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ORIGINAL PAPERS

Intramuscular route of progesterone administration increases pregnancy rates during non-downregulated frozen embryo transfer cycles

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Abstract
Objective The optimal route of progesterone (P4) administration in embryo transfer (FET) cycles remains to be determined. The objective of this study is to compare the pregnancy outcomes between intramuscular (IM) and vaginal progesterone (PV) administration for endometrial preparation in non-donor FET cycles.
Study design A retrospective clinical study in a private practice infertility setting.
Results No significant differences in patient demographics and embryo characteristics were noted between the two groups. The clinical pregnancy rate as well as the live birth rate were significantly higher in the IM arm compared to the PV arm (38.2% vs 28%, 34.5 % vs 22.8%, respectively).
Conclusion Although both routes of progesterone administration had similar rates of initial positive pregnancy tests, the IM route had a significantly higher live birth rate. The exact reason for this difference remains to be determined.

Keywords Frozen embryos · Progesterone · Cryopreservation · Frozen embryo transfer

Introduction

Preparation of an artificial endometrium for FET has evolved dramatically over the past 15 years. The initial regimens involved the use of GnRH analogues for pituitary suppression followed by the administration of exogenous estrogens to stimulate the endometrium [1, 2]. This was followed by progesterone (P4) supplementation for 3 to 6 days before ET [3]. Several studies have questioned the need for GnRH analogues for the pituitary suppression of endogenous ovulation [4–6]. Indeed, "natural cycle override" regimens have been used in which high doses of exogenous estrogens are administered during the follicular phase of the natural cycle to prevent a premature LH surge followed by progesterone supplementation for luteal phase support when the endometrium is of adequate thickness([7]. Both regimens have been shown to have comparable pregnancy rates.

Progesterone used for luteal phase support can be administered using any one of three main routes: oral, intramuscular (IM), and vaginal. The oral route of administration has been clearly shown to be inferior to the other routes in the promotion of a secretory endometrium favorable to implantation [8]. The comparison between the intramuscular and vaginal routes has been more controversial. Although several studies have shown that cycles in which vaginal progesterone was used had comparable outcomes to IM progesterone in both fresh IVF and FET cycles [8–10], other researchers have recommended IM progesterone based on better outcome compared to vaginal progesterone in IVF cycles [11].

In our institution, both the IM and vaginal routes of P4 have been used with good success in FET cycles. The purpose of this study was to perform a retrospective chart review to compare the pregnancy rates for each route of administration as well as to determine the efficacy of the "natural cycle override" regimen.

Materials and methods

A retrospective chart review was performed on all non-donor ET cycles that were performed between January 2003

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and December 2004 from a local private infertility practice. Institutional Review Board approval was obtained before the data collection. During the calendar year 2003, the center's protocol for luteal support for FET cycles was the use of IM P4. The protocol was changed in the calender year 2004 when luteal support was shifted to vaginal administration, in an effort to decrease the number of injections for patients. All patients undergoing FET in the years 2003 and 2004 were included in this study. The cohort size was 199 in the IM arm and 211 in the vaginal administration arm. The following information was collected: patient age at the time of egg retrieval, infertility diagnosis, gravidity and parity, mean embryo cell number and grade, stage at embryo freezing and transfer, the number of embryos transferred, duration of the artificial proliferative phase, endometrial thickness (EC) in the late proliferative phase, cycle cancellation due to poor endometrial response or spontaneous ovulation, P4 level 2 weeks after the day of ET, day of ET after starting P4 supplementation, positive serum hCG, clinical pregnancy rates, number of gestational sacs identified on ultrasound, implantation rate, live birth rate and route of P4 administration. The identification of fetal heart beat by transvaginal ultrasound defined a clinical pregnancy.

