NEW DEBATE

What is the ‘ideal’ duration of progesterone supplementation before the transfer of cryopreserved–thawed embryos in estrogen/progesterone replacement protocols?

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Different studies dealing with the start of progesterone supplementation in assisted reproduction treatment cycles have shown that the problem apparently is the correct timing. We therefore would like to discuss the data on: (i) the start of progesterone replacement in oocyte donation programmes; (ii) the start of progesterone replacement in frozen–thawed hormone-supplemented cycles; (iii) the problem of too early a rise of progesterone in fresh IVF cycles as a model of too early an administration of progesterone; and (iv) the benefit of high progesterone levels on the day of embryo transfer in fresh IVF cycles. From the data reviewed in this paper it seems to be appropriate to start progesterone administration before transfer in oocyte donation programmes as well as transfer of cryopreserved/thawed cells as soon as the endometrium is developed sufficiently (≥8 mm, trilaminar pattern), and to perform the embryo transfer not before day 3–4 of progesterone treatment, i.e. embryo development on day 2–3. Studies dealing with the influence of too early a rise of progesterone in fresh IVF cycles have shown different results. In fact high progesterone levels seem to reflect a high response but not a lower probability of conception. Furthermore, high progesterone levels on the day of embryo transfer in fresh IVF cycles could lower myometrial contractility and therefore increase implantation rates. Since the experience from oocyte donation programs shows the benefit of a longer preparation time using progesterone, and high progesterone levels seem to have a benefit during embryo transfer, this would suggest extending progesterone administration before transfer. However, we have to find the optimal individual transfer protocol after mock cycles, for example with pinopode detection or other methods applicable in routine IVF programmes. We need more studies to be sure whether reproductive outcome after transfer of cryopreserved–thawed cells in estrogen/progesterone supplement cycles is influenced by the duration of progesterone pretreatment. If this is so, we must look for practicable methods to modify the protocols according to the individual patient, the embryonic developmental stage during transfer and other variables.

Key words: cryopreservation/embryo/progesterone/replacement protocol

Introduction

There are many reports dealing with the recommended type and dosage of estrogen and progesterone supplementation in artificial endometrial preparation before the transfer of frozen–thawed embryos (for an overview see Devroey and Pados, 1998). We know from oocyte donation programmes that a maximum flexibility is necessary to synchronize the recipient until oocytes are available. The aim is an open so-called ‘window of implantation’ with a highly receptive-appearing endometrium at the time of embryo transfer. This period lasts a maximum of 48 h (Psychoyos, 1986). At the end of endometrial preparation should be an overlapping between the ‘window of transfer’, during which a transfer is planned, and this ‘window of implantation’.

The problem of progesterone in assisted reproduction treatment cycles apparently is the correct timing. We therefore would like to discuss the data on: (i) the start of progesterone replacement in oocyte donation programmes; (ii) the start of progesterone replacement in frozen–thawed hormone-supplemented cycles; (iii) the problem of too early a rise of progesterone in fresh IVF cycles as a model of too early an administration of progesterone; and (iv) the benefit of high progesterone levels on the day of embryo transfer in fresh IVF cycles. Taking all information together, we will suggest
a protocol for the optimal preparation of frozen–thawed cycles in IVF patients.

**Importance of an adequate period of estrogen administration before starting progesterone supplementation**

It was shown in donor programmes that especially the length of estrogen administration could be varied and delayed, but different results exist with regard to the limits of variation. Michalas et al. (1996) reported that the pregnancy rate per cycle was comparable when estradiol was administered from 6 to 11 days before progesterone addition but it dropped significantly thereafter. On the other hand, lower serum estradiol concentrations and higher early abortion rates were observed in patients after short estrogen exposure (5–10 days) (Navot et al., 1991).

