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ASSISTED REPRODUCTION TECHNOLOGIES

Transfer of cryopreserved - thawed embryos in hCG induced natural or clomiphene citrate cycles yields similar live birth rates in normo-ovulatory women

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Abstract
Introduction The purpose of this retrospective analysis is to compare the efficiency of hCG-induced natural and Clomiphene citrate (CC) cycles in normovulatory patients undergoing frozen embryo transfer (FET).
Materials and methods It was retrospectively conducted in the Dutchspeaking Free University of Brussels and covered the period from April 2003 to August 2006. In particular, 428 day-three FET cycles belonging to the two comparative groups were recruited. Of these FET cycles, 261 were hCG-induced natural and 167 clomiphene citrate-induced cycles.
Results No statistically significant difference was observed in live birth rate between CC and natural group (22.2% versus 22.6%), respectively (P=0.708). Except for the number of embryos transferred (1.72±0.46 for CC group versus 1.63±0.48 for natural group, P=0.045), no other parameters seem to influence the outcome.
Discussion To our knowledge, this is the first attempt to investigate which of the above mentioned regimens is optimal for normo-ovulatory women in FET cycles. A similar delivery outcome was observed for hCG–induced natural and CC-induced cycles used for endometrial preparation in FET.

Keywords FET · Clomiphene citrate · hCG · Natural cycle · Live birth

Capsule Similar delivery outcome between hCG–induced natural and CC-induced cycles used for endometrial preparation in normo-ovulatory women in FET.

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Introduction

Since the first report of a successful pregnancy following a transfer of the first thawed human pre-embryo in 1983 by Trounson and Mohr [1] and a successful pregnancy following a thawed human blastocyst in 1985 by Cohen et al. [2], the cryopreservation of embryos has become a useful tool in in-vitro fertilization (IVF) treatments. Transfer of frozen embryos has been a widely practiced technique, because of the relatively low cost, the absence of the ovarian hyperstimulation syndrome (OHSS) and reduction of multiple pregnancies [3]. Additionally, deliveries of healthy babies [4, 5] have confirmed the safety of the technique.

However, the pregnancy rate after FET is lower than in fresh cycles [6] but the cumulative pregnancy rate per oocyte recovery [7] and the cumulative live birth rate per cycle are increased [8].

To carry out a transfer with frozen embryos, synchronization between the development of the embryo and the endometrium must be present [9, 10] to ensure a proper and successful implantation.

For this purpose, different protocols have been described including the spontaneous ovulatory cycle (natural cycle), ovulation induction with drugs as clomiphene citrate (CC) or

the artificial cycle where the endometrium is prepared with exogenous estradiol and progesterone hormones [10, 11].

The natural cycle is one of the most common protocol used in FET [10].

Most of the time, ovulation is induced by the physiological rise of luteinizing hormone (LH). The fact that patients do not use medications, makes it very interesting and reduces the cost of the treatment [10, 12]. In spite of this, more frequent transvaginal ultrasounds and monitoring of the LH surge [13] are needed to know the date of the ovulation and planning the frozen-embryo transfer which signifies an inconveniency for the patients.

In a natural cycle, in presence of a mature follicle and satisfactory endometrial development, exogenous human chorionic gonadotrophin (hCG) can also be used to induce final oocyte maturation and ovulation in order to mimic the function of LH [14]. It is considered as the standard of care for patients who are undergoing FET in a natural cycle.

Moreover, the use of hCG for triggering the ovulation instead of spontaneous LH surge, significantly reduces the number of monitoring visits.

A study published by Weissman et al. [15] shows that each patient who receives hCG to trigger the ovulation, saves one visit at the clinic in preparation for FET and it is as efficient as serial monitoring until the ovulation detection in terms of implantation, pregnancy and live birth rates.

Ovulation induction using CC is another option in FET cycles. However, it is not widely used, probably due to the anti-estrogenic effects on the endometrium [16, 17]. The final maturation of the oocyte may also be performed using exogenous hCG in FET cycles using CC.

Regarding the effectiveness of different protocols used in FET cycles, a recent Cochrane review by Ghobara and Vandekerckhove [10] showed that, at the present time, there is insufficient evidence to support the use of the natural or artificial cycle in patients undergoing a FET.

However, to our knowledge, there are no published studies comparing the natural cycle and the use of CC in FET using exogenous hCG to induce final oocyte maturation.

We decided to retrospectively analyze the results obtained with these two regimens in patients undergoing FET.

Materials and methods

Patient population

Data from 721 natural and clomiphene citrate-induced FET cycles from April 2003 to August 2006 were evaluated. All the cycles were performed at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University.

