Infertility

Progesterone replacement with vaginal gel versus i.m. injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts

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Submitted on March 4, 2014; resubmitted on April 11, 2014; accepted on April 28, 2014

STUDY QUESTION: Does the type of luteal support affect pregnancy outcomes in recipients of vitrified blastocysts?

SUMMARY ANSWER: Luteal support with vaginal progesterone gel or i.m. progesterone (IMP) results in comparable implantation and pregnancy rates in IVF patients receiving vitrified blastocysts.

WHAT IS KNOWN ALREADY: In fresh IVF cycles, both IMP and vaginal progesterone have become the standard of care for luteal phase support. Due to conflicting data in replacement cycles, IMP is often considered to be the standard of care.

STUDY DESIGN, SIZE, DURATION: Retrospective analysis of 920 frozen embryo transfer (FET) cycles between 1 January 2010 and 1 September 2012.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients from a large, private practice undergoing autologous and donor FET using IMP or vaginal progesterone gel for luteal support were included in the analysis. IMP was used for luteal support in 682 FET cycles and vaginal progesterone gel was used in 238 FET cycles. Standard clinical outcomes of positive serum hCG levels, implantation, clinical pregnancy, spontaneous abortion and live birth were reported.

MAIN RESULTS AND THE ROLE OF CHANCE: The IMP and vaginal progesterone gel groups had similar patient demographics for all characteristics assessed. Implantation rates (46.4 versus 45.6%, P = 0.81), clinical pregnancy rates (61.7 versus 60.5%, P = 0.80) and live birth rates (49.1 versus 48.9%, P > 0.99) were not significantly different between IMP and vaginal progesterone gel, respectively.

LIMITATIONS, REASONS FOR CAUTION: This study is limited by its retrospective design and by its lack of randomization to the type of luteal support. In addition, because no a priori expected rates of success could be provided for this retrospective investigation, it was not possible to estimate statistical power associated with the various outcomes presented.

WIDER IMPLICATIONS OF THE FINDINGS: With the recent trends toward single embryo transfer (SET) and use of vitrified blastocysts in FET cycles, our data with ~40% of cycles being SET and use of exclusively vitrified blastocysts are more relevant to current practices than previous studies.

STUDY FUNDING/COMPETING INTEREST(S): Support for data collection and analysis was provided by Actavis, Inc. D.S. has received honoraria for lectures and participation in Scientific Advisory Boards for Actavis, Inc. J.P. is an employee of Actavis, Inc. N.E. has received payment from Actavis, Inc., for her time for data collection. H.H. has received payment from Actavis, Inc., for statistical analyses. Z.P.N. has nothing to disclose.

Key words: frozen embryo transfer / luteal phase support / progesterone gel / vaginal progesterone / i.m. progesterone
Introduction

The first human pregnancy following transfer of cryopreserved embryos occurred 30 years ago (Trounson and Mohr, 1983). Since then, the use of frozen embryo transfer (FET) has been increasing and now almost 25% of all cycles in the USA are FET (SART, 2012). One of the reasons for the increased use of FET in recent years is related to the advances in cryopreservation. The use of vitrification was shown to have greater post-thawing survival rates of both cleavage stage embryos and blastocysts compared with slow freezing in one meta-analysis (Loutradi et al., 2008). Another meta-analysis showed higher clinical pregnancy rates and live birth rates with vitrification compared with slow freezing (Abdel-Hafez et al., 2010). Pregnancy rates with FET comparable with fresh IVF can now be achieved as reported in a recent meta-analysis (Roque et al., 2013).