Successful implantation also was observed in an extreme situation even after 100 days of unopposed estradiol valerate administration (Remohi et al., 1995). Ovulatory patients in this study received a GnRH analogue simultaneously. ‘Break-through bleeding increasingly appeared according to the duration of estrogen replacement. These clinical observations provide evidence that the concept of ‘prolonged follicular phase’ estrogen replacement for ovum donation can be maintained, at least as long as 15 weeks.’ Because of the high incidence of break-through bleeding after 9 weeks (>44%), the authors recommended stopping estrogen replacement after this time. Yaron et al. (1995) extended uterine preparation with estradiol as long as 5 weeks without significantly decreased pregnancy rates.

It was suggested that shorter and lower dosage protocols of estradiol priming of the endometrium could result in higher abortion rates. This indicates an optimal endometrial proliferation which is necessary to enable optimal development of progesterone receptors and subsequent transformation into an endometrium receptive to the transferred embryo (Navot et al., 1991). Neither endometrial thickness nor serum estradiol were able to predict optimal receptivity and therefore outcome in oocyte donation (Remohi et al., 1997). Banz et al. (2002) concluded from their results in cryopreservation cycles that monitoring is unnecessary in a protocol with estradiol patches and progesterone vaginal gel.

In cases outside donation programmes we do not have the problem of asynchrony because the patients’ own cells are cryopreserved. Devroey and Pados (1998) presumed that an adequate period of estrogen administration is necessary in order to achieve a subsequent normal secretory endometrium. They have summarized different studies and demonstrated that it is possible to vary the length of the follicular phase and still achieve satisfactory reproductive outcome.

**Endometrial pinopodes as one method to individualize progesterone pretreatment and to optimize endometrial receptivity before transfer**

Despite the frequently observed glandular–stromal asynchrony in endometrial biopsies taken on day 21 of estrogen/progesterone-supplemented cycles (Devroey and Pados, 1998), other studies presented some evidence that adequate endometrial morphology does not always imply normal endometrial receptivity (Younis et al., 1996). Furthermore endometrial morphology differs according to the hormone replacement preparation and route of administration (Sauer et al., 1991).

One may speculate that an individualized endometrial preparation—especially individualized duration of progesterone pretreatment—could improve implantation rates. Pantos et al. (2004) investigated endometrial pinopodes—characteristic protrusions on the surface of the endometrial epithelial cells during the luteal phase—using scanning electron microscopy (SEM). Pinopodes as markers of endometrial receptivity appear to correspond to the ‘window of implantation’. In a mock cycle, endometrial samples were taken on days 6 and 8 (P6 and P8) after starting progesterone administration for the detection of pinopodes using SEM. Transfer of day 3 embryos was standard in the authors’ centre. In nearly 74% a modified transfer cycle was suggested (following pinopode detection) and applied by altering and extending the duration of progesterone pretreatment to synchronize day 3 embryos in P5 endometrium. They achieved significantly higher pregnancy and delivery rates comparing cycles with and without modification. This is in agreement with other studies reporting delayed pinopode formation in artificial cycles compared with natural or IVF cycles (Nikas et al., 1995). In contrast, long stimulation protocols with GnRH agonist and gonadotrophins led to accelerated pinopode development by 1–2 days compared with natural cycles (Develioglu et al., 1999).

Nikas et al. (1995) reported that assessing the time of pinopode expression can prove helpful in defining endometrial receptivity, because pregnancy rates have been shown to improve with adjustment of the day of transfer (Nikas and Psychoyos, 1997).

**Studies evaluating the duration of progesterone administration before embryo transfer in oocyte donation programmes with estradiol/progesterone-supplemented recipient cycles**

Beside the variable duration of estradiol administration, the additional progesterone extension could allow a maximum flexibility in oocyte donation programmes until good quality oocytes are available. It was shown that the variation in progesterone administration between 2 and 4 days before embryo transfer did not affect pregnancy outcome (Michalas et al., 1996). Navot et al. (1991) have revealed that pregnancies can occur with the transfer of day 2 and day 3 embryos on day 1–6 after starting progesterone in donor oocyte recipients.

