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ASSISTED REPRODUCTION TECHNOLOGIES

Disparities in reproductive outcomes according to the endometrial preparation protocol in frozen embryo transfer

The risk of early pregnancy loss in frozen embryo transfer cycles

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Abstract
Purpose The purpose of this study was to determine the effect of stimulated and artificial endometrial preparation protocols on reproductive outcomes in frozen embryo transfer (FET) cycles.
Methods We performed a retrospective study of 1926 FET cycles over a 3.5-year period in the Fertility Unit at a University Hospital. Stimulated and artificial protocols were used for endometrial preparation. The embryos for FET were obtained from either in vitro fertilization or intracytoplasmic sperm injection cycles. Live birth rate and early pregnancy loss rates were retrospectively compared.

In artificial protocols, oral or vaginal administration of oestradiol 2 mg two or three times a day was followed by vaginal supplementation with progesterone 200 mg two or three times a day. In stimulated protocols, recombinant follicle-stimulating hormone was administered from day 4 onward. Vaginal ultrasound was used for endometrial and ovarian monitoring. A pregnancy test was performed 14 days after FET. If it was positive, oestradiol and progesterone were administered up until the 12th week of gestation in artificial cycles. We defined early pregnancy losses as biochemical pregnancies (preclinical losses) and miscarriages.
Results Data on 865 artificial cycles (45% of the total) and 1061 stimulated cycles (55%) were collected. Early pregnancy loss rate was significantly lower for stimulated cycles (34.2%) than for artificial cycles (56.9%), and the live birth rate was significantly higher for stimulated cycles (59.7%) than for artificial cycles (29.1%).
Conclusion In frozen embryo transfer, artificial cycles were associated with more early pregnancy loss and lower live birth rate than stimulated cycles.

Keywords Frozen embryo transfer · Reproductive outcomes · Endometrial preparation

Introduction

According to the 15th World Report on Assisted Reproductive Technology [1], the number of frozen embryo transfer (FET) cycles increased by 27.6% between 2008 and 2010. The early pregnancy loss (EPL) rates for FET were 28.9% in 2008, 25.4% in 2009, and 25.2% in 2010. The delivery rates for single embryo transfer (SET) with a frozen embryo were 18.1% in 2008, 19.6% in 2009, and 20.5% in 2010; these values were similar to those for SET with a fresh embryo [1].

According to the European Society of Human Reproduction and Embryology's latest report, the pregnancy rate for FET cycles across Europe was 23.1% in 2015 vs. 21.3% in 2011 [2].

There is no consensus on the optimal endometrial preparation protocol for FET cycles. It is crucial to identify the implantation window, i.e. the time period during which the endometrium is receptive for embryo implantation. FET is

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increasingly used in in vitro fertilization and intracytoplasmic sperm injection cycles; when compared with fresh embryo transfer, FET appears to be associated with higher pregnancy and implantation rates [3].

Various cycle protocols are used for the preparation of the endometrium in FET: modified natural, artificial and stimulated cycles.

In modified natural cycles, no medications are used for ovarian stimulation; ovulation is induced during the course of a natural cycle.

An artificial cycle is a hormone-replacement cycle where endometrium is prepared with administration of exogenous oestrogen followed by progesterone administration before embryo transfer.

In stimulated cycles, the follicular development is induced and controlled with gonadotropins then ovulation is triggered with recombinant-human chorionic gonadotropin (r-HCG) or HCG once the ovulation criteria are met.

Scientific work found no consensus concerning the optimal protocol to use.