Progesterone is critical in achieving endometrial-embryo synchrony (Nawroth and Ludwig, 2005) and maintaining early pregnancy especially in FET cycles where there is minimal endogenous progesterone production. In fresh IVF cycles, both i.m. progesterone (IMP) and vaginal progesterone have become the standard of care for luteal phase support (Practice Committee of ASRM, 2008) due to multiple prospective (Schoolcraft et al., 2000; Dal Prato et al., 2008; Kahraman et al., 2010; Yanushpolsky et al., 2010; Silverberg et al., 2012) and retrospective (Mitwally et al., 2010) studies and meta-analyses (Zarutskie and Phillips, 2009; van der Linden et al., 2011) demonstrating that IMP and vaginal progesterone are associated with comparable pregnancy outcomes. In FET cycles, the preferred route of administration for progesterone is an area of active research. While Crinone 8% is currently the only treatment option approved by the US Food and Drug Administration for progesterone replacement (FET and donor cycles) in ART, a large number of US programs still consider IMP to be the standard of care (Kaser et al., 2012). In Europe, IMP is typically avoided because of the potential for severe side effects (Fatemi, 2009). Several small, retrospective (Williams et al., 2000; Berger and Phillips, 2008, 2012) and prospective (Gibbons et al., 1998; Jobanputra et al., 1999) studies in replacement cycles reported comparable pregnancy rates with vaginal progesterone gel compared with IMP. However, one recent retrospective study observed that Day 3 FET cycles with vaginal progesterone gel for luteal support had lower odds of clinical and live birth compared with those with IMP support (Kaser et al., 2012). With these conflicting results, the best progesterone formulation and protocol for luteal phase support in FET cycles is still unclear.

The objective of our study was to assess the efficacy of vaginal progesterone gel versus IMP for luteal support in FET cycles. While previous FET studies have primarily examined Day 3 embryos cryopreserved with slow freeze, our study examined a more relevant patient population receiving vitrified blastocysts.

Materials and Methods

Study design

This study was approved by Schulman Associates Institutional Review Board. All autologous and egg donor FET cycles with blastocyst stage embryos performed at Reproductive Biology Associates from 1 January 2010 to 1 September 2012 were reviewed for the type of progesterone used for luteal phase support. Patient characteristics, FET cycle parameters, embryo quality and clinical outcomes were compared between those supported with IMP (50 mg once daily) and those supported with twice daily vaginal progesterone gel Crinone 8% (90 mg; Watson Pharma, Inc., Parsippany, NJ, USA). The following exclusion criteria were applied: cryopreservation stage other than blastocyst, donated embryo, no luteal phase support (i.e. natural cycle), IMP dose other than 50 mg once daily, Crinone dose other than 90 mg twice daily and progesterone formulations other than IMP or Crinone.

Clinical and laboratory protocols

Clinical and laboratory protocols and procedures were uniform during the period under evaluation. Controlled ovarian hyperstimulation was performed using one of two protocols: GnRH agonist long protocol or GnRH antagonist protocol with FSH (Gonal-F, EMD Serono, USA; Follistim, Merck, USA; Bravelle, Ferring, USA). Recombinant hCG (Ovidrel, EMD Serono) was administered to trigger nuclear maturation of oocytes when two or more follicles had reached 18 mm diameter. Oocyte retrieval was performed 36 h after hCG administration. The partner’s sperm was prepared by density gradient centrifugation and ICSI fertilization was performed as previously described (Van Steirteghem et al., 1993). Eighteen hours after ICSI, oocytes were assessed for the presence of pronuclei and switched to Quinn’s advantage cleavage medium (Cooper/Sage, Bedminster, NJ, USA) with 15% serum protein substitute (SPS; Cooper/Sage) for further culture (Van Steirteghem et al., 1993). Embryos were evaluated on Days 3, 5 and 6, and were assessed for development and quality. Blastocyst stage embryo assessment was performed according to the SART embryo grading system (Racowski et al., 2010).

