Optimization of cryocycles by using pinopode detection in patients with multiple implantation failure: preliminary report

I Sudoma a, b, *, Y Goncharova a, V Zukin a, b

a Clinic of Reproductive Medicine 'Nadiya', Kyiv, Ukraine; b National Medical Academy for Postgraduate Education named after P. Shupyk, Ministry of Health of Ukraine, Ukraine

* Corresponding author. E-mail address: i.sudoma@ivf.com.ua (I Sudoma).

Abstract The aim of this study was to investigate pinopode formation patterns in patients with a history of multiple IVF failures and to evaluate if their detection with subsequent modification of protocols using frozen–thawed embryos could help to increase the pregnancy and live-birth rates in these patients. The study included 55 women with at least three implantation failures. On-time pinopodes were present in only 12.7% of cases, the rest showed acceleration, delay, arrest or asynchronization of pinopode formation. Patients with anomalies of pinopode development were divided into two subgroups, one of which had the standard protocol and the other a modified protocol, in which the duration of progesterone administration before embryo transfer was changed according to pinopode formation pattern. In nine patients with the modified protocol, with asynchronized and arrested pinopodes, simultaneous transfer of embryos of different ages was fulfilled. The implantation, pregnancy and take-home-baby rates for the modified and standard protocols were 28.0% versus 6.0%, 52.4% versus 12.0% and 47.6% versus 12.0%, respectively. Detection of inappropriately timed pinopode formation with subsequent synchronization of embryonic development and endometrial maturation allowed improvements in the effectiveness of programmes using frozen–thawed embryos in women with multiple implantation failure.

Introduction The results of IVF cycles after years of experience remain suboptimal. Only one of four attempted IVF cycles results in a live birth and in one-third of failed attempts no identifiable cause is found (Andersen et al., 2005). The high fertilization rates continue to contrast with the low implantation rates and it seems that the reason for this, at least partly,
lies at the level of endometrial receptivity (Margalioth et al., 2006). Investigations in animal models by Psychoyos (1973, 1976, 1986) and Denker (1994) showed that the short phase of endometrial receptivity — known as the implantation window — is preceded and followed by a non-receptive state. Hatched blastocysts attach non-selectively to endometrial stromal cell cultures (Carver et al., 2003) as well as to tissue culture dishes, indicating that it is the endometrial epithelium that has the unique property to be resistant to attachment in other phases but the receptive one. The implantation window is described as the period in the mid-luteal phase from day 19 to day 24 (Dominguez et al., 2003) and is time-limited (Wilcox et al., 1999).

During the implantation window, plasma membranes of the luminal endometrial epithelium cells loose the microvilli and the apical cell surface develops pinopodes or uterine spikes (Lopata et al., 2002; Murphy, 2000). With the use of scanning electron microscopy (SEM), pinopodes were found in 78% of endometrial biopsies on post-ovulatory day 6 in normally cycling women (Nikas, 1999).

The role of pinopodes in the implantation process remains unclear. Pinopodes may influence the concentration of endometrial fluids near the implantation site, thus facilitating the process of adhesion and invasion. Such abilities were proven in rodents (Parr and Parr, 1977), but no signs of pinocytosis were found in humans (Adams et al., 2002). Pinopodes may also enhance the implantation surface towards the embryo (Aplin, 2006) or they could release some implantation-facilitating or promoting molecules (Kabir-Salmani et al., 2005). Pinopode appearance coincides with other implantation markers: loss of steroid receptors, maximal expression of integrin, osteopontin, leukemia inhibitory factor and its receptor (Nikas and Aghayanova, 2002) and heparin-binding epidermal growth factor-like growth factor (Stavreus-Evers et al., 2002).

Synchronization of endometrial maturation and embryonic development could be very important for the success of implantation. There is evidence that endometrial receptivity may be adversely affected by ovarian stimulation (Basir et al., 2001; Bourgain and Devroey, 2003; Martel et al., 2003). Elevated estrogen concentrations in ovarian stimulation cycles may result in advanced endometrial maturation with reduced pinopode formation at the time of embryo implantation (Kolb and Paulson, 1997). Accelerated pinopode formation in ovarian stimulation cycles was demonstrated by Nikas et al. (1999). Patients with higher concentrations of progesterone (>6 ng/ml) on the day after human chorionic gonadotrophin administration exhibited the greatest prematurity of morphological features (Devioglu et al., 1999) Accelerated endometrial development may lead to embryo—endometrial asynchrony and reduced implantation rates. Another investigation by Murata et al. (2005) also showed the significance of synchronization of the implantation window and embryo stage. In this study the authors demonstrated that slower growing embryos may not implant because they do not coincide with the implantation window and cryopreservation of such embryos and subsequent synchronization of embryo transfer with endometrial status could rescue implantation. In that sense, programmes using frozen—thawed embryos are good for the purpose of modifying the endometrium to achieve optimal results.