In Groenewoud et al.'s randomized controlled trial, artificial cycles were not found to be superior to modified natural cycles in terms of clinical pregnancy and live birth rates [4]. A retrospective study by Jouan et al. demonstrated the superiority of clomiphene citrate cycles over artificial cycles—notably with higher overall pregnancy rates (24.3 vs. 20.8%, respectively). Clomiphene citrate cycles were also associated with a significantly higher ongoing pregnancy rate (18.6%) [5]. Tomas et al. retrospectively compared the pregnancy loss rates associated with three types of endometrial preparation protocol prior to FET: a natural cycle with luteal phase support from progesterone; a natural cycle with human chorionic gonadotropin (HCG) for ovulation triggering; and an artificial cycle (oestradiol + progesterone) [6]. The researchers concluded that the clinical pregnancy rate and live birth rate per ET were similar for all three protocols but that the preclinical and clinical pregnancy loss rates were significantly ($p < 0.0001$) higher for artificial cycles. In FET cycles, the type of endometrial preparation protocol was the only factor independently correlated with the pregnancy loss rate [6].

A 2008 Cochrane Collaboration review of protocols used for endometrial preparation in FET concluded that there was insufficient evidence to support the use of one protocol over another with regard to live birth and clinical pregnancy rates [7]. Likewise, a meta-analysis published in 2013 found that natural cycles, modified natural cycles (ovulation triggered by HCG) and artificial cycles did not differ significantly in terms of clinical pregnancy rates or live birth rates [8].

Overall, there are few data [8] to suggest that stimulated protocols are of benefit for endometrial preparation in FET cycles. Hence, the objective of the present study was to evaluate the live birth rate and EPL rates associated with artificial and stimulated endometrial preparation protocols used for FET cycles.

Material and methods

Study design

We performed a retrospective, single-centre study of 1926 FET cycles over a 3.5-year period (from January 2012 to June 2015) in the fertility unit at a University Hospital.

Participants

Patients in the study had previously undergone fresh in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles and were generally good responders but failed to have ongoing pregnancy/live birth after fresh embryo transfer. Frozen embryos used were derived from these IVF/ICSI cycles and were vitrified mainly at day 2 or 3.

Methods

Standard, regular monitoring consisted of vaginal ultrasound (to evaluate endometrial thickness and follicular development) and blood hormone assays (including oestradiol, progesterone and LH plasma levels). A pregnancy test was performed 14 days after ET.

Variables

Reproductive outcomes (live birth rate and EPL rate) for artificial and stimulated endometrial preparation protocols were compared. Early pregnancy loss was defined as a biochemical pregnancy (defined as a preclinical loss with a detection of serum human chorionic gonadotrophin (HCG) with no development into a clinical pregnancy) [9] or a miscarriage (defined by the French College of Gynaecologists and Obstetricians (*College National des Gynécologues et Obstétriciens* Français) as the loss of pregnancy within 14 weeks of confirmation of a clinical pregnancy) [10]. The patients' baseline characteristics (including age, body mass index (BMI), duration of infertility, aetiology of infertility and number of embryos transferred) were recorded.

Endometrial preparation protocols

Patients were allocated to artificial or stimulated cycles according to the physician's judgement and experience. In artificial cycles, patients received oral or vaginal oestradiol 2 mg two or three times daily from day 1 of the cycle onwards. When the endometrial thickness reached 7 mm, we added vaginal supplementation with progesterone 200 mg two or three times a day. The embryo was transferred according to its development stage at the time of freezing.

In stimulated cycles, patients received a daily subcutaneous injection of recombinant gonadotrophin (37.5–100 IU) from day 4 of the cycle onwards. The dose was adjusted according to the BMI, the ovarian reserve and any previous ovarian response to stimulation. When the ovulation criteria were met (one follicle ≥ 16 mm and peak plasma oestradiol level > 200 pg/ml), we triggered ovulation with a subcutaneous injection of HCG (5000 IU) or recombinant HCG (250 μg). These patients had no intercourse on ovulation day. The adequacy of the luteal phase was evaluated by measuring blood progesterone levels 3 days after ovulation had been triggered. If the progesterone level 3 days after ovulation triggering exceeded 3 ng/ml, FET was implemented (depending on the embryo's development stage at the time of freezing). Stimulated cycles were not supplemented with progesterone.

Statistical analysis

A chi-squared test was used for intergroup comparisons of qualitative variables. The threshold for statistical significance was set to $p < 0.05$.

Ethical approval

The study was approved by Institutional Review Board, and the study data completely excluded the identification of subjects.

