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Vaginal progesterone supplementation has no effect on ongoing pregnancy rate in hCG-induced natural frozen–thawed embryo transfer cycles

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ABSTRACT

Objective: The purpose of this study is to assess the effect of luteal phase supplementation (LPS) on pregnancy rates in human chorionic gonadotropin (hCG)-induced natural frozen–thawed (FET) cycles.
Study design: All performed hCG-induced natural FET cycles from January 2006 until August 2007 were retrospectively identified. The study group consisted of 452 cycles: 243 supplemented with progesterone administration (600 mg natural micronized progesterone in three separate doses) and 209 without progesterone. Analysis was limited to cycles where embryos were cryopreserved on day 3. Final oocyte maturation was achieved by hCG when endometrial thickness of ≥7 mm and a follicle of 17 mm were present on ultrasound.
Results: No statistically significant differences were observed in ongoing pregnancy rate between the two groups (22% versus 21%, $p = 0.8$; difference +1%; 95% confidence interval (CI): −6.5 to +8.7). The non-significant effect of the presence or not of luteal support on pregnancy rate was confirmed by logistic regression (odds ratio (OR): 0.9, 95% CI: 0.54–1.47, $P = 0.64$). A previous pregnancy following fresh embryo transfer (OR: 6.04, 95% CI: 3.63–10.02, $P = 0.001$) and increased endometrial thickness (OR: 1.25, 95% CI: 1.11–1.41, $P = 0.001$) significantly affected the achievement of ongoing pregnancy, whereas the association between embryo score and achievement of pregnancy was marginally significant (OR:0.28, 95% CI: 0.08–0.97, $P = 0.05$).
Conclusion: There is no convincing evidence to support the use of LPS in hCG-induced natural FET cycles, since there is no luteal phase defect. Further prospective randomized studies are necessary to confirm these findings.

1. Introduction

The first successful pregnancy following frozen–thawed embryo transfer (FET) was described in 1983 by Trounson and Mohr [1]. However, the question which of cycle regimens should be advocated for the preparation of the endometrium remains unanswered. Different approaches with regard to endometrial preparation with gonadotropins/GnRH agonists, clomiphene citrate, exogenous administered estrogens and progestatives for controlled cryo-thawed embryo transfer have been described around the world [2,3]. The most common modalities for FET are natural cycle or endometrial preparation with exogenous estrogen and progesterone, with or without the addition of a GnRH agonist [4–7]. In the recent Cochrane review by Ghobara and Vandekerckhove [3], no conclusions could be drawn due to the lack of sufficient prospective randomized trials assessing the different methods of endometrial preparations prior to FET.

Different IVF centres around the world using the natural cycle for FET, administer human chorionic gonadotropin (hCG) to induce the ovulation for planning the FET. Administration of hCG for FET timing has been accepted as the standard of care for patients who are undergoing FET in natural cycle.

Studies in menopausal women have demonstrated that LH values were reduced after an hCG injection [8]. As LH is essential for the maintenance and normal steroidogenic activity of the human corpus luteum [9] abnormal LH secretion may account for a defective luteal phase in ovarian stimulation.

The human chorionic gonadotropin (hCG) administered for the final oocyte maturation could potentially cause a luteal phase defect by suppressing the LH production via a short-loop feedback mechanism [8]. However, the administration of hCG did not downregulate the LH secretion in the luteal phase of normal, unstimulated cycles in normo-ovulatory women [10].

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The objective of this paper is to evaluate whether the vaginal administration of natural micronized progesterone would improve the pregnancy rates in hCG-induced natural cryo-thawed embryo transfer cycles as compared with hCG-induced natural cycles without luteal phase support (LPS).

2. Materials and methods

2.1. Patients

All performed hCG-induced natural FET cycles from January 2006 until August 2007 at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University were identified. Analysis was limited to cycles where embryos were cryopreserved on day 3 after conventional IVF or intracytoplasmic sperm injection (ICSI). The study group consisted of 452 cycles: 243 supplemented with progesterone administration (600 mg natural micronized progesterone in three separate doses) and 209 without progesterone. Patients were included in the study only once.

Inclusion criteria for participation in the study were: (i) maternal age ≤37 years old (on the day of embryo freezing), (ii) regular menstrual cycle (25–34 days) [11]. Exclusion criteria were: (i) the use of testicular sperm for ICSI (ejaculated sperm only), (ii) basal follicle stimulating hormone (FSH) levels ≥12 IU/l, (iii) American Fertility Society (AFS) grades ≥III for endometriosis, (iv) body mass index (BMI) ≥29.