It is believed, that in artificial cycles, which are used for transfer of frozen—thawed or donor embryos/oocytes in assisted reproduction treatment, the implantation window is postponed in comparison with natural cycles (Nikas et al., 1999). Currently there is not enough data concerning the optimal length of progesterone administration before embryo transfer (Nawroth and Ludwig, 2005). In most of the studies analyzed by Nawroth and Ludwig, the beginning of progesterone administration coincided with embryonic day 0 (Banz et al., 2002; Dal Prato et al., 2002; Muasher et al., 1991; Pattinson et al., 1992,1994; Queenan et al., 1997a, 1997b; Schröder et al., 2002; Seelig et al., 2002) or day 1 (pronucleus stage) (Boldt et al., 2003; Lelaidier et al., 1995; Revel et al., 2004; Simon et al., 1998, 1999).

Obviously, the use of a marker that could give information about the opening and the closure of the implantation window in each patient could be very helpful in optimizing the transfer of frozen or donated ova in artificial cycles. One such promising marker appears to be the detection of pinopodes in endometrial biopsies examined by SEM. This pinopode examination, originally proposed by Nikas (1999), is performed in a mock cycle preceding the transfer cycle where two endometrial biopsies are taken in the mid-luteal phase. Following SEM examination, the most receptive day is determined as the day when fully developed pinopodes are present. In a subsequent cycle the embryos are transferred such that a day-6 embryo coincides with the most receptive day.

A similar approach was used by Adams and Murphy (2001) and more recently by Pantos et al. (2004). In the latter study, pinopode formation pattern was examined in 46 candidate embryo recipients with previous multiple implantation failure. Two consecutive endometrial biopsies on days 6 and 8 of progesterone administration (P6 and P8, respectively) were taken in a mock cycle and investigated by SEM and the day was determined that showed fully developed pinopodes for each woman. Progesterone commencement in the transfer cycle was modified in such a way, that the day of fully developed pinopodes coincided with the day-6 embryo. In the majority of patients (73.9%) a modified protocol was indicated and applied. Significantly better results were obtained in the modified protocol as compared with patients in which pinopode formation did not indicate any modification and the standard protocol was used: the pregnancy rate was 76.47% for the modified, and 33.33% for the standard protocols.

However, other investigators were unable to confirm these data. Pinopodes were found in two separate biopsies taken a full 7 days apart in some patients (Acosta et al., 2000). Usadi et al. (2003) looked at fertile women and found that pinopodes were consistently present from luteal day 5 to luteal day 14 with no apparent rise in scores around the expected window of receptivity. Furthermore, Quinn et al. (2007) found that pinopodes are present throughout the luteal phase of the menstrual cycle, up to week 11 of pregnancy and in the endometrium of women on gonadotrophin-releasing hormone agonists and hormone therapy. The authors concluded that pinopodes can be detected in the progesterone-exposed endometrium for an extended period of time, and therefore questioned the perception that they are markers for the implantation window in the human endometrium.
The aim of the present study was to investigate the pinopode formation pattern in patients with a history of multiple IVF failure and to test if pinopode detection can help to modify individually and successfully the timing of embryo transfer in relation to progesterone administration in frozen–thawed IVF cycles.

Materials and methods

The study included 55 women with at least three implantation failures after transfer of two or more good-quality embryos in each cycle. All these women were planning to have a frozen–thawed embryo cycle in the reproductive clinic Nadiya (Kyiv, Ukraine) during the period from July 2006 to December 2007. The first part of this study investigated pinopode formation in all these women before the treatment cryocycle.

In the second part of the study, women with anomalies in pinopode formation (48 women) were divided into two subgroups. One group was treated with the standard protocol (25 women), which is normally used in the clinic. According to the standard protocol, a 5-day-old blastocyst is transferred on day 7 of progesterone administration. Another group had a modified protocol (23 women): the endometrial exposure to progesterone was different, depending on pinopode formation in such a way that the most receptive day (the day of fully developed pinopodes) corresponded to embryonic day 6. This was achieved by altering the progesterone commencement day of the embryo transfer cryocycle.