Embryos were cryopreserved as previously described (Chang et al., 2008). The basal medium used for embryo cryopreservation was HEPES-buffered embryo culture medium (Cooper/Sage) supplemented with 20% (v/v) SPS (Cooper/Sage). Briefly, the embryos were equilibrated in equilibration medium [basal medium with 7.5% (v/v) ethylene glycol and 7.5% (v/v) dimethylsulfoxide (DMSO)] at room temperature for 15 min. Embryos were transferred into the vitrification medium (basal medium with 15% (v/v) ethylene glycol, 15% (v/v) DMSO and 0.5 mol/l sucrose) at room temperature for 45–60 s. The cryoprotectant-treated embryos were placed onto a fine propylene strip (Cryotop; Kitazato Bio Pharma Co., Japan), which was then submerged into liquid nitrogen and ready for storage. Embryos were warmed by direct immersion of the propylene strip with vitrified embryos into 5.0 ml of warming solution [HEPES-buffered embryo culture medium with 20% (v/v) SPS and 1.0 mol/l sucrose] at 37°C for 1 min. Embryos were then picked up and transferred into 1.0 ml of the dilution solution [HEPES-buffered embryo culture medium with 20% (v/v) SPS and 0.5 mol/l sucrose] for 3 min at room temperature. The embryos were subsequently washed in 1.0 ml washing solution [HEPES-buffered embryo culture medium with 20% (v/v) SPS] for 10 min at room temperature.

Uterine preparation was carried out using either daily graduated oral (Estrace®, Warner Chilcott US, LLC, Rockaway, NJ, USA) or transdermal (Vivelle-Dot®; Noven Pharmaceuticals, Inc., Miami, FL, USA) estrogen or 6 mg i.m. estradiol valerate administered every 3 days. Transvaginal ultrasound was performed between Days 10 and 14 of estrogen priming. Crinone 8% (90 mg twice a day) or IMP (50 mg once daily) was started on Day 15 of estrogen therapy. Blastocyst transfer was performed on Day 6 of progesterone therapy. Pregnant recipients continued to receive the same dosages of progesterone and estrogen until 10 weeks’ estimated gestational age.

Outcome variables

The following patient demographics and cycle characteristics were collected: patient age at cryopreservation and transfer, BMI of embryo recipient, parity, number of prior cycles and spontaneous abortions, number of prior failed fresh and FET cycles, final measured endometrial thickness, percentage of...
cycles with assisted hatching, use of a gestational carrier, percentage of biopsied embryos and percentage of embryos from previously frozen oocytes. A prior failed cycle was defined as any fresh or frozen cycle that did not result in a positive hCG test. In cycles derived from donor eggs, the age of the egg donor at cryopreservation was used for patient age and the age of embryo recipient was used for uterine age.

Standard clinical outcomes of positive serum hCG levels, implantation, clinical pregnancy, spontaneous abortion and live birth were reported. Implantation rate was defined as the percentage of embryos implanting successfully relative to the total number of embryos transferred. Clinical pregnancy rate was defined as the proportion of transfers that resulted in at least one intrauterine gestational sac documented by ultrasound. Spontaneous abortion was defined as a loss of a clinical pregnancy before 20 weeks’ gestation. Live birth rate was defined as the number of transfers that resulted in live birth. Delivery outcome was unknown for seven cycles that had documented clinical pregnancies; these cycles were not included in the analysis of live birth.

Statistical analyses

All data summaries and statistical analyses were undertaken using SAS® Software Version 9.3. A value of \( P < 0.05 \) was considered significant. For each treatment group, the total number of FET cycles across subjects served as the denominator for all evaluations. Clinical outcomes were evaluated using the appropriate statistical methods for continuous and categorical data. Statistical testing consisted initially of fitting a Poisson regression model for implantation rate and a logistic regression model for each clinical outcome as a function of treatment, parity, subject age at transfer, autologous versus donor egg type and whether the subject was a gestational carrier. Since none of the potential covariates was found to be a statistically significant contributor to either model, the resultant analysis was based on a parsimonious model that evaluated the direct effect of treatment group with no additional, required adjustment for concomitant variables. For implantation rate (range 0–100%), treatment groups were compared using a two-sample t-test (parametric) and Wilcoxon rank sum test (non-parametric), the latter to account for potential deviations from normality. Implantation rate data were also summarized using appropriate statistics for continuous data. For confirmed biochemical pregnancy, clinical pregnancy, live birth and spontaneous abortion rates, the two treatment groups were compared by estimating the odds ratio (OR; Crinone versus IMP) and its exact 95% confidence interval (CI). In retrospective studies, the OR can provide a reasonable estimate of relative risk (or, in the case of this study, relative benefit). An OR = 1 implies comparable rates between the two treatment groups; a 95% CI that includes an OR = 1 provides an estimate of the precision of the OR estimate.