Results

One thousand nine hundred twenty-six patients who had previously undergone fresh IVF or ICSI cycles but failed to conceive were included in the study from January 2012 and June 2015 and received frozen embryos derived from the fresh cycles. The mean age of the patients was 33.5 years old, and their mean BMI was 23.7. Patients had a mean infertility duration of 4.11 years. There were no significant differences between artificial cycles (n = 865) and stimulated cycles (n = 1061) in terms of the baseline characteristics (including age, BMI, the duration and aetiology of infertility and the number of embryos transferred; Tables 1 and 2).

The EPL was significantly higher for artificial cycles than for stimulated cycles (53.2 vs. 29%, respectively), whereas the live birth rate was significantly lower (29.6 vs. 59.9% respectively) (Table 3). Similar results were observed in the subgroup of cycles with single embryo transfer (Table 4) that consisted of 971 FET.

Discussion

A total of 1926 FET cycles were performed between January 2012 and June 2015 at the Fertility Unit at a University Hospital. Our analysis showed that stimulated cycles were associated with a significantly higher live birth rate and a significantly lower EPL rate, relative to artificial cycles.

Table 1 Age distribution of patients with stimulated and artificial cycles

Age (years)	Stimulated protocols (n = 1061)	Artificial protocols (n = 865)	p value
20–25	n = 17 (1.1%)	n = 18 (2%)	0.09 NS
26–30	n = 222 (21%)	n = 211 (24.4%)	0.08 NS
31–35	n = 443 (41.9%)	n = 345 (39.9%)	0.36 NS
36–40	n = 314 (29.7%)	n = 231 (26.7%)	0.14 NS
> 40	n = 65 (6.1%)	n = 60 (6.9%)	0.49 NS

NS not significant

The present study had a number of strengths. The inclusion of a large patient population (n = 1926) at a single institution meant that the protocols for assisted reproductive techniques were relatively homogeneous. The study also had limitations. Firstly, the study's retrospective nature may have introduced selection or information bias. Secondly, we lacked data on treatment compliance at home; despite the best efforts of the care team and frequent reminders, some patients may not have fully complied with the 12-week course of progesterone supplementation. This (amongst other factors) might have led to a higher EPL rate for artificial cycles.

Few studies have compared the reproductive outcomes in stimulated and artificial cycles. Wright et al.'s prospective randomized, comparative trial of artificial and stimulated protocols in 194 patients reported similar implantation rates (8.5 vs. 7.3%, respectively), pregnancy rates (16 vs. 13%), cancellation rates (23% for both) and mean ± standard deviation endometrial thickness (8.7 ± 1.1 vs. 8.7 ± 1.0 mm, measured by ultrasound on the day of progesterone initiation) [11]. However, the sample size was relatively small.

When considering live birth and clinical pregnancy rates for natural cycles, modified natural cycles, artificial cycles and clomiphene citrate protocols, a Cochrane Collaboration review by Ghobara et al. and a meta-analysis by Groenewoud et al. concluded that there was insufficient evidence to support the use of one protocol over another [7, 8]. Some studies have reported that artificial protocols have a higher EPL rate than

Table 2 BMI distribution for patients with stimulated and artificial cycles

BMI (kg/m^2)	Stimulated protocols (n = 1061)	Artificial protocols (n = 865)	p value
≤ 30	n = 954 (89.9%)	n = 756 (87.4%)	0.34
> 30	n = 107 (10.1%)	n = 109 (12.6%)	0.27

Table 3 Reproductive outcomes for patients with stimulated and artificial cycles

	Stimulated protocols (n = 1061)	Artificial protocols (n = 865)	p value
Positive pregnancy test	n = 192 (18.1%)	n = 152 (17.6%)	0.76 NS
Early pregnancy loss	n = 57 (29%)	n = 81 (53.2%)	0.0001
Biochemical pregnancy	n = 26 (13.55%)	n = 27 (17.7%)	0.28
Missed abortion < 14 GW	n = 31 (16.1%)	n = 54 (35.5%)	0.0001
Live birth rate	n = 115 (59.9%)	n = 45 (29.6%)	0.0001
Other events[a]	n = 20 (11.1%)	n = 26 (17.2%)	NS

[a] Ectopic pregnancy, interruption of pregnancy or intrauterine foetal death

natural and modified natural cycles [6]. Even though artificial protocols are more convenient for patients and physicians, their superiority over other endometrial preparation protocols used for in FET has not been demonstrated. Hence, we consider that the present study is the first to provide evidence in favour of stimulated cycles over artificial cycles.

