cycles is normalized with progesterone or human chorionic gonadotropin (hCG) supplementation in the late luteal phase[21]. A study on pinopodes further supports this shift in endometrial maturation in IVF cycles[14]. Albeit that the implantation rates are lower in IVF compared with natural cycles, the observed altered endometrial development in IVF and hormone replacement cycles is thought to have no major impact on actual endometrial receptivity, the endometrium playing only a generally permissive role[1,22].

Adequate proliferative and secretory changes are necessary for successful implantation to occur. Endometrial thickness can be regarded as a marker for proliferation and is easily measurable using an ultrasound scan. Conflicting reports exist concerning possible relationships between endometrial thickness and treatment outcome in IVF cycles[23]. The existing data suggest that endometrial thickness has no predictive value on the cycle outcome[23]. However, most studies have concluded that pregnancy rates drop when the endometrial thickness is less than 7 mm[6].

LESSONS FROM OOCYTE DONATION AND FROZEN-THAWED EMBRYO TRANSFER CYCLES

Endometrial proliferation is necessary to enable optimal progesterone receptor function and transition to receptive endometrium[24]. Neither endometrial thickness nor serum estradiol has been shown to predict optimal receptivity and

outcome in an oocyte donation program[25]. In oocyte donation cycles the length of estrogen administration can be varied between the extremes of 6 and 100 days before progesterone addition. However, the miscarriage rate increases below 11 days and beyond 9 weeks[24]. Different durations of progesterone exposure between 1 and 9 days before day 2–3 embryo transfer have been reported[24]. Pregnancies have been achieved after 1–6 days of progesterone before day 2–3 embryo transfers in oocyte donation cycles[26]. Rosenwaks et al.[27] found that in recipients of donor oocytes, day 2 embryos are best transferred to the uterus on day 3 or 4 of progesterone exposure.

In estradiol/progesterone supplemented frozen-thawed embryo transfer cycles varying durations, preparations, and dosages of hormone administration have been reported[24]. It has been suggested that it is appropriate to start progesterone administration as soon as the endometrium is developed sufficiently, i.e. at least 8 mm in thickness with a trilaminar ultrasound appearance, and to perform the embryo transfer not before 3–4 days of progesterone treatment[24].

FORMS OF DRUGS USED FOR ENDOMETRIAL PREPARATION

The various forms of estrogen and progesterone delivery used in oocyte donation programs have been extensively discussed in a review by Devroey and Pados[10]. The oral route of estrogen administration results in less fluctuation in serum estrogen concentrations but a lower estradiol:estrone ratio than the transdermal route of delivery. Both are equally effective in terms of pregnancy rates[10]. Native progesterone, which is more effective in luteal support for implantation and pregnancy than other progesterone derivatives, can be administered orally, intravaginally, or intramuscularly[10]. The vaginal micronized progesterone suppositories and i.m. injections have been shown to be the most effective and best tolerated routes of progesterone delivery. A meta-analysis of five prospective randomized trials suggests that there is no difference in pregnancy rates between luteal support with i.m. progesterone or i.m. hCG[28]. It is recommended that the luteal supplementation should be performed using progesterone rather than hCG, given the higher risk of ovarian hyperstimulation syndrome (OHSS) with hCG use[1]. The comparison between the delivery of native progesterone by the i.m. or vaginal route gives a relative ratio of 1.33 in favor of i.m. delivery[28]. The significance of this finding needs to be further evaluated. So far, there does not seem to be any major change in the clinical practice of luteal support in IVM programs. This may be due to the greater ease and simplicity of vaginal progesterone administration.

HOMORNE SUPPLEMENTATION IN IVM CYCLES

Optimal thickness of the endometrium

In a retrospective analysis of frozen-thawed embryo transfers in spontaneous cycles, Loh and Leong[29] showed better pregnancy rates if the endometrium thickness was at least 11 mm. In IVF cycles the consensus has been that the thicker the endometrium, the better the pregnancy rate. In natural cycles the opposite seems to be true. The mid proliferative and preovulatory endometrial thickness has been shown to be less in conception cycles compared with non-conception cycles[30,31]. An increased preovulatory endometrial thickness seems unfavorable for spontaneous conception, with a cut-off thickness of 12 mm and a mean thickness of 7.8 mm for pregnant and 9.1 mm for non-pregnant cycles with a leading follicle of 19 mm in diameter[31].