Analysis was limited to cycles where embryos were cryopreserved on day 3 after conventional IVF or intracytoplasmic sperm injection (ICSI). Therefore, the study group finally consisted of 428 cycles. Of these FET cycles, 261 were hCG induced natural and 167 clomiphene citrate — induced cycles.

All patients included were 37 years old (on the day of embryo freezing) with a body mass index (BMI) <29, basal follicle stimulating hormone (FSH) levels <12 IU/l and had a regular menstrual cycle (25–34 days) [18].

The primary end-point was the live birth rate. Secondary outcome measure was the detection of ongoing pregnancy defined as a pregnancy beyond the 12th week of gestation and implantation rate (i.e. the division of the total number of foetal hearts observed by ultrasound with the total number of embryos transferred).

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

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. In one group, patients received a daily dose of 50 mg of Clomiphene citrate (Clomid; Sanofi-Aventis, Brussels, Belgium) during five consecutive days (from day 3 until day 7). In the other group, patients underwent a natural cycle. Based on the fact that 10% to 18% of regular cycles are anovulatory the assignment for expectant management was done according to the attending physician's preference at outpatient clinic. Monitoring of serum hormonal levels and ultrasound examination started from day 8 of the cycle and was repeated when necessary. Final oocyte maturation for the two groups was achieved by administration of 5,000 IU of hCG (Pregnyl;NV Organon) when endometrial thickness of 7 mm or more was reached and a follicle of 17 mm was present on ultrasound. One day after hCG administration, serum progesterone and estradiol were assessed to confirm ovulation.

All 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.

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.

Timing of the FET

All embryos were frozen on day 3. The cryopreserved embryo transfer was planned 5 days after the hCG administration. The embryo transfers were done without ultrasound guidance [19] using a standard embryo transfer catheter (K-soft 5100, Cook). The serum hCG level was measured 12 days after the embryo transfer.

Embryo freezing and thawing

IVF and ICSI treatments were carried out as described by Van Landuyt et al. [20]. 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 with ≤20% fragmentation were present. Embryos with >20% but <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 were performed as described in detail previously [21].

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 2 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. The choice of the number of embryos transferred was not associated with any other interfering factors (i.e.parity,prior twin pregnancy).

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 that further cleaved overnight were selected for transfer.

Statistical analysis

Statistical tests Continuous variables of the two groups were compared using Student's *t* test for independent samples or Mann-Whitney *U* test for variables with normal or not normal distributions respectively. Normality was checked using Kolmogorov-Smirnov with Liliefors correction. Categorical variables were compared using chi-squared or Fisher's exact test, where appropriate.

The crude odds ratio for the live birth rate with the corresponding 95% confidence intervals (CIs) was calculated by using logistic regression analysis. So, as to control for potential confounding effects, two multivariate logistic regression models were calculated. One regression model used all available variables as independent covariates and the other used only variables that presented statistically significant differences between the two comparison groups. Adjusted odds ratios were calculated for the live birth rate for each model.

All statistical tests used a two-sided alpha of 0.05. Analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL).

Results

Demographic, FET cycle characteristics and hormonal values between the two groups are presented in Tables 1 and 2. Patients in the CC group were younger compared with the patients of the Natural group (difference 1.21 years, 95%CI, 0.53–1.89). There were more patients in the Natural group that had only one follicle on the day of hCG compared with the patients in the CC group (difference 15.3%, 95%CI, 9.8–21.7). In addition, a significant difference was observed between CC and Natural group on LH level [8(6–12) IU/l] versus [10.3(7.1–18) IU/l] $P<0.001$ and estradiol level [450 (285–670) pg/ml] versus [237 (172–324) pg/ml] $P<0.001$ on hCG day, respectively.

In the Natural group there were more patients that had only one embryo transferred compared to the CC group (difference 9.1%, 95%CI, 0.1–17.7). Significant difference was also observed between CC and Natural group on endometrial thickness (8.06±2.45) versus (8.66±2.24), $P=0.004$. Moreover, comparing the indication for treatment of the two groups, patients in the CC were treated more frequently than the ones in the Natural group for a male (49.7% vs. 36.0%, difference 13.7%, 95% C.I.: 4.1%–23.0%) or idiopathic factor (43.7% vs. 29.1%, difference 14.6%, 95% C.I.: 5.3%–23.8%) and less frequently for a tubal factor (6.6% vs. 34.9%, difference 28.3%, 95% C.I.: 20.9%–34.9%).