Results

Patient demographics and embryo characteristics

Between 1 January 2010 and 1 September 2012, a total of 920 FET cycles were available for analysis. IMP was used for luteal support in 682 FET cycles and vaginal progesterone gel was used in 238 FET cycles. The two groups had similar demographic characteristics except for parity (Table I), where a significantly smaller proportion of FET cycles was obtained from parous women in the vaginal progesterone group (39.1%) compared with the IMP group (49.9%). Oocyte age at the time of embryo cryopreservation and age of embryo recipient at transfer were not significantly different between patients treated with IMP or vaginal progesterone gel. Other parameters, such as BMI and reason for infertility, were comparable between treatment groups.

<table>
<thead>
<tr>
<th>Table I Patient demographics for frozen embryo transfer (FET) cycles using i.m. progesterone (IMP) versus vaginal progesterone gel (Crinone) for luteal support.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP ((n = 682))</td>
</tr>
<tr>
<td>Oocyte age at embryo cryopreservation</td>
</tr>
<tr>
<td>Age of embryo recipient at transfer</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>≥ 1</td>
</tr>
<tr>
<td>No. prior failed cycles</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>≥ 1</td>
</tr>
<tr>
<td>No. prior SAB</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>≥ 1</td>
</tr>
<tr>
<td>BMI (kg/m²) of embryo recipient</td>
</tr>
<tr>
<td>No. of obese embryo recipients</td>
</tr>
<tr>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Advanced reproductive age</td>
</tr>
<tr>
<td>Anovulation</td>
</tr>
<tr>
<td>Diminished reserve</td>
</tr>
<tr>
<td>Endometriosis</td>
</tr>
<tr>
<td>Gestational carrier</td>
</tr>
<tr>
<td>Male factor</td>
</tr>
<tr>
<td>Tubal factor</td>
</tr>
<tr>
<td>Uterine factor</td>
</tr>
<tr>
<td>Unexplained</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

Values represent mean ± SD or n (%). FET, frozen embryo transfer; SAB, spontaneous abortion.

\( a \) IMP versus Crinone using a 95% CI test (parametric).

\( b \) Failed cycle refers to any fresh or frozen cycle without a positive hCG.

\( c \) Obesity defined as BMI ≥ 30 kg/m².

As shown in Table II, the number of embryos transferred and the number of good quality embryos transferred were similar between groups with ~40% of FET cycles overall being single embryo transfers (SET). Embryos were derived from autologous oocytes in 75% of all cycles and from donor oocytes in 25% of all cycles. A greater percentage of donor cycles were supplemented with IMP than vaginal progesterone gel (27.0 versus 18.5%, respectively) because Crinone was not first line for cryopreserved donor cycles in our practice until late 2012 and our data collection ended in September of 2012. In addition, final measured endometrial thickness was comparable (9.5 versus 10.0 mm).

Clinical outcomes

Implantation rates, positive serum hCG, clinical pregnancy rates, spontaneous abortion rates and live birth rates were not significantly different for FET recipients treated with IMP and those treated with vaginal progesterone gel (Table III). The odds of an FET resulting in a clinical
pregnancy were not significantly different for the IMP versus vaginal progestrone gel group (61.7 versus 60.5%, OR 0.95, CI 0.69–1.30;  P = 0.80). Similarly, the odds of an FET resulting in a live birth were also not significantly different in the IMP versus vaginal progesterone gel group (49.1 versus 48.9%, OR 0.99, CI 0.73–1.35;  P > 0.99). The potential effect of the following covariates: subject age at cryopreservation, parity, embryos derived from autologous versus donor oocyte and gestational carrier were evaluated, but the difference between the vaginal progesterone gel and the IMP treatment groups remained non-significant. As a result, statistical outcomes based on the parsimonious model testing the effect of treatment alone were retained for presentation.

