Does a frozen embryo transfer ameliorate the effect of elevated progesterone seen in fresh transfer cycles?

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Objective: To compare the effect of progesterone (P) on the day of trigger in fresh assisted reproduction technology (ART) transfer cycles versus its effect on subsequent frozen embryo transfer (FET) cycles.
Design: Retrospective cohort study.
Setting: Large private ART practice.
Patient(s): Fresh autologous and FET cycles from 2011–2013.
Intervention(s): None.
Main Outcome Measure(s): Live birth.
Result(s): A paired analysis of patients who underwent both a fresh transfer and subsequent FET cycle and an unpaired analysis of data from all fresh transfer cycles and all FET cycles were performed. We analyzed 1,216 paired and 4,124 unpaired cycles, and P was negatively associated with birth in fresh but not FET cycles in all analyses. Interaction testing of P and cycle type indicated P had a different association with birth in fresh versus FET cycles. When P was $\geq$2 ng/mL at the time of trigger, live birth was more likely in FET versus fresh cycles in the paired analysis (47% vs. 10%), in the unpaired analysis (51% vs. 14%), and in unpaired, good blastocyst only transfer subgroup (51% vs. 29%). Live birth was similar in FET cycles, with P $\geq$2 ng/mL versus P $<$ 2 ng/mL (51% vs. 49%). Conversely, live birth was lower in fresh cycles, with P $\geq$2 ng/mL versus P $<$2 ng/mL (15% vs. 45%).
Conclusion(s): Elevated P levels on the day of trigger during the initial fresh cycle were negatively associated with live birth in the fresh transfer cycles but not in subsequent FET cycles. Freezing embryos and performing a subsequent FET cycle ameliorates the effect of elevated P on live-birth rates. (Fertil Steril® 2016;105:93–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: Elevated progesterone, fresh transfer versus FET cycles, live birth

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The negative effect of elevated progesterone (P) on the day of ovulation trigger during in vitro fertilization (IVF) cycles has been well documented (1–5), with decreasing clinical pregnancy rates as P rises. Despite the increased use of gonadotropin-releasing hormone (GnRH) analogues for pituitary suppression to prevent premature luteinizing hormone (LH) surges, early rises in P are still present in 5% to 38% of all assisted reproduction technology (ART) cycles (1, 6–8). Elevated P before the trigger has been associated with multiple normally developing follicles that each produce P (5, 9), high doses of exogenous follicle-stimulating hormone (FSH), and high levels of serum estradiol on the day of ovulation trigger (1, 5).

Over the past several decades, there has been long-standing debate of the

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causative relationship between P and pregnancy rates. Some studies have concluded elevated P adversely affects oocyte quality and thus results in negative effects on fertilization rates, embryo quality, and pregnancy rates (10, 11). If an elevated level of P affects the oocyte and thus embryo quality, the pregnancy rates should be affected in all the cycles in which the embryo is transferred. In other words, an elevated P during the fresh cycle should have a carryover effect on pregnancy in subsequent sibling frozen embryo transfer (FET) cycles. However, studies have demonstrated that elevated P has no negative effect on oocyte quality in donor recipients (12, 13). Yang et al. (14) published the first and only prospective randomized clinical study evaluating FET versus fresh embryo transfer. The FET cycles had statistically similar live births compared with fresh cycles (38.1% vs. 13.9%) if P was $\geq$1.88 ng/mL on day of HCG trigger. This statistically similar outcome was likely due to the small size of the study.

The more accepted association between elevated P and pregnancy is a deleterious effect on the endometrium during the window of implantation (9, 13, 15, 16). Analyses of endometrial gene expressions have been performed in IVF cycles with elevated P and demonstrate advancements in the endometrium leading to endometrium–embryo asynchrony (17–19). Thus, this advancement in the endometrium may alter the window of implantation or the period in which the endometrial epithelium acquires the ability to functionally support blastocyst adhesion (20).