The following protocol was used in each cycle: With the onset of menses, micronized estradiol (Estrace; Warner Chilcott, Rockaway, NJ) was administered at a dose of 2 mg TID for an average of 14 days. A single transvaginal ultrasound was performed 1 day before initiation of exogenous P4 to evaluate the endometrial thickness (EC) (minimum thickness=6 mm). A serum P4 level was concurrently drawn to ensure that spontaneous ovulation had not occurred. After documenting an adequate EC, P4 was initiated using one of two regimens: during the year 2003, 50 mg of IM progesterone in oil was administered the first day followed by 100 mg daily thereafter. During the year 2004, vaginal micronized progesterone 200 mg (Prometrium; Solvay Pharmaceuticals, Marietta, GA) TID was utilized. Estradiol (E2) was continued throughout the first 10 weeks using the same daily dose. Embryo transfer was performed on day 4 or 6 of progesterone administration depending on the stage of the embryo; non-blastocyst embryos were transferred on day 4 and blastocysts were transferred on day 6. Serum β-HCG and P4 levels were obtained 2 weeks after the transfer. If the serum β-hCG confirmed a pregnancy, a transvaginal ultrasound was performed 2 weeks thereafter to document a gestational sac as well as a fetal heart beat. Ongoing pregnancies were supported with continued hormonal treatment (both E2 and P4) until the 10th gestational week.

Embryo cryopreservation and thawing were performed as previously described [12–14]. The thawing procedures were done the day of the ET, except for embryos that were cryopreserved at the 2PN stage, where the thawing was done 1 day before the ET.

Statistical analysis was carried out using the Student's *t*-test and Chi square. A *p*-value of <0.05 was considered to be significant.

Results

A total of 410 cycles were retrospectively analyzed, 199 in the IM arm and 211 in the PV arm of the study. Patient and embryo characteristics are shown in Table 1. Both groups were similar in age at retrieval, gravidity, parity, and infertility diagnosis (except for male factor: 38% in the IM arm versus 27% in the PV arm, $p=0.03$). Embryo characteristics at the time of cryopreservation, thawing, and transfer were also not significantly different between the two groups. Cycle characteristics and outcomes are shown in Table 2. Progesterone levels 2 weeks after ET were higher in the IM arm compared to the PV arm (44.12 vs 10.79, $p<0.001$). No differences were seen in the initial positive pregnancy tests between the two arms. The IM group showed a trend toward higher implantation rates although this did not reach statistical significance (21.5% vs 16.5%, $p=0.087$). However, the ongoing clinical pregnancy rates were significantly higher in the IM arm (38.2% vs 28.0%, $p=0.035$). Live births in the IM arm were significantly higher compared to the PV arm (34.5 % vs 22.8%, $p=0.040$). Conversely, biochemical pregnancy rate was significantly higher in the PV arm (10%) compared to the IM arm (2.5%, $p=0.002$). A biochemical pregnancy was defined as a positive pregnancy test without development of a gestational sac. The cancellation rate was 2.7% ($n=11$); 2/11 were due to premature ovulation, 5/11 were due to thin endometrium (< 6 mm), and 4/11 were due to vaginal bleeding. No comparison between the cancellation rates of the two arms was done because cancellations occurred in the proliferative phase and were therefore independent of the route of P4 administration. Within each arm, no differences in P4 level or EC were seen between patients who conceived and those who did not.

Discussion

Our results indicate that a "natural cycle override" for FET cycles can be successfully achieved using a fixed E2 dose to simulate the proliferative phase with subsequent excellent clinical pregnancy rates as well as live birth rates. The goal of much subsequent research has been to find an optimal route of P4 administration for luteal phase support that maintains good pregnancy rates while limiting side effects and increasing the ease of administration for the

Table 1 Patient and embryo characteristics

	IM	PV	*p* value[a]
Age	31.7	31.7	NS
Gravidity	1.29	1.29	NS
Parity	0.71	0.71	NS
Diagnosis[b]			NS
Tubal factor	23.6%	24.2%	NS
Endometriosis	34.7%	39.3%	NS
PCOS	26.6%	26.5%	NS
Unexplained	8.5%	10.4%	NS
Male factor	37.7%	27.0%	0.03
Other	2.5%	1.9%	NS
Stage at freezing[b]			
2 PN	6.5%	6.6%	NS
Cleaved	77.4%	74.9%	NS
Blastocyst	15.1%	17.5%	NS
Mean embryo grade at ET[c]	2.47	2.2	NS
Mean cell number at ET[c]	5.53	5.61	NS
Stage at transfer			
Cleaved	84.4%	82%	NS
Non-expanded blast	7.0%	6.6%	NS
Expanded blastocyst	8.5%	11.4%	NS