In different studies dealing with oocyte donation, the transfers were performed on the second (Abu-Musa et al., 1998; Zegers-Hochschild et al., 2000), third (Remohi et al., 1997; Zegers-Hochschild et al., 2000), fourth (Jobanputra et al., 1999; Tesarik et al., 2003) and fifth (Ben-Nun and Shulman, 2003) days.
1997) day of progesterone administration or even later (Navot et al., 1986, 1991) (Table I).

In one study, 4–5 days of progesterone administration were optimal for embryo transfer comparing results after transfers between day 2 and day 7 of progesterone administration (Navot et al., 1986). Rosenwaks (1987) reported best results after transfers on day 3–5 of progesterone supplementation.

Prapas et al. (1998) performed an interesting retrospective study on the association between the ‘window of embryo transfer’ and the duration of progesterone therapy. They transferred day 2 embryos (4–6-cell) after 2, 3, 4, 5 and 6 days following initiation of endometrial exposure to progesterone. Their results indicate that the window of implantation depends on the duration of progesterone treatment. It begins ~48h after starting progesterone administration and lasts for ~4 days. Highest pregnancy rates were achieved after 5 days (48.3%), with lower rates after 4 days (40%), 6 days (20.4%) and 3 days (12%). No pregnancies were observed after 2 days of progesterone administration.

Studies evaluating the duration of progesterone administration before frozen–thawed embryo transfer in estradiol/progesterone-supplemented cycles

To our knowledge, prospective studies comparing different durations of progesterone supplementation before transfer of cryopreserved–thawed cells with regard to treatment outcome have not yet been performed.

A search of the related literature from estradiol/progesterone-supplemented cycles to prepare endometrium for cryopreserved–thawed day 2–3 embryos shows varying days, preparations and dosages of progesterone administration before transfer of cryopreserved–thawed non-fertilized oocytes, pronucleated oocytes or embryos (Table II). It is not possible to find a rational guideline for when to start progesterone before transfer. In most published studies the transfers of day 2–3 embryos were performed on the third day of progesterone administration. Lelaidier et al. (1995) have shown high pregnancy rates after transferring blastocysts on the fifth day of progesterone administration and concluded

Table I. Some studies dealing with estrogen/progesterone supplementation to prepare recipient endometrium in oocyte donation programmes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transfer of:</th>
<th>Estrogen preparation by:</th>
<th>Progesterone preparation by:</th>
<th>Days of progesterone exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Navot et al., 1986</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate or oral estradiol and estriol (2:1)</td>
<td>Intramuscular progesterone</td>
<td>2–7</td>
</tr>
<tr>
<td>Rosenwaks, 1987</td>
<td>Day 2–3 embryos</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>3–5</td>
</tr>
<tr>
<td>De Ziegler and Frydman, 1990</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Micronized progesterone</td>
<td>3–5</td>
</tr>
<tr>
<td>Navot et al., 1991</td>
<td>Day 2–3 embryos</td>
<td>Transdermal estradiol</td>
<td>Intramuscular progesterone</td>
<td>1–6</td>
</tr>
<tr>
<td>Michalis et al., 1996</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Intramuscular progesterone</td>
<td>2–4</td>
</tr>
<tr>
<td>Ben-Nun and Shulman, 1997</td>
<td>No information</td>
<td>Subcutaneous estradiol implants</td>
<td>Intramuscular progesterone</td>
<td>5</td>
</tr>
<tr>
<td>Remohi et al., 1997</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Abu-Musa et al., 1998</td>
<td>Day 2 embryos</td>
<td>Conjugated estrogens</td>
<td>Dehydrogesterone</td>
<td>2</td>
</tr>
<tr>
<td>Prapas et al., 1998</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Vaginal progesterone</td>
<td>2–6</td>
</tr>
<tr>
<td>Jobanputra et al., 1999</td>
<td>Day 3 embryos</td>
<td>Estradiol patches</td>
<td>Vaginal progesterone</td>
<td>4</td>
</tr>
<tr>
<td>Zegers-Hochschild et al., 2000</td>
<td>Day 2–3 embryos</td>
<td>Estradiol</td>
<td>Intramuscular progesterone or vaginal progesterone ring</td>
<td>2–3</td>
</tr>
<tr>
<td>Tesarak et al., 2003</td>
<td>Day 3 embryos</td>
<td>Estradiol valerate</td>
<td>Vaginal progesterone</td>
<td>4</td>
</tr>
<tr>
<td>Pantos et al., 2004</td>
<td>Day 3 embryos</td>
<td>Estradiol valerate</td>
<td>Vaginal progesterone</td>
<td>6–9</td>
</tr>
</tbody>
</table>