In all cases, cleavage-stage (day-2 and day-3) frozen embryos were thawed and cultured to the blastocyst stage and then transferred. In some patients, different-age embryos were transferred simultaneously in the same catheter. If this type of transfer was planned, the embryos were thawed on different days. Some embryos cultured to the blastocyst stage and others at cleavage stages were mixed together and transferred to the uterus in one catheter. The embryo quality on day 5 was assessed according to the criteria of Gardner et al. (2001). This scoring system is based on the formation and degree of expansion (from early blastocyst to fully expanded blastocyst (score ranged from one to six), the development of the inner cell mass (C, B, A) and the development of the trophectoderm (C, B, A). All patients had at least one blastocyst with high scores (three to five, A-B) per transfer. In all cases, except one (in which four embryos were transferred), two or three embryos were transferred.

Pinopode investigation protocol

All subjects underwent a mock cycle, identical to the standard one used for cryopreserved–thawed embryo transfer cycles. Pituitary down-regulation was achieved with the gonadotrophin-releasing hormone agonist triptorelin embonate, administered on cycle days 19–24 (Diphereline 3.75 mg; IPSEN, France). After 14–16 days from the start of down-regulation (but not earlier than the 3rd day of menstrual bleeding) oestradiol therapy was started with oestradiol valerate (Proginova; Bayer Schering Pharma, AG, Germany) in an increasing daily dosage from 2 to 6 mg (5 days 2 mg; next 5 days 4 mg; and next 5 days 6 mg). After 15 days, transvaginal ultrasound was used for evaluation of endometrial thickness. If the endometrium was less than 7 mm, the oestradiol dosage was increased to 8 mg per day. After endometrial thickness reached 7 mm or more, dydrogesterone acetate (Duphaston; Solvay Pharmaceuticals, Netherlands) was administered at a constant daily dosage of 60 mg together with oestradiol valerate at a dosage of 2 mg per day until the day of biopsy.

In all women a double biopsy was performed on days P7 and P9 of dydrogesterone administration. The endometrial tissue was taken by an endometrial sampler (Wallace, UK) from anterior (first biopsy) and posterior (second biopsy) uterine walls to avoid repeated sampling from the same place. The biopsy tissues were rinsed in saline, immersed in a 2.5% glutaraldehyde phosphate-buffered solution and kept in this solution for 24 h. Then the specimens were rinsed several times in phosphate buffer, fixed in 4% osmium phosphate-buffered solution, dehydrated in an acetone solution in distilled water at increasing concentrations (from 20% to 100%) and kept in 100% acetone. Then the samples were dried in a critical point drier with carbon dioxide, mounted and coated with gold (150–200 angstrom) and examined by SEM (Superprobe 733 JEOL, Japan) at ×2000 magnification.

Evaluation of pinopode development

Endometrial tissue was examined under the SEM. One hundred randomly selected fields of endometrial epithelium were examined per sample. The same two observers evaluated all biopsies. The stage of pinopode development was estimated according to the description presented by Nikas et al. (1999), who distinguished developing, fully developed and regressing pinopodes. If in one biopsy the majority (60% or more) of pinopodes were fully developed, this day was stated as the most receptive. If in the first biopsy the pinopodes were developing, and in the second regressing, the day of fully developed pinopodes was estimated between these 2 days, and if in the first biopsy the pinopodes were regressive, the previous day was stated as the day of fully developed pinopodes. And, if in the second biopsy the pinopodes were developing, the next day was thought to be the most receptive one (Nikas, 1999). If approximately equal quantities of pinopodes were in different stages of development, several receptive days (2 days, as a rule) were suggested.

Standard protocol

The standard protocol was performed for the frozen–thawed embryo transfer cycles as described earlier for the mock cycle in which the biopsies were performed. The oestradiol priming in all studied patients went on for 15–28 days. Dydrogesterone acetate and oestradiol valerate administration was continued until the day of human chorionic gonadotrophin testing.

Modified protocol

Pituitary downregulation and endometrial preparation with oestrogens was fulfilled as in the standard protocol.
Progesterone (dydrogesterone acetate) was then administered in accordance to the pinopode formation pattern. The day of fully developed pinopodes should coincide with a day-6 embryo. If several days of fully developed pinopodes were stated, embryos of different ages matching to these days were placed into one catheter. In women with arrested pinopodes, day-3 and day-5 embryos were transferred on day P7.