No significant differences were present between the two groups regarding BMI, type of the previous treatment, rank of trial, number of days until hCG administration, progesterone and FSH level on the day of hCG and the quality of the embryos transferred.

Table 3 illustrate the cycle outcomes after transfer of frozen embryos according to the treatment. No significant differences were observed between two groups in terms of live birth rate (22.6% vs.22.2%).

Table 1 Baseline characteristics and embryological data according to treatment

	Natural group (n=261)	CC group (n=167)	P value
Age (years)	32.52±3.42	31.31±3.63	0.001[a]
Body Mass Index-BMI (kg/m^2)	23.24 ±1.71	23.17 ±2.87	0.760[a]
Indication for treatment			<0.001[b]
Andrological	94 (36.0)	83 (49.7)	
Tubal	91 (34.9)	11 (6.6)	
Idiopathic	76 (29.1)	73 (43.7)	
Treatment type			0.056[c]
IVF	77 (29.5)	35 (21.0)	
ICSI	184 (70.5)	132 (79.0)	
Rank of trials	1.94±0.98	1.87±1.16	0.152[d]
Number of follicles on the day of hCG >17 mm			0.001[d]
1	257 (98.5)	139 (83.2)	
2	4 (1.5)	19 (11.4)	
3	0(0)	8 (4.8)	
4	0(0)	1(0.6)	
Endometrium thickness (mm)	8.66±2.24	8.06±2.45	0.004[d]
Number of days until hCG administration	12.83 ±2.05	12.71 ±2.05	0.429[d]
Number of transferred embryos	1.72±0.46	1.63±0.48	0.045[d]
Embryo score[e]			0.094[a]
1 (top quality)	241 (92.3)	145 (87.3)	
2 (good quality)	20 (7.7)	21 (12.7)	

All cases are presented as mean ± SD or cases (percentages)

[a] P value obtained with independent samples Student t test

[b] P value obtained with chi-squared test

[c] P value obtained with Fisher's exact test

[d] P value obtained with Mann-Whitney U test

[e] All embryos were frozen on day 3. A top-quality embryo means: >=7 cells on day 3, <=10% fragmentation, no multinucleation, similar cell size in accordance to the cleavage stage. A good quality embryo means: 6 cells with fragmentation 0% up to 20% or >=7 cells with fragmentation >10% up to 50%

Group sample sizes of 261 in the normal group and 167 in the CC group achieve 5% power to detect a difference between the group proportions of −0.004 (22.6% vs. 22.2%, as observed). In order to achieve 80% power to detect such a low difference (0.4%) as significant, more than 100,000 patients should be included in each group.

Considering live birth the outcome of interest and all other outcomes (stillborns, elective termination, no pregnancy) failure, the crude OR for women receiving CC was 0.974 (95% CI: 0.611–1.553) compared to women in the Natural cycle . When all variables presented in Tables 1 and 2 were entered in the logistic regression analysis as additional covariates, the adjusted OR was 1.140 (95% CI: 0.609–2.135). When only those variables that were significantly different (i.e. age, indication for treatment, number of follicles, number of transferred embryos, LH and estradiol on day of hCG, endometrium thickness) were entered in the model as covariates the results did not change substantially (adjusted OR: 1.067, 95% CI:0.585–1.949).

In both models of logistic regression, the number of transferred embryos was a significant predictor of live birth rate with OR: 1.811, 95% CI: 1.062–3.088, P=0.029 for the first model and OR: 1.740, 95% CI: 1.037–2.921, P=0.036 for the second model. More transferred embryos were associated with a higher probability of live birth.

Table 2 Hormonal profile on the day of hCG according to treatment

	Natural group (n=261)	CC group (n=167)	P value
FSH (IU/l)	5.1 (4.3-7.6)	5.2 (3.9–7.1)	0.187
LH (IU/l)	10.3 (7.1–18.0)	8.0 (6.0–12.0)	<0.001
Estradiol (pg/ml)	237 (172–324)	450 (285–670)	<0.001
Progesterone (ng/ml)	0.60 (0.44–0.90)	0.67 (0.46–0.90)	0.725

All cases are presented as median (interquartile range). Kolmogorov-Smirnov with Liliefors correction showed that all hormones deviated from the normal distribution, so Mann-Whitney's U test was used

Table 3 Outcome measures between the study groups

	Natural group (n=261)	CC group (n=167)	P value
Ongoing pregnancy rate (per cycle)	60 (23.0)	39 (23.4)	1.000[a]
Implantation rate (per ET)	76 (17.9)	57 (19.8)	0.557[a]
Number of pregnancies			0.892[b]
Singletons	50 (19.2)	31 (18.6)	
Twins	10 (3.8)	8 (4.8)	
Delivery outcome			0.708[a]
Live births	59 (22.6)	37 (22.2)	
Stillborn	1 (0.4)	1 (0.6)	
Elective termination	0 (0.0)	1 (0.6)	