Table II. Some studies dealing with estrogen/progesterone supplementation to prepare endometrium before transfer of cryopreserved–thawed embryos

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transfer of:</th>
<th>Estrogen preparation by:</th>
<th>Progesterone preparation by:</th>
<th>Days of progesterone exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muasher et al., 1991</td>
<td>Day 2 embryos</td>
<td>Estradiol patches</td>
<td>Intramuscular progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Pattinson et al., 1992</td>
<td>Day 2 embryos</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Pattinson et al., 1994</td>
<td>Day 2 embryos</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Leclaidier et al., 1995</td>
<td>Blastocysts</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>5</td>
</tr>
<tr>
<td>Queenan et al., 1997a</td>
<td>Day 2 embryos</td>
<td>Estradiol patches</td>
<td>Intramuscular progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Queenan et al., 1997b</td>
<td>Day 2 embryos</td>
<td>Estradiol patches</td>
<td>Intramuscular progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Horne et al., 1997</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Vaginal progesterone</td>
<td>4</td>
</tr>
<tr>
<td>Simon et al., 1998</td>
<td>Day 2–3 embryos</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>2–3</td>
</tr>
<tr>
<td>Simon et al., 1999</td>
<td>Day 2–3 embryos</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>2–3</td>
</tr>
<tr>
<td>Banzi et al., 2002</td>
<td>Day 2 embryos</td>
<td>Estradiol patches</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Seelig et al., 2002</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Schröder et al., 2002</td>
<td>Day 2 embryos</td>
<td>Estradiol patches</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Dal Prato et al., 2002</td>
<td>Day 2 embryos</td>
<td>Estradiol valerate</td>
<td>Intramuscular progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Boldt et al., 2003</td>
<td>Day 3 embryos</td>
<td>Estradiol</td>
<td>Intramuscular progesterone</td>
<td>3</td>
</tr>
<tr>
<td>Revel et al., 2004</td>
<td>Day 3 embryos</td>
<td>Estradiol</td>
<td>Vaginal progesterone</td>
<td>3</td>
</tr>
</tbody>
</table>
that the implantation window was brought forward in time in the case of blastocysts.

But the really optimal duration of progesterone supplementation is still a matter of debate. Comparing the above-mentioned studies in regard to oocyte donation, it seems that there is a trend towards shorter progesterone supplementation period before transfer of cryopreserved-thawed embryos. There are unconfirmed reports from animal studies about a 12 h delayed reactivation of thawed embryos in relation to its morphological stage and endometrial phase. This could justify a shorter progesterone pretreatment. We do not know the real reason. One can assume that it is the result of absent prospective randomized studies addressing the question of optimal length of progesterone pretreatment in this situation.

One possibility could be to transfer the experiences of donation programmes, but this would mean extending progesterone administration before transfer and/or finding the optimal individual transfer protocol after mock cycles with pinopode detection or other methods applicable in routine IVF programmes.

The level of progesterone in the late follicular phase of IVF cycles—a similar problem?

Progesterone is a critical factor in the late follicular phase of fresh IVF cycles. We are aware of the never-ending debate on the question of whether a subtle, late follicular phase, pre-hCG rise of progesterone above a certain threshold has an impact on the outcome of treatment in IVF cycles (Table III). Also there is some controversy regarding the effect of such a critical rise, which is now arbitrarily chosen to be in the range of ~1.0 ng/ml. Taking all information together, there seems to be no effect on oocyte and embryo quality, but more on the endometrium, and this especially in poor-responder patients. However, too high a progesterone level seems to have detrimental effects.