[a] By Student's *t*-test, except as noted
[b] By Chi square
[c] For non-blastocyst embryos only

patient. Disadvantages seen in oral administration include alterations in absorption influenced by food intake, metabolic inactivation due to the hepatic first-pass effect, and drowsiness [15–17]. Intramuscular P4 is uncomfortable to administer, requires daily injections to maintain appropriate serum concentration, and can lead to inflammation of the injection site. Therefore, much research has been done to evaluate the efficacy and side effect profile of vaginal progesterone since this form is easy to administer and has few side effects with minimal systemic absorption.

Vaginal P4 has been shown to be superior to oral progesterone in producing in-phase secretory endometrium and having greater bioavailability [18]. The comparison between vaginal and IM progesterone has been more controversial. Similar pregnancy outcomes have been found in one prospective randomized study comparing the two routes in FET cycles [10]. In this study, patients were randomized to FET cycles in which IM progesterone or PV was used for endometrial preparation. Compared to our study, lower pregnancy rates were noted in both arms (15.9% in the IM arm and 16.8% in the PV arm), and therefore a larger number would be required in order to detect a difference in outcomes in their study. A power analysis of their study showed at 31,000 patients would be needed in each arm in order to detect a significant difference. This may explain the difference in outcomes between our study and theirs. Although our study was a retrospective one, bias was minimized due to the fact that route of administration was arbitrarily distributed by year.

In contrast, IM progesterone has been demonstrated to have greater pregnancy outcomes in comparison to PV progesterone in fresh IVF cycles [11]. It has been postulated that higher local endometrial levels of progesterone seen with vaginal progesterone compared to IM progesterone (although serum levels do not reflect this) are the culprit [19]. Whether this difference plays a role in the relative implantation inhibition seen with the use of PV remains to be determined.

The cumulative pregnancy rate in our study (combining both routes of P4 administration) is 28.5%. This rate is in the higher end of what is published in the SART data as well as the literature. When examining the published literature, some studies show no difference in outcomes between non-downregulated FET cycles vs GnRH agonist down-regulated ones [20, 21], while others show superiority of GnRH down-regulated cycles [22]. Although the randomized study by Toukhy and colleagues showed that

Table 2 Cycle characteristics and outcomes

	IM	PV	*p* value[a]
Number of embryos transferred	2.86	2.82	NS
Duration of proliferative phase	14.66	14.43	NS
Endometrial thickness	9.43	9.51	NS
Progesterone level	44.12	10.79	<0.001
Pregnancy outcomes[b]			
2 week pregnancy test	43.7%	40.8%	NS
Clinical pregnancy rate	38.2%	28.0%	0.035
Implantation rate	21.5%	16.5%	0.087
Biochemical pregnancy rate	2.5%	10%	0.002
Ectopic rate	1.5%	0%	0.069
Live birth rate	34.5%	22.8%	0.004

[a] By Student's *t*-test, except as noted
[b] By Chi square

the live birth rate is higher in the GnRH down-regulated FET cycles (20% for GnRH suppression vs 8.5% for high dose E2 suppression), their rates were significantly lower than our data, where our combined live birth rate for a similar non-downregulated proliferative phase is 28.5% (vs 8.5% in their study). Their choice of luteal support (P4 pessaries) may be a contributing factor.

In conclusion, our study showed that the use of a "natural cycle override" regimen appears to be an adequate substitute to GnRH analogue suppression cycles with the added benefits of shorter cycle length, less injections for the patients, and lower costs. Although both routes of P4 administration result in acceptable pregnancy rates, IM progesterone use results in significantly higher clinical pregnancy and live birth rates compared to PV. Therefore, consideration should be given to preferentially using IM progesterone in FET cycles and restricting the use of PV to patients who are intolerant to the IM route of administration. Based on the FET rates in the SART database as well as the published literature, we also conclude that the use of a "natural cycle override" regimen appears to be an adequate substitute to GnRH analogue suppression cycles with the added benefits of shorter cycle length, less injections for the patients, and lower costs.

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