IVM is performed in a natural cycle either in the mid follicular phase of an ovulatory cycle or in the proliferative phase of an anovulatory cycle. By the time of IOC, the endometrium has started to proliferate, but has not yet reached

the thickness of a mature preovulatory endometrium. Therefore the ultrasonographic data on optimal endometrial thickness from stimulated IVF, oocyte donation, and frozen-thawed embryo transfer cycles may not be relevant to IVM. Many women with PCOS have a thin endometrium on the day of IOC, yet comparable pregnancy rates to IVF have been reported[32,33]. Child et al.[33] found a small but significant difference in the mean endometrial thickness on the day of embryo transfer between conception and non-conception cycles in 155 IVM patients (10.2 versus 9.4 mm, respectively). However, there was no difference in the mean endometrial thickness on the day of IOC between pregnant (6.5 mm) and non-pregnant women (6.6 mm). Figure 20.1 shows the thickness of the endometrium on the day of IOC in 171 cycles with embryo transfer performed at the Infertility Clinic of the Family Federation of Finland in women with normal or polycystic ovaries[34]. No correlation between endometrial thickness and cycle outcome was found. An ongoing pregnancy and live birth was achieved when the endometrium was 3 mm on the day of oocyte retrieval.

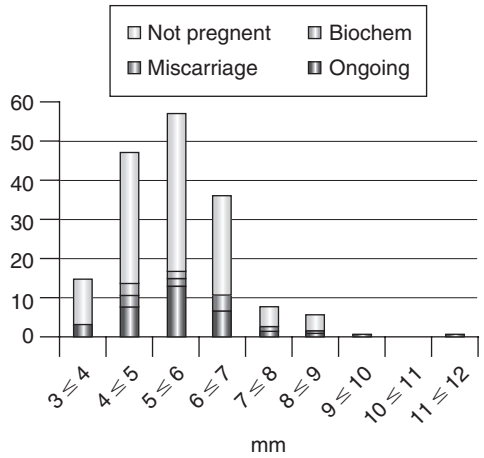

Figure 20.1 The number of ongoing pregnancies, miscarriages, biochemical pregnancies and non-pregnant cycles according to the endometrial thickness on the day of IOC

The effect of gonadotropin priming on the endometrium

The challenge with IVM is to retrieve immature oocytes before ovulation, usually in mid to late follicular phase, and then return a cleaving embryo a few days later into the uterus. The use of minimal FSH stimulation during the early follicular phase could potentially increase the endometrial thickness before IOC and thus improve the outcome. However, there does not seem to be any benefit of early follicular phase FSH priming in women with regular cycles and normal ovaries[35]. On the other hand, in PCOS patients with no spontaneous follicle development and subsequent endometrial proliferation, there were more pregnancies in the FSH primed group compared to the non-primed group, albeit no difference in the endometrial thickness at IOC between the groups was observed[36]. Others have not found any beneficial effect of FSH priming in PCOS patients[32,37].

In a case report, Barnes et al.[38] described a successful IVM cycle in the presence of an 18 mm dominant follicle; 1000 IU hCG was administered at the time of oocyte recovery followed by progesterone pessaries 300 mg/day 48 h later. Ultrasound assessment revealed a corpus luteum and a secretory endometrium of 9 mm at the time of embryo transfer. They speculated that the administration of hCG prior to IOC may enhance synchronization between the uterus and embryo. Administration of hCG has been shown to increase the endometrial thickness and implantation rate in recipients of donated oocytes[39]. The possible role of in-vivo administration of hCG on the uterus remains to be elucidated[40].

Start, dose, and duration of hormone supplementation

In IVM, the uterus has to be prepared and synchrony between the endometrium and embryo achieved in a greatly accelerated time schedule

compared to other types of ART. At the time of IOC, the endometrium is still relatively thin owing to the low levels of estradiol secretion from the small antral follicles in the early to mid follicular phase. The start of progesterone administration at the time of insemination coincides with the rise of serum progesterone after ovulation in a natural cycle[35]. The first protocol used for endometrial preparation consisted of estradiol valerate 2–4 mg daily from the day of IOC and progesterone intravaginal suppositories 200–300 mg per day started 48 h later at the time of insemination[3,38].

Concerns for the short duration of estradiol priming and data from donor oocyte recipient studies suggesting that at least 6 days of estrogen priming before embryo transfer are needed to prepare the endometrium for implantation prompted Russell et al.[4] to compare two regimens of endometrial priming: an early follicular phase priming using 17β-estradiol 2 mg twice a day starting on cycle day 2 or 3 and a mid follicular priming of 2 mg of 17β-estradiol starting on cycle day 6 and increasing by 1–2 mg per day depending on the endometrial thickness on ultrasound scan. Both groups were continued on 8 mg per day from the day of IOC. Intramuscular progesterone 50 mg was started on the day following IOC and continued by 100 mg on the second day until the pregnancy test. Both estradiol and progesterone were continued, if the test was positive, until 70 days' gestation[4]. They concluded that a significant decrease in the maturation and cleavage rates was identified with immature oocytes exposed to early exogenous estrogens[41].