The cause of this progesterone rise is controversially discussed. Mio et al. (1992) suggested that fluctuating levels of endogenous LH in non-agonist cycles with CC/HMG might be responsible. Since, however, the phenomenon also is observed in GnRH analogue cycles, this cannot be the only cause. Saadat et al. (2004) confirmed elevated progesterone levels in the late follicular phase and accelerated endometrial maturation in the subsequent luteal phase, but there was no significant difference with regard to this phenomenon between cycles using GnRH agonists or antagonists.

In comparison to the influence of increasing endogenous progesterone, the administration of progesterone before oocyte retrieval was associated with a lower pregnancy rate than the administration after oocyte retrieval (Sohn et al., 1999). The question is, however, whether this negative effect results from an effect on the oocytes or the endometrium.

The most common explanation of increasing progesterone level is based on the correlation of a subtle progesterone rise with a high response (Fanchin et al., 1993; Givens et al., 1994; Ubaldi et al., 1996; Lindheim et al., 1999). Likewise it was discussed whether excessive granulosa cell sensitivity due to supraphysiological levels of FSH may lead to activation of LH receptors even in the presence of low LH levels, since LH receptors are expressed as a consequence of estradiol and FSH (Harada et al., 1995; Ubaldi et al., 1995a).

Ubaldi et al. (1995a) reported significantly higher LH levels in a high progesterone group as compared to controls, but no correlation with pregnancy rates, suggesting that low LH levels can luteinize granulosa cells without ovulation. No correlation was shown with immunological or bioactive LH levels, either in a short protocol (Harada et al., 1995) or with elevated LH levels (Abuzeid and Sasy, 1996; Fanchin et al., 1997a). Adonakis et al. (1998) could not show a correlation with additional administration of hMG.

Finally, circadian ACTH variations and an adrenal contribution of progesterone were suggested, since dexamethasone was found to suppress early progesterone rise and androgens show a concomitant peak with progesterone (Eldar-Geva et al., 1997; Fanchin et al., 1997b).

The effects found to be associated with an early progesterone rise are shown in Table III. Mostly, endometrium quality seems to drop. The detrimental effect, however, is counterbalanced by the positive effect of the high response or the fact that those patients with an early progesterone rise are mostly patients with a good prognosis to become pregnant. This high response in those patients is at least partly confirmed by the observation that a post-hCG increase of progesterone correlates with a positive cycle outcome (Gonen et al., 1993; Prien et al., 1994). Fanchin et al. (1997a) confirmed this theory when they analysed 1012 patients who underwent 1189 cycles. They did a subanalysis according to poor, intermediate and good response. A subtle progesterone rise only in poor-response patients had a significant influence on pregnancy rates (Fanchin et al., 1997a).

Develioglu et al. (1999) have shown that higher levels of progesterone the day after hCG are the most predictive feature of early pinopode detection correlating with endometrial prematurity after GnRH agonist/gonadotrophin stimulation.

Ubaldi et al. (1995b) demonstrated a case report of a patient with repeated IVF cycles, in which progesterone levels were elevated to >4 ng/ml. The patient became pregnant twice, experienced one abortion and one delivery. This group confirmed their results, that a rise in progesterone does not have any negative impact after that in a large cohort of >1200 ICSI cycles (Ubaldi et al., 1995a). It may be hypothesized that the exclusive use of ICSI in this cohort influenced the results—not by ICSI itself, but by the cohort of pre-selected patients. It may be that in an IVF cohort, patients are more sensitive to those subtle changes, since the female factor is the predominant one in this group—in contrast to patients treated by ICSI.

Results of the study by Moffitt et al. (1997) confirmed no significant differences in the progesterone levels on the day of hCG between patients who conceived in both fresh and cryopreserved cycles.