Because a lower proportion of FET cycles among parous women involved treatment with vaginal progesterone gel (39.1%) compared with IMP (49.9%), the association between progesterone support and cycle outcome was also analyzed after excluding all FET cycles in parous women. When only nulliparous women were analyzed, there was no significant difference in the implantation rate (44.6 versus 48.8%,  P = 0.30), clinical pregnancy rate (62.0 versus 66.2%,  P = 0.44), spontaneous abortion rate (14.9 versus 13.8%,  P = 0.87) or live birth rate (48.7 versus 53.1%,  P = 0.43) between IMP and vaginal progesterone gel, respectively.

Since only 18.5% of FET cycles using donor embryos were supplemented with vaginal progesterone gel compared with 27.0% supplemented with IMP, the association between progesterone support and cycle outcome was also analyzed after excluding all FET cycles using donor embryos. When only cycles with autologous embryos were analyzed, the clinical outcomes were consistent with the main analysis. There was no significant difference in the implantation rate (45.9 versus 47.5%,  P = 0.66), clinical pregnancy rate (62.4 versus 64.9%,  P = 0.60), spontaneous abortion rate (14.1 versus 11.9%,  P = 0.53) or live birth rate (49.6 versus 53.4%,  P = 0.42) between IMP and vaginal progesterone gel, respectively.

With the recent trend toward SET to reduce the risk of multiple pregnancies, an analysis of only SET cycles was conducted. The clinical pregnancy rates were slightly lower overall, but findings were consistent with the main analysis. There was no significant difference in the implantation rate (55.6 versus 51.6%,  P = 0.50), clinical pregnancy rate (55.6 versus 51.6%,  P = 0.58), spontaneous abortion rate (11.1 versus 12.6%,  P = 0.81) or live birth rate (43.1 versus 38.9%,  P = 0.56) between IMP and vaginal progesterone gel, respectively.

### Discussion

Our large retrospective study examined luteal support with IMP versus vaginal progesterone gel in FET cycles and was unique in that we evaluated vitrified blastocysts, and most IVF centers are moving toward vitrification of blastocysts for their FET cycles. The findings of our study were consistent with the results of the majority of other published studies showing similar pregnancy rates in replacement cycles when comparing IMP and vaginal progesterone gel. Three small retrospective studies examined IMP or vaginal progesterone gel in either donor or FET cycles (Williams et al., 2000; Berger and Phillips, 2008, 2012). Berger and Phillips (2008) conducted a single-center, retrospective analysis of 279 women receiving either vaginal progesterone gel (90 mg twice daily) or IMP (50 mg once daily) as luteal support for FET cycles and reported no significant difference in implantation, clinical pregnancy or delivery rates. Berger and Phillips (2012) also reported no significant difference in pregnancy outcomes between vaginal progesterone gel and IMP in a retrospective analysis of 225 recipients of donor oocytes. Williams et al. (2000) conducted a retrospective analysis of 96 women receiving vaginal progesterone gel (90 mg twice daily) or vaginal progesterone tablet (200 mg three times daily) or IMP (50 mg once daily) and reported no significant difference in pregnancy rates between the three formulations. Two small, prospective studies showed equivalence of IMP and vaginal progesterone gel in donor

### Table II Embryo characteristics for FET cycles using IMP versus Crinone for luteal support.

<table>
<thead>
<tr>
<th></th>
<th>IMP (n = 682)</th>
<th>Crinone 8% (n = 238)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of embryos transferred</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>No. of good quality embryos transferred</td>
<td>1.6 ± 0.7</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>SET</td>
<td>288 (42.2)</td>
<td>95 (39.9)</td>
</tr>
<tr>
<td>Autologous versus donor embryos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>498 (73.0)</td>
<td>194 (81.5)</td>
</tr>
<tr>
<td>Donor</td>
<td>184 (27.0)</td>
<td>44 (18.5)</td>
</tr>
</tbody>
</table>

Values represent mean ± SD or n (%).