**Statistical analysis**

Statistical analysis was performed using the Student’s t-test and Mann Whitney U-test for evaluating the means of data (age, quantity of previous treatment cycles, grade and quantity of embryos, implantation rate, take-home baby rate) in the groups, which were compared. The chi-squared and Fisher tests were used for evaluating the dichotomously distributed variables (e.g. pregnancy versus non-pregnancy). $P < 0.05$ was considered as significant.

**Results**

In all endometrial samples investigated pinopodes were identified. The time of fully developed pinopodes is presented in Table 1. In these women the on-time (P8) pinopodes were present only in seven (12.7%) cases. The pinopode formation was accelerated to P6 and P7 in six cases (10.9%), delayed in 21 (38.2%) patients and the same stage of developing pinopodes in both biopsies (arrested pinopodes) were seen in 15 (27.3%) women.

In six cases (10.9%) a different pattern of pinopode development was seen in the same biopsy and was therefore defined as asynchronized pinopodes. In these cases, approximately equal numbers of pinopodes were in different stages of growth, so the days of fully developed pinopodes were different for them. In these patients 2 days were determined when fully developed pinopodes were present (Table 2). In seven patients with on-time (P8) fully developed pinopodes, the standard protocol was used and pregnancy occurred in two women. Women with retardation, more rapid, arrested and asynchronized development of pinopodes (48 women) were divided into two subgroups, one of which had the standard protocol and the other had the modified protocol. In nine patients with asynchronized and arrested pinopodes, embryo transfer of different-age embryos was fulfilled.

The more precise data on pinopode formation, transfer day, embryo number and results for the nine patients who underwent the modified protocol are summarized in Table 3.

The pregnancy rate in multiple implantation failure women with pinopode formation anomalies (46 patients) depending on the protocol used is presented in Table 4. Two women from the modified protocol group had no blastocysts and embryo transfer was cancelled.

Thus, in patients where the modified protocol was used, the implantation, pregnancy and take-home-baby rates were significantly higher in comparison with the standard protocol group.

**Discussion**

All patients with multiple implantation failure examined using SEM showed well-formed pinopodes. However only seven patients had on-time pinopodes on day P8 while the other 48 patients showed aberrations in the timing of pinopode formation, with fully developed pinopodes detected early on day P6 and P7 or late on days P9 and P10. Eleven out of 21 patients in the modified protocol group became pregnant after the first attempt of a synchronized embryo transfer following pinopode investigation. In contrast, only three out of 25 patients in the standard protocol became pregnant following the standard protocol. The plausible explanation for this significant difference is a better synchronization between embryonic development and endometrial maturation achieved by pinopode detection in these patients with untimed pinopode formation. The synchronization based on the concurrence of a day-6 blastocyst with an endometrium containing fully developed pinopodes, as first introduced by Nikas (1999), works well in clinical

---

**Table 1** Pinopode development in mock cycles.

<table>
<thead>
<tr>
<th>No. (%) of women (n = 55)</th>
<th>P7</th>
<th>P9</th>
<th>Day of fully developed pinopodes or other results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (5.5)</td>
<td>R</td>
<td>–</td>
<td>P6</td>
</tr>
<tr>
<td>3 (5.5)</td>
<td>FD</td>
<td>–</td>
<td>P7</td>
</tr>
<tr>
<td>7 (12.7)</td>
<td>D</td>
<td>R</td>
<td>P8</td>
</tr>
<tr>
<td>4 (7.3)</td>
<td>–</td>
<td>FD</td>
<td>P9</td>
</tr>
<tr>
<td>17 (30.9)</td>
<td>D</td>
<td>–</td>
<td>P10</td>
</tr>
<tr>
<td>15 (27.3)</td>
<td>D</td>
<td>D</td>
<td>Arrested pinopodes</td>
</tr>
<tr>
<td>6 (10.9)</td>
<td>Different combinations</td>
<td>Asynchronized pinopodes</td>
<td>–</td>
</tr>
</tbody>
</table>

D = Developing; FD = Fully developed; P = day of progesterone administration; R = Regressing.

**Table 2** Days of fully developed pinopodes in patients with an asynchronized pinopode pattern.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>P7</th>
<th>P9</th>
<th>Days of fully developed pinopodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D</td>
<td>FD, R</td>
<td>P8, P9</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>FD, R</td>
<td>P8, P9</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>D, FD</td>
<td>P9, P10</td>
</tr>
<tr>
<td>4</td>
<td>FD, R</td>
<td>–</td>
<td>P6, P7</td>
</tr>
<tr>
<td>5</td>
<td>FD, R</td>
<td>–</td>
<td>P6, P7</td>
</tr>
<tr>
<td>6</td>
<td>D, FD</td>
<td>R</td>
<td>P7, P8</td>
</tr>
</tbody>
</table>

D = Developing; FD = Fully developed; P = day of progesterone administration; R = Regressing.
practice. This synchrony is based on data from in vivo conception cycles (Hertig et al., 1956).