2.2. Data collection

Required information was retrieved from the electronic database of our centre. According to Belgian law, Institutional Review Board was not required for this retrospective study. No conflicts of interest were reported.

2.3. Procedures

On day 2 or 3 of the cycle all patients underwent transvaginal ultrasound and serum hormone analysis for FSH, LH, estradiol and progesterone levels. Monitoring of serum hormonal levels and ultrasound examination started from day 8 of the cycle and was repeated as necessary. Final oocyte maturation was achieved by 5000 IU of hCG (Pregnyl; NV Organon) when endometrial thickness of 7 mm or more and a follicle of 17 mm were present on ultrasound. The day after the hCG administration, serum progesterone and estradiol were assessed to confirm ovulation. The decision to give or not to give luteal phase support was based on the discretion of the physician. Two hundred and forty-three patients received daily vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan; Besins, Brussels, Belgium) starting from the day after hCG administration and continued until 7 weeks of gestation if pregnancy was achieved.

2.4. Hormonal measurements

Serum LH, FSH, hCG, E_2, and P were measured with the automated Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra-assay and interassay coefficients of variation (CVs) were <3% and <4% for LH, <3% and <6% for FSH, <5% and <7% for hCG, <5% and <10% for E_2, and <3% and <5% for P, respectively.

2.5. Timing of the FET

All embryos were frozen on day 3. The cryopreserved embryo transfer was planned 5 days after the hCG administration. The center works on a 7-day schedule, thus allowing ETs on day 5. The embryo transfers were done without ultrasound guidance [12] using a standard embryo transfer catheter (K-soft 5100, Cook). The serum hCG level was measured 12 days after the transfer.

2.6. Embryo freezing–thawing

IVF and ICSI treatments were carried out as described by Van Landuyt et al. [13]. Embryo selection for transfer or freezing was done in the morning of the day of transfer. Fresh cleavage-stage embryos were selected for transfer on day 3 if the embryo had at least 5 cells with <50% anucleated fragments or 4 cells if the embryo originated from a 2-cell on day 2 of development. Embryos were selected for freezing if at least 6 blastomeres were present with ≤20% fragmentation. Embryos with >20% but with <50% fragmentation were frozen if they had reached the 8-cell stage. Embryos with >50% of the blastomeres multinucleated were not selected for transfer or freezing. The freezing and thawing procedure was done as described in detail previously [14].

Cleavage-stage embryos were evaluated for morphological survival immediately after thawing and were further cultured overnight in sequential media. The next morning, further cleavage was evaluated.

According to the Belgian IVF legislation (KB 1 July 2003), a maximum of two embryos could be replaced per frozen embryo transfer. Therefore, for each patient planned for a thawed embryo replacement, only one straw containing up to two embryos was thawed. If at least one embryo survived with all cells intact there was no further thawing. If not, a second straw was thawed if available.

For day 3 embryos, the morphological survival rate was scored by counting the number of blastomeres that were intact upon the total number of blastomeres of the embryo at the moment of selection for freezing. Frozen–thawed embryos were suitable for transfer when at least 50% of the blastomeres were intact. Preferentially, 100% intact embryos which further cleaved overnight were selected for transfer.

2.7. Outcome measures

Primary outcome measure was detection of ongoing pregnancy defined as pregnancy beyond the 12th week of gestation. Secondary outcome measure was implantation rate (determined as the division of the total number of fetal hearts observed by ultrasound with the total number of embryos transferred).

2.8. Statistical analysis

Proportions were compared with the Fisher's exact test or the chi-square test where appropriate. Continuous variables were compared with the *t*-test for independent samples or the Mann–Whitney test depending on the normality of their distribution. Multivariable logistic regression was specified to evaluate association between ongoing pregnancy after FET and variables of interest. A *P*-value of <0.05 was considered statistically significant.

3. Results

There were no significant differences between the groups in the demographic, cycle or embryologic characteristics (Table 1).

The pregnancy outcomes of the two groups are shown in Table 2. Ongoing pregnancy rate was similar in the LPS group compared with the no-utrogestan group (21% versus 22%, $P = 0.8$).

The effect of the parameters (mentioned in Table 1) on ongoing pregnancy achievement after frozen embryo transfer was examined by robust logistic regression. Variables that entered the model were selected by means of univariate comparisons between