### Table III Pregnancy outcomes for FET cycles using IMP versus Crinone for luteal support.

<table>
<thead>
<tr>
<th></th>
<th>IMP (n = 682)</th>
<th>Crinone 8% (n = 238)</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation rate</td>
<td>46.4 ± 42.0</td>
<td>45.6 ± 42.5</td>
<td>0.90 (0.64–1.27)</td>
<td>0.58</td>
</tr>
<tr>
<td>Positive serum HCG</td>
<td>496 (72.7)</td>
<td>168 (70.6)</td>
<td>0.95 (0.69–1.30)</td>
<td>0.80</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>421 (61.7)</td>
<td>144 (60.5)</td>
<td>0.87 (0.53–1.38)</td>
<td>0.62</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>91 (13.3)</td>
<td>28 (11.8)</td>
<td>0.99 (0.73–1.35)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Live birth</td>
<td>332 (49.1)</td>
<td>116 (48.9)</td>
<td>0.99 (0.73–1.35)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

Values represent mean ± SD or n (%). CI, confidence interval.

* ^Treatment groups were compared using a two-sample t-test. Wilcoxon rank sum test was also evaluated and P-value = 0.79.
* ^Based on 676 IMP and 237 Crinone cycles with known delivery outcomes.
oocyte programs (Gibbons et al., 1998; Jobanputra et al., 1999). Gibbons et al. (1998) randomized 54 patients to vaginal progesterone gel (90 mg twice daily) and 18 patients to IMP (100 mg once daily) and reported no differences in implantation or ongoing pregnancy rates. Likewise, Jobanputra et al. (1999) prospectively followed 44 patients on vaginal progesterone gel (90 mg once daily) and 42 patients on IMP (100 mg once daily) and reported no differences in implantation or ongoing pregnancy rates.

Our results were inconsistent with a recent retrospective study by Kaser et al. (2012) that reported significantly higher clinical pregnancy and delivery rates with IMP versus vaginal progesterone gel. Kaser et al. (2012) hypothesized that the differences in pregnancy rates between IMP versus vaginal progesterone gel observed in their study may be due to the timing of the progesterone administration, since higher endometrial concentrations of progesterone following vaginal progesterone gel may cause premature closure of the implantation window. The authors suggested that delaying the initiation of vaginal progesterone gel in FET cycles may prevent premature closure of the implantation window based on the findings of a previous study in fresh IVF cycles that delayed initiation of vaginal progesterone gel (Yanushpolsky et al., 2010). Reproductive outcomes with different vaginal progesterone start days in replacement cycles were evaluated in a small study (Escribá et al., 2006). Vaginal progesterone was initiated on the day before donor oocyte retrieval, day of donor oocyte retrieval or 1 day after donor oocyte retrieval in Day 3 embryo transfers and no difference in pregnancy outcomes was observed between the groups except for a higher biochemical pregnancy rate when progesterone was initiated the day before oocyte retrieval. In our standard FET protocol with vitrified blastocysts, we do not delay the initiation of vaginal progesterone gel compared with IMP and did not see a difference in pregnancy outcomes. Further research is needed to better understand the impact of the timing of initiation of vaginal progesterone on the window of implantation and what other factors may play a role.