The current results are in accordance with those of Pantos et al. (2004) who showed that a better synchrony achieved by pinopode detection may improve implantation rates of the recipients in an embryo donation setting. Taking biopsies and scratching the endometrium in a cycle prior to embryo transfer has been shown to enhance implantation (Narvekar et al., 2010). However, this cannot account for the sharp difference in pregnancy rates found in the current study between the standard protocol and modified protocol groups, since double biopsies were taken in both groups.

Regarding pinopode formation patterns, the current results seem to differ from those of Usadi et al. (2003) who detected pinopodes from luteal day 5 to the end of the cycle, and of Quinn et al. (2007) who detected pinopodes from day 15 to the end of the cycle and in pregnancy, concluding that pinopodes are not a useful marker of the implantation window. In the current study, pinopodes in most cases were confined to P6–P10 and their detection proved to be useful for a better timing of embryo transfer. However, there was a group of patients with arrested/asynchronized pinopodes where pinopodes were detected in both P7 and P9 biopsies. Since further biopsies were not performed later in the cycle in this group, it is not known whether pinopodes would disappear later or persist to the end of the cycle, corresponding to the findings of these investigators.

Successful synchronization of the embryo transfer cycle based on SEM findings in a previous mock cycle provides indirect evidence that the endometrium of a given patient shows a similar response in two subsequent artificial cycles under the same protocol. This seems to contrast with the findings of Ordi et al. (2003) who found poor reproducibility and high variability of pinopode patterns from cycle to cycle in three consecutive cycles. However, there was a group of patients with arrested/asynchronized pinopodes where pinopodes were detected in both P7 and P9 biopsies. Since further biopsies were not performed later in the cycle in this group, it is not known whether pinopodes would disappear later or persist to the end of the cycle, corresponding to the findings of these investigators.

Successful synchronization of the embryo transfer cycle based on SEM findings in a previous mock cycle provides indirect evidence that the endometrium of a given patient shows a similar response in two subsequent artificial cycles under the same protocol. This seems to contrast with the findings of Ordi et al. (2003) who found poor reproducibility and high variability of pinopode patterns from cycle to cycle in three consecutive cycles. However, these investigators studied spontaneous cycles and took only one biopsy per cycle, while the current work studied artificial cycles and took two biopsies per cycle. Moreover, in previous studies, the pinopode formation pattern showed good intrapatient consistency in different (natural, stimulated and artificial) cycles (Sudoma, 2005).

Table 3 Modification of protocol on frozen–thawed embryo transfer in women with asynchronized and arrested pinopodes.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days of fully developed pinopodes</th>
<th>Day of embryo transfer</th>
<th>Days of embryo development</th>
<th>No. of embryos</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 P8, P9</td>
<td>P7</td>
<td>5/4*</td>
<td>1/1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2 P9, P10</td>
<td>P8</td>
<td>5/4*</td>
<td>1/1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3 P6, P7</td>
<td>P5</td>
<td>5/4*</td>
<td>2/2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4 Arrested</td>
<td>P7</td>
<td>5/3</td>
<td>1/1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5 Arrested</td>
<td>P7</td>
<td>5/3</td>
<td>1/1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6 Arrested</td>
<td>P7</td>
<td>5/3</td>
<td>2/1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7 Arrested</td>
<td>P7</td>
<td>5/3</td>
<td>1/2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8 Arrested</td>
<td>P7</td>
<td>5/3</td>
<td>2/1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>9 Arrested</td>
<td>P7</td>
<td>5/3</td>
<td>2/1</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

*aThe embryos of day 5 and day 4 were transferred in one catheter.

Table 4 Pregnancy rate in multiple implantation failure women with pinopode formation anomalies.

<table>
<thead>
<tr>
<th>Day of fully developed pinopodes</th>
<th>Standard protocol</th>
<th>Modified protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6</td>
<td>1/1</td>
<td>1/2</td>
</tr>
<tr>
<td>P7</td>
<td>1/1</td>
<td>–</td>
</tr>
<tr>
<td>P9</td>
<td>0/3</td>
<td>1/1</td>
</tr>
<tr>
<td>P10</td>
<td>0/8</td>
<td>3/9</td>
</tr>
<tr>
<td>Asynchronized pinopodes</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Arrested pinopodes</td>
<td>1/9</td>
<td>3/6</td>
</tr>
<tr>
<td>Total (%)</td>
<td>3/25 (12.0)</td>
<td>11/21 (52.4)*</td>
</tr>
</tbody>
</table>

Values are n/total no. of women, unless otherwise stated. *Two women had no blastocysts for transfer.