The benefit of high progesterone levels on the day of embryo transfer in fresh IVF cycles

Fanchin et al. (1998) showed by M-mode sonography of the myometrium that higher contractility of the myometrium was
<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>Protocol</th>
<th>Patients (n)</th>
<th>Cycles (n)</th>
<th>Definition of progesterone rise</th>
<th>Incidence of progesterone rise (%)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mio et al., 1992</td>
<td>Retrospective</td>
<td>CC (day 3–7), hMG (day 5 onwards, every other day)</td>
<td>101</td>
<td>170</td>
<td>1.0–2.0 ng/ml (day 7 up to 24 h before hCG)</td>
<td>31.7 (32/101 patients) 20.5 (36/170 cycles)</td>
<td>Incidence of progesterone rise (%) Oocyte number ↓ (4.2 ± 2.5 versus 5.2 ± 3.5) (P &lt; 0.05)</td>
</tr>
<tr>
<td>Fanchin et al., 1993</td>
<td>Prospective</td>
<td>Long (phase?) agonist/hMG (n = 349) Short agonist/hMG (n = 236)</td>
<td>518</td>
<td>585</td>
<td>&gt;0.9 ng/ml (pre-hCG)</td>
<td>17 (100/585 all cycles) 20 (69/349 long protocol cycles) 13 (31/236 short protocol cycles)</td>
<td>Fertilization rate (49.0 versus 61.4%) (P &lt; 0.01) 12 ongoing pregnancies (only cycles without progesterone rise) Estradiol ↓ (2.488 ± 973 versus 2.069 ± 833 pg/ml) (P &lt; 0.05)</td>
</tr>
<tr>
<td>Legro et al., 1993</td>
<td>Prospective</td>
<td>Long luteal agonist/hMG</td>
<td>n.a.</td>
<td>114</td>
<td>≥1.2 ng/ml (pre-hCG)</td>
<td>29 (33/114)</td>
<td>Pregnancy rate/ET ↓ (18 versus 33%) (P &lt; 0.01)</td>
</tr>
<tr>
<td>Givens et al., 1994</td>
<td>Retrospective</td>
<td>Long luteal agonist/hMG</td>
<td>171</td>
<td>189</td>
<td>≥0.9 ng/ml (pre-hCG)</td>
<td>71 (134/189)</td>
<td>Ongoing pregnancy rate/ET ↓ (14 versus 28%) (P &lt; 0.01)</td>
</tr>
<tr>
<td>Harada et al., 1995</td>
<td>Prospective?</td>
<td>Short agonist/hMG</td>
<td>97</td>
<td>116</td>
<td>1.0–2.0 ng/ml (day 7 up to 24 h before hCG)</td>
<td>23.3 (27/116)</td>
<td>Pregnancy rate/ET ↓ (16 versus 28%) (P &lt; 0.01)</td>
</tr>
<tr>
<td>Ubaldi et al., 1995a</td>
<td>Retrospective</td>
<td>Long luteal agonist/hMG (ICSI)</td>
<td>Not available</td>
<td>1275</td>
<td>&gt;1.0 ng/ml</td>
<td>4.5 (53/1222)</td>
<td>Ongoing pregnancy rate/cycle ↓ (12 versus 24%) (P &lt; 0.01)</td>
</tr>
<tr>
<td>Abuzeid and Sasy, 1996</td>
<td>Retrospective</td>
<td>Long luteal agonist/hMG</td>
<td>54</td>
<td>63</td>
<td>&gt;0.9 ng/ml (pre-hCG)</td>
<td>71 (45/63)</td>
<td>Oocyte number ↑ (2967 ± 1125 M versus 2133 ± 859) (P &lt; 0.001)</td>
</tr>
<tr>
<td>Fanchin et al., 1996</td>
<td>Retrospective</td>
<td>Long follicular agonist/hMG</td>
<td>102</td>
<td>106</td>
<td>&gt;0.9 ng/ml (pre-hCG)</td>
<td>21 (34/162)</td>
<td>Oocyte number ↑ (11.9 versus 8.8) (P &lt; 0.01)</td>
</tr>
<tr>
<td>Ubaldi et al., 1996</td>
<td>Prospective</td>
<td>Multiple dose antagonist/hMG</td>
<td>24</td>
<td>24</td>
<td>≥1.1 ng/ml</td>
<td>20 (5/24)</td>
<td>Fertilization rate ↓ (55.3 versus 64.1%) (P &lt; 0.002)</td>
</tr>
<tr>
<td>Moffitt et al., 1997</td>
<td>Retrospective</td>
<td>Long luteal agonist/FSH, hMG</td>
<td>333</td>
<td>333</td>
<td>&gt;0.9 ng/ml (pre-hCG)</td>
<td>13 (44/333)</td>
<td>Estradiol values ↑ (2967 ± 1125 M versus 2133 ± 859) (P &lt; 0.001)</td>
</tr>
<tr>
<td>Fanchin et al., 1997</td>
<td>Retrospective</td>
<td>Long follicular agonist/hMG</td>
<td>131</td>
<td>153</td>
<td>&gt;0.9 ng/ml (pre-hCG)</td>
<td>26.8 (41/153)</td>
<td>Estradiol values ↑ (2.488 ± 973 versus 2.069 ± 833 pg/ml) (P &lt; 0.05)</td>
</tr>
</tbody>
</table>