Based on the current literature, many ART programs are recommending cryopreservation of embryos when P is elevated during the initial fresh IVF cycle. A subsequent FET cycle would allow better endometrial synchronization with the embryo. Surprisingly, few studies have evaluated the effect of elevated P in the initial fresh IVF cycle with embryo performance in the subsequent FET cycles. Theoretically, a FET cycle would ameliorate the effects of the elevated P if decreased pregnancy rates during the initial fresh cycle were due to endometrium–embryo asynchrony. We compared the outcomes of patients who underwent an autologous IVF cycle with fresh transfer who had a known elevated P on day of trigger with the outcomes of their subsequent FET cycles.

MATERIALS AND METHODS

Study Design

Our retrospective cohort analysis of fresh autologous IVF and FET cycles from 2011 to 2013 was performed at Shady Grove Fertility Reproductive Science Center in Rockville, Maryland. During this time period, serum progesterone levels were obtained on all autologous fresh cycles on the day of ovulation trigger. The P level on the day of human chorionic gonadotropin (hCG) or GnRH agonist trigger in the fresh cycle was counted as the P level for both that fresh and any subsequent sibling FET cycles resulting from that original fresh cycle. Only cycles with an embryo transfer were included. Two main analyses were performed. Analysis 1 included patients who underwent both a fresh autologous IVF cycle with embryo transfer and a subsequent FET cycle within the study period. In this paired analysis, only the first FET cycle was included and matched with the fresh IVF cycle. Analysis 2 was an unpaired comparison of data from all fresh transfer cycles with known P levels on day of trigger compared with all FET cycles with a known P level from its fresh cycle that generated those vitrified embryos. In the unpaired analysis, multiple fresh and FET cycles from the same patient could be included. A subgroup analysis of the unpaired cohort was also performed of all patients receiving a good blastocyst transfer (21). The study was approved by the institutional review board.

Patients

All patients who underwent a fresh or frozen autologous embryo transfer during the periods in which serum P levels were measured on the day of trigger were included in the analysis. There were no fresh transfers in patients with a P level >3.5 during the study period. Exclusion criteria were donor oocyte cycles or no embryo transfer cycles.

Stimulation Protocol

For ovarian stimulation, mixed FSH/LH protocols under GnRH antagonist or GnRH agonist pituitary suppression (22) were used. In general, oral contraceptive treatment was initiated 21 days before stimulation. For GnRH antagonist cycles, the antagonist (Ganirelix) was initiated when the lead follicle was 14 mm in size. For GnRH agonist cycles, 20 units of leuprolide acetate (Lupron) were initiated during the last 3 days of oral contraceptives. The leuprolide acetate dose was decreased to 5 units when ovarian suppression was confirmed with ultrasound and serum estradiol <5 pg/mL. Ovarian stimulation was achieved by employing recombinant FSH and human menopausal gonadotropin (hMG). When the lead follicle was $\geq$18 mm, final oocyte maturation was triggered with 10,000 IU of hCG or with 4 mg of GnRH agonist in some of the GnRH antagonist cycles as indicated. Serum P levels were obtained on the day of trigger. Oocyte retrieval occurred 36 hours later, and fertilization was achieved with conventional IVF or intracytoplasmic sperm injection, as clinically indicated.

Over the duration of the study, all embryo cryopreservation and thawing at our center were performed via a vitrification-warming method (14). For endometrial preparation in FET cycles, patients underwent ovarian and uterine suppression using combined hormonal oral contraceptive pills. After baseline hormone assessment and a transvaginal ultrasound to document no functional ovarian cysts and a thin endometrium, the patients were started on intramuscular estradiol valerate at 4 mg every third day. When the serum estradiol level had reached >200 pg/mL and the endometrial thickness was >8 mm on transvaginal ultrasound, the patients were started on 50 mg daily of intramuscular progesterone in oil.

Ultrasound guided embryo transfer was performed on either day 3 or on day 5. Day-5 transfers were performed if there were three or more high-quality embryos available on day 3 to wait until a day-5 transfer. Otherwise, the embryos were transferred on day 3. Embryos were graded as good, fair, or poor according to the simplified Society for Assisted Reproductive Technology (SART) scoring system (21). Serum hCG levels were assessed at 4 weeks' gestational age followed