Table 5 Characteristics of patients and treatment cycles in the standard protocol and modified protocol groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Standard protocol</th>
<th>Modified protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>34.6 ± 5.2</td>
<td>34.6 ± 3.2</td>
</tr>
<tr>
<td>Number of previous IVF failures</td>
<td>3.7 ± 1.0</td>
<td>3.3 ± 1.6</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
<td>2.7 ± 0.8</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Embryo quality (% of at least stage-3 blastocysts)*</td>
<td>45 ± 6</td>
<td>44 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SD unless otherwise stated. There were no statistically significant differences between the two groups. *According to the criteria of Gardner et al. (2001).

Table 6 Outcomes in women according to treatment protocol.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Standard protocol</th>
<th>Modified protocol</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation</td>
<td>4/67 (6.0)</td>
<td>14/50 (28.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>3/25 (12.0)</td>
<td>11/21 (52.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Take-home-baby</td>
<td>3/25 (12.0)</td>
<td>10/21 (47.6)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are n/total no. of women (%) unless otherwise stated.
yet what mechanisms were involved in the cases of successful implantations in these women. During the early events of implantation the direct cell-to-cell communication of the trophectoderm with the endometrial luminal epithelial surface takes place (Thie et al., 1998). It is considered as an initial key process, when the human embryo is activated and the trophectoderm of the blastocyst differentiates to the invasive phenotype of trophoblast (Fujiwara et al., 2003). The blastocyst expresses many molecules, such as cadherins, selectins and integrins which are proposed to be related to this process of preimplantation relations between the embryo and endometrium (Campbell et al., 1995; Carver et al., 2003). So, it is also possible that prolonged embryonic interaction with the endometrium (due to more continuous exposure of endometrium to blastocyst-produced substances) could be the main (or an additional) reason for successful implantation in these women.

But it is more likely that, in some cases, pinopode formation does not occur simultaneously and pinopodes become mature on different days after the beginning of progesterone administration. So on the one hand these patients have several receptive days, but on the other hand the quantity of areas with fully developed pinopodes in these women can be diminished and insufficient for embryonic implantation. That is why the different-age embryos, one of which reaches day 6 at the moment when endometrium manifests mature pinopodes, could improve implantation. There is a single case report (Esfandiari et al., 2008) with a similar approach devoted to a successful experience of double-frozen embryo transfer in a patient with repeated failed intrauterine insemination and IVF cycles. The authors’ concept was to place embryos at the same developmental stage (eight cells) into the uterus at two different times to maximize the likelihood of synchronization with the window of implantation.

It is believed that the proposed method of pinopode investigation with subsequent modification of progesterone exposure is useful for the asynchronized group as well as in other patients with delayed or accelerated pinopode formation, because it gives the possibility to take into account the individual implantation window peculiarities in preparing for transfer of frozen—thawed embryos. Certainly, one should remember that the results are very much dependent on the specialist who performs the pinopode detection.

In conclusion, firstly in patients with multiple implantation failures, the severe anomalies of pinopode formation (shift in pinopode ripening for more than 1 day (P6, P10), asynchronized development, arrested pinopodes) occur in 74.5% of cases (41 women). Secondly, the modification of progesterone administration and the use of simultaneous transfer of embryos of different age in the cases of inappropriately timed pinopode development allow improvement in the effectiveness of the programmes using frozen—thawed embryos in women with multiple implantation failures.

References
Kolb, B.A., Paulson, R.J., 1997. The luteal phase of cycles utilizing controlled ovarian hyperstimulation and the possible impact of

Cryocycles by use of pinopode formation in multiple implantation failure 595


Queenan, J.T., Veeck, L.L., Toner, J.P., et al., 1997b. Cryopreservation of all prezygotes in patients at risk of severe hyperstimulation does not eliminate the syndrome, but the chances of pregnancy are excellent with subsequent frozen—thaw transfers. Human Reproduction 12, 1573–1576.


Declaration: The author reports no financial or commercial conflicts of interest.

Received 1 December 2009; refereed 21 January 2011; accepted 2 February 2011.