**Table III.** Summary of studies reporting an early progesterone rise and their outcomes

- **Reference:** List of references for each study.
- **Design:** Type of study (Retrospective, Prospective).
- **Protocol:** Details of the treatment protocol.
- **Patients (n):** Number of patients in the study.
- **Cycles (n):** Number of cycles in the study.
- **Definition of progesterone rise:** Threshold for progesterone measurement.
- **Incidence of progesterone rise (%):** Percentage of patients with a progesterone rise.
- **Outcome:** Summary of outcomes including pregnancy rates and other relevant statistics.
associated with a lower pregnancy rate. They also showed that the rate of contractions was correlated with serum progesterone levels: the higher serum progesterone levels on the day of embryo transfer, the lower the myometrial contractility and the higher the pregnancy rates. This was confirmed by data on the start of administration of a vaginal progesterone gel on the day of oocyte retrieval as compared to the day of embryo transfer (Fanchin et al., 2001). With an earlier start of progesterone, contractility was suppressed and pregnancy rates rose.

Ayoubi et al. (2001) also demonstrated a lower contractility as early as 3 days after starting administration of 45 mg micronized progesterone transvaginally.

Conclusion

What is the exact timing of progesterone start? What is the best time-point for embryo transfer in frozen–thawed hormone-supplemented cycles?

The data from fresh IVF cycles show that neither too early nor too late a progesterone administration is beneficial. The method of pinopode detection is not appropriate in routine work but the question is: how can we individualize the protocols in our daily practice?

We think that the paper by Pantos et al. (2004) could stimulate awareness that there is work to do. Perhaps further studies will show that we have a broad range of equally effective protocols with different lengths of progesterone pretreatment before the transfer of cryopreserved–thawed cells, as is suggested from the studies in Table I with regard to oocyte donation.

From the data reviewed in this paper it seems to be appropriate to start progesterone administration before transfer of cryopreserved–thawed cells as soon as the endometrium is developed sufficiently (≥ 8 mm, trilaminar pattern, and to perform the embryo transfer not before day 3–4 of progesterone treatment, i.e. embryo development on day 2–3. One has to take into account that hCG administration in fresh cycles will already have led to an increase in progesterone levels and that therefore endometrium will progress quickly compared with frozen–thawed cycles which are hormone supplemented.

At the moment this question is open for discussion. We need more studies to be sure whether reproductive outcome after transfer of cryopreserved–thawed cells in estrogen/progesterone-supplemented cycles is influenced by the duration of progesterone pretreatment. If this is so, we must look for practicable methods to modify the protocols according to the individual patient, the embryonic developmental stage during transfer and other variables.

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