The hypothesis of potentially inadequate endometrial preparation in the IVM cycle by the Trounson method[3] was addressed in a study by Suikkari et al.[37] in which IVM embryos at both pronucleus and cleaved stage were cryopreserved to be replaced in either a natural or hormone supplemented frozen-thawed embryo transfer cycle. Unfortunately, the cryosurvival of the IVM embryos was found to be significantly decreased compared to conventional IVF and ICSI embryos and no conclusions on the efficacy of endometrial preparation could be drawn[37].

Prospective studies comparing different protocols for optimal endometrial preparation in IVM cycles are lacking. Based on the insights into the physiology of endometrial preparation from oocyte donation and frozen-thawed embryo transfer programs, the groups performing IVM with embryo transfers have conjured slightly different estrogen/progesterone supplementation protocols, shown in Table 20.1. The differences in the pregnancy results are likely to be due to factors other than the endometrial priming protocols used. Figure 20.2 shows a schematic presentation of the timing of hormone supplementation in IVM cycles used by several groups[33–35]. In general, the administration of oral estrogen is started on the day of IOC and the dose is adjusted to the thickness of the endometrium on the day. Vaginal progesterone pessaries are commenced at the time of insemination or intracytoplasmic sperm injection (ICSI). The time of embryo transfer may vary between day 2 and day 5 after insemination or ICSI. Both estrogen and progesterone are continued after a positive pregnancy test until 7–12 weeks of gestation.

Mikkelsen et al.[42] have used a standard protocol for endometrial preparation in all their studies including women with regular cycles and normal ovaries and women with polycystic ovaries with or without follicular phase FSH

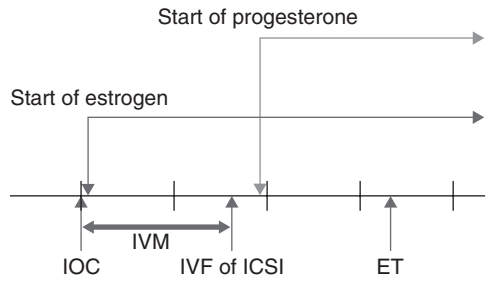

Figure 20.2 Protocol for endometrial preparation in IVM cycle (see color plate section)

Table 20.1 Endometrial preparation protocols for IVM in the patient's own transfer cycle

Reference	*Estrogen preparation*	*Dose, route*	*Start*	*Progesterone preparation*	*Dose, route*	*Start*	*Continued until*	*PR/ET (%)*
Trounson et al.[3], 1994	Estradiol valerate	2 mg/d, oral	Day of IOC	Progesterone pessaries			Not identified	Not identified
Barnes et al.[38], 1996	Estradiol valerate	4 mg/d, oral	Day of IOC	Progesterone pessaries	300 mg/d, vaginal	48 hours after IOC at the time of insemination	Not identified	Not identified
Russell et al.[4], 1997	Estradiol valerate	4 mg/d or incremental increase from 2 mg/d, oral	Cycle day 2–3 or 6	Progesterone	50 mg increasing to 100 mg on the second day, intramuscular	Day after IOC	10 gw	1/14 (7)*
Mikkelsen et al.[35], 1999	Estradiol valerate	6 mg/d, oral	Day of IOC	Micronized progesterone	300 mg/d, vaginal	2 days after IOC	7 gw	5/20 (25)*
Child et al.[43], 2001	Estradiol valerate	6–10 mg/d, oral	Day of IOC	Progesterone	400 mg/d, vaginal	Day of ICSI	12 gw	34/169 (20)*
Lin et al.[32], 2003	Estradiol valerate	6–10 mg/d, oral	Day of IOC	Micronized progesterone	800 mg/d, vaginal	Day of ICSI	12 gw	23/68 (34)*
Le Du et al.[46], 2005	Estradiol hemihydrate	6–10 mg/d, oral	Day of IOC	Progesterone	Not identified	Day of IOC	12 gw	9/40 (23)*
Son et al.[47], 2005	Estradiol valerate	6 mg/d, oral	Day of IOC	Progesterone cream	100 mg/d, vaginal	Day of IOC	9–10 gw	24/47 (51)*
Söderström-Anttila et al.[34], 2005	Estradiol valerate	6 mg/d, oral	Immediately after IOC	Micronized progesterone capsules	600 mg/d, vaginal	Evening of the day of insemination or ICSI	9–10 gw	49/184 (27)*

* percentage of pregnancies/embryo transfer
PR/ET, pregnancies/embro transfer; gw, gestation week.

priming. The administration of 17β-estradiol 2 mg orally three times daily was started on the day of oocyte retrieval. If endometrial thickness was <6 mm at ultrasound on the day of aspiration, the cycle was cancelled. Two days later the luteal phase was supported by vaginal micronized progesterone suppositories 100 mg three times daily. Both estrogen and progesterone were continued until the pregnancy test, and if it was positive hormone supplementation was continued until 50 days, i.e. 7 weeks of gestation.