Our results highlight the benefits of more "natural" hormonal support via the corpus luteum in stimulated cycles, with probable advantages in terms of the reproductive outcomes of FET cycles. Even in the subgroup of polycystic ovarian syndrome (PCOS) patients, ovarian hyperstimulation was not a significant or relevant issue in stimulated cycles. To the best of our knowledge, there are no randomized, controlled studies of reproductive outcomes for artificial cycles vs. stimulated cycles in patients with polycystic ovarian syndrome. A recent meta-analysis could not find robust data on live birth, ongoing pregnancy and clinical pregnancy rates to favour stimulated or artificial endometrial preparation protocols prior to FET for patients with PCOS [12].

A recent retrospective study by Kofinas et al. sought to determine the optimal progesterone level at the time of FET for euploid single embryo transfer (SET) after artificial endometrial preparation protocols. The researchers found a significantly lower overall pregnancy rate and live birth rate and a higher spontaneous abortion rate and biochemical pregnancy rate when the serum progesterone level exceeded 20 ng/dl [13]. However, the serum progesterone level does not necessarily reflect the endometrial concentration or the implantation window [14]. In fact, the implantation rate is correlated with endometrial patterns (anatomical changes linked to the menstrual cycle) rather than endometrial thickness. For example, the implantation rate is low for type 3 endometrial patterns (mid-late secretory, homogeneous hyperechoic endometrium) [15].

Vaginal absorption of exogenous progesterone is variable, and the peak serum progesterone level is reached 6 to 8 h after administration. This means that the uterine peak was reached even earlier. The LH activity provided by HCG after stimulation in the presence of a corpus luteum leads to more constant circulatory concentration of progesterone. These arguments are in favour of a local CL production of progesterone [16] without exceeding the level of 20 ng/dl which is associated with an increased risk of abortion [13].

When exogenous progesterone is administered intramuscularly or subcutaneously and even vaginally, there is variable, limited endometrial exposure with earlier uterine peak, leading to less synchronization between embryo and endometrial development [17]. In fact, exposure to progesterone will induce specific changes in endometrium and expression of some protein and biochemical markers of receptivity that are appropriate for successful implantation, according to the stage of embryo development. Alterations in these mechanisms lead to alterations in the implantation window, less endometrial receptivity and implantation [3].

In conclusion, stimulated cycles seem to be associated with higher live birth rate and lower early pregnancy loss than

Table 4 Reproductive outcomes for SET after stimulated or artificial cycles

	Stimulated protocol n = 555	Artificial protocol n = 416	p value
Positive pregnancy test	n = 89 (16%)	n = 61 (14.7%)	0.56 NS
Early pregnancy loss	n = 32 (35.9%)	n = 33 (54.1%)	0.02
Biochemical pregnancy	n = 14 (15.7%)	n = 13 (21.3%)	0.2 NS
Missed abortion < 14 GW	n = 18 (20.2%)	n = 20 (32.8%)	0.08 NS
Live birth rate	n = 49 (55%)	n = 11 (18%)	0.0001
Others[a]	n = 8 (9.1%)	n = 17 (27.9%)	NS

[a] Ectopic pregnancy, interruption of pregnancy or intrauterine foetal death

artificial cycles. A prospective, randomized study is now required to accurately assess the efficacy of each type of endometrial preparation protocol.

Compliance with ethical standards The study was approved by Institutional Review Board, and the study data completely excluded the identification of subjects.

Conflict of interest The authors declare that they have no conflict of interest.

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