There are many factors that may influence endometrial–embryo synchrony in FET cycles. Endometrial factors such as duration of progesterone before embryo transfer (Nawroth and Ludwig, 2005), length of estrogen administration (Nawroth and Ludwig, 2005) and lack of ovarian stimulation with gonadotrophins as in fresh IVF-embryo transfer (Shapiro et al., 2008) may play a role. Embryonic factors, including the embryonic development stage at transfer and cryopreservation method, are also important (Cercas et al., 2012). Several of these factors may play a role in the different pregnancy outcomes observed in our study compared with the study by Kaser et al. (2012). Our study evaluated vitrified Day 5 blastocyst transfers, whereas Kaser et al.’s (2012) looked at primarily Day 3 embryos that were cryopreserved via slow freeze. Some authors have observed a higher rhythm of cell division in vitrified/warmed embryos compared with frozen/thawed embryos and this may impact the window of implantation and the length of progesterone treatment needed to synchronize the endometrium (Cercas et al., 2012). Further research is needed to better understand the effects of cryopreservation method and stage of embryonic development on the window of implantation.

The strengths of this study include the sample size (920 FET cycles analyzed) that included a wide variety of typical infertility patients presenting for FET. Since many IVF centers are moving toward vitrification of blastocysts, our patient population receiving vitrified blastocysts is more relevant than previous studies. Also, with the recent trend toward SET to reduce the risk of multiple pregnancies (Maheshwari et al., 2011), our data with ~40% of FET cycles being SET are relevant to current practice. In addition, FET provides a good model for evaluating the efficacy of progesterone replacement, since recipients of frozen embryos have no functioning corpora lutea and therefore produce no endogenous progesterone. Several studies have suggested lesser quality embryos may be transferred in FET cycles, especially with embryos derived from autologous oocytes, and that more intense progesterone support may be required in these cycles (Kaser et al., 2012; Feinberg et al., 2013).

Our study showed that even in FET cycles derived from autologous oocytes, vaginal progesterone gel and IMP resulted in similar clinical pregnancy rates (IMP versus Crinone; 62.4 versus 64.9%, P = 0.60) and live birth rates (IMP versus Crinone; 49.6 versus 53.4%, P = 0.42).

This study is limited by its retrospective design and by its lack of randomization to the type of luteal support. Selection of progesterone type was left to the discretion of the physician and this may have resulted in biases as to who received IMP or vaginal progesterone gel. In addition, because no a priori expected rates of success could be provided for this retrospective investigation, it was not possible to estimate statistical power associated with the various outcomes presented. A prospective, randomized study, while ideal, would take years to complete if standardized freezing protocols were used, since patients often do not use their cryopreserved embryos until several years later.

In summary, this study demonstrated that vaginal progesterone gel was comparable to IMP as progesterone replacement in terms of pregnancy outcomes within a single FET program utilizing embryos derived from autologous and donated oocytes. While these results are consistent with solid clinical evidence that vaginal and i.m. regimens have comparable efficacy in providing luteal phase support in IVF-embryo transfer, as determined by pregnancy outcomes, only adequately powered prospective studies can establish equivalence of progesterone regimens. Vaginal progesterone gel also offers the advantages of ease of use and avoidance of painful i.m. injections.

**Authors’ roles**

D.S. and J.P. designed the study, interpreted the data, drafted and revised the manuscript; N.E. performed data collection and revised the manuscript; H.H. conducted data analysis, interpreted the data and revised the manuscript; Z.N. interpreted the data and revised the manuscript. All of the authors read and approved the final version to be submitted for publication.

**Acknowledgments**

The authors thank Lynn Hummer RN, Krista Gilbert RN BSN, Lisa Lobuglio RN and Karen Feagin RN for their assistance with data collection.

**Funding**

Support for data collection and analysis was provided by Actavis, Inc. Funding to pay the Open Access publication charges for this article was provided by Actavis, Inc.

**Conflict of interest**

D.B.S. has received honoraria for lectures and participation in Scientific Advisory Boards for Actavis, Inc. J.A.P. is an employee of Actavis, Inc. N.M.E. has received payment from Actavis, Inc. for her time for data.
collection. H.H. has received payment from Actavis, Inc. for statistical analyses. Z.P.N. has nothing to disclose.

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