The McGill group in Montreal has used individualized protocols adjusted to the endometrial thickness at IOC. The patients have been given estradiol valerate in divided doses, starting on the day of oocyte retrieval. If the endometrial thickness on the day of oocyte retrieval was less than 6 mm, a 10 mg dose of estradiol was given; if it was more than 6 mm, a 6 mg dose was given[43,44]. Luteal support was provided by 200 mg intravaginal progesterone twice daily, starting on the day of ICSI and continued along with estradiol until 12 weeks of gestation[43]. On the day of embryo transfer, the endometrial thickness was measured again and if it was less than 7 mm, the couples were offered embryo cryopreservation and transfer in a subsequent cycle[45]. Other groups around the world working closely with the McGill group have adopted slightly different estrogen/progesterone treatment regimens[46,47]. The French group reported their results in infertile women with PCOS. They used an endometrial thickness of 5 mm as a cut-off to give 10 mg of estradiol, otherwise they gave 6 mg estradiol per day. Unlike in other programs, progesterone was started on the day of IOC. Their results showed an endometrial thickness of 8–13 mm at embryo transfer in all patients and a clinical pregnancy rate of 23% (9/40) per embryo transfer[46]. A group in South Korea has published high pregnancy and implantation rates (51% and 24%, respectively) after blastocyst transfers in patients with a risk of OHSS in previous IVF cycles. For endometrial preparation they gave estradiol valerate 6 mg and progesterone 100 mg daily from the day after IOC. The medication was continued until 9–10 weeks of pregnancy[47].

After our preliminary study[37], we have adopted the estrogen/progesterone supplementation protocol described by Mikkelsen et al.[35] Endometrial proliferation is enhanced by commencing oral estradiol valerate 6 mg per day (Progynova, Schering, Finland) immediately after IOC to ensure as long an estrogen exposure as possible before the start of progesterone. Vaginal micronized progesterone 300 mg twice daily (Lugesteron, Leiras, Finland) is commenced in the evening of the following day, which is the day of insemination or ICSI. Both estrogen and progesterone are continued until the first pregnancy ultrasound at 7 weeks of gestation. If a healthy pregnancy is found, the estrogen and progesterone doses are tapered over the following 2 weeks and discontinued at 9–10 weeks of gestation[34].

IVM combined with natural cycle IVF

The combination of a natural cycle IVF and IVM offers an interesting alternative to improve the pregnancy rates of conventional IVM. Thornton et al.[48] found that at the oocyte pick-up in unstimulated IVF cycles, immature oocytes were observed in 48% of the cases. These metaphase I and GV oocytes could be matured and fertilized in vitro, resulting in 'extra' embryos available for transfer. In a natural cycle there is a cohort of follicles developing alongside the dominant follicle. These follicles have been thought to undergo atresia under the increasing steroid hormone secretion from the dominant follicle[49]. This hypothesis is being questioned by Chian et al.[50], who found that developing follicles of about 12 mm in diameter seemed to be able to respond to exogenous hCG administration and mature oocytes could be retrieved from these follicles. As the endometrial thickness increases with increasing estradiol levels from the growing follicles, exogenous hormonal supplementation may not be necessary in these cases[50].

SUMMARY AND CONCLUSIONS

In an IVM cycle, the physiologic periovulatory hormonal and endometrial changes of a natural cycle are missing at the time of oocyte retrieval. Adequate endometrial proliferation has to be achieved in just 2 days before the start of progesterone administration in order to open the window of implantation for the embryo. Basically, identical protocols for endometrial preparation have been used both in women with regular cycles and normal appearing ovaries and in women with polycystic ovaries with or without follicular phase priming with FSH or hCG[34–36,42].

There are only minor differences in the estrogen/progesterone supplementation protocols between the groups performing clinical IVM today. In general, estrogen is commenced on the day of IOC because of the evidence that excessive estrogen exposure in the early follicular phase may be deleterious to the oocytes[41]. In order to allow as long an estrogen exposure as possible before the progesterone is administered, it is advisable to start estrogen tablets immediately after the oocyte pick-up. Progesterone is usually started 36–48 h later. Both estrogen and progesterone are continued until 7–12 weeks of gestation (Table 20.1). The pregnancy rates vary between 20 and 51% in different reports[32,34–36,43–47]. It is likely that other factors such as patient selection, hormonal priming, oocyte aspiration technique, and culture method have more impact on the results than the current endometrial preparation protocols. However, to further increase the efficacy of IVM, it is important to focus on ways of improving the endometrial preparation.

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