All cases are presented as cases (percentages)

[a] P value obtained with Fisher's exact test

[b] P value obtained with Mann-Whitney U test

A subgroup analysis, only for single embryo transfer (SET), was performed between the two groups (Table 4). Implantation and ongoing pregnancy rate was significantly higher in the Natural group in comparison with the CC group (22.7% vs. 8.5%, P=0.040; 21.6% vs. 6.4%, P=0.030).

Discussion

To the best of our knowledge, this is the first retrospective analysis that compares clomiphene citrate versus hCG induced natural cycles in FET in women with regular spontaneous ovulatory cycles. The live birth rate was similar between the two groups, with a crude OR: 0.974 (95% CI: 0.611–1.553), which reflects a non-significant difference between both groups. Except for the number of embryos transferred in favor of CC group, no other parameters seem to influence this outcome.

Because of the risk of multiple gestations, legislation as well as common practice in many countries, a reduction of the number of embryos transferred in fresh cycles is applied [22, 23]. Cryopreservation of supernumerary embryos has become a common procedure in advanced reproductive technologies [24]. Cryopreservation has been shown to increase the pregnancy rate per follicle aspiration cycle while offering a more convenient and less demanding treatment [25]. A recent Cochrane review comparing the different treatment regimens used in FET concludes that there is insufficient evidence to support the use of one menstrual regimen over another [10].

In ovulatory patients, frozen embryo transfer is commonly performed during a natural cycle. This offers the advantage of using the physiological process of endometrial preparation for implantation without using medical intervention [15]. However, FET in a natural cycle needs numerous monitoring visits for blood and ultrasound testing's until the detection of ovulation [13] and has an obvious practical drawback for those centers that do not perform embryo transfer during the weekend.

In order to reduce the above mentioned disadvantages of the natural regimen, ovulation induction agents (either CC or gonadotrophins or a combination of both) are used [26]. Another approach that also simplifies the monitoring process is the administration of hCG in a natural cycle in order to trigger ovulation in the presence of a mature follicle and a sufficient endometrial development. In the Cohrane review [10], the only studies with CC concern CC versus artificial cycle [27] and the association of CC with human menopausal gonadotrophin (HMG) versus HMG [28]. No difference was observed on assessing the clinical pregnancy rates for the first comparison. On the other hand, the comparison between these last two ovulation induction regimens showed that the pregnancy rate was significantly higher in the HMG group and this difference was attributed to the anti-estrogenic effect of clomiphene on the endometrium.

On the other hand, a retrospective study by Weismann et al. [15] comparing a natural cycle with a hCG-induced natural cycle, reported similar results on implantation, clinical pregnancy and live birth rate.

Until now, no study comparing hCG-induced natural cycle and CC cycle in FET has ever been conducted. The lack of studies assessing these two cycle regimens maybe attributed to the fact that CC in FET is infrequently used due to its effect on the endometrium. In the present retrospective analyze , a decreased endometrium thickness was observed in CC group. However, the statistical analysis showed that it did not interfere with the outcomes. While in clinical practice ,endometrial thickness tends towards 5–6 mm using CC, in our results the mean of endometrial thickness was 8,06 mm .This fact could be explained by the small dose of CC (50 mg) that it was administrated. Previous studies have shown that the anti-estrogenic effect of CC is linked to its long half-life [17], manifested as a thinner endometrium [29, 30] and is negatively correlated with the clomiphene dose [16]. We should also take into consideration the possibility that the administration of hCG in a natural cycle as well as in CC cycle could have an impact on endometrial receptivity due to the presence of hCG receptors in the endometrium [31].

It is known that age at the time of cryopreservation is more important than age at the time of embryo transfer [32]. In large series of studies in which the age limit was 40 years [33, 34], it was demonstrated that the lower the age of the patients, the higher the probability of achievement of an ongoing pregnancy. In the current retrospective analyze, no correlation was found between age at the time of transfer and live birth and pregnancy rate, probably because women included were up to 37 years old.

No relationship between hormonal profile on the day of hCG administration and the outcome of an ensuring FET cycle was found. The presence of a higher estradiol level on this day during CC cycle has no impact in predicting pregnancy and delivery outcomes.

Table 4 Outcome measures for SET between the study groups

	Natural group (n=97)	CC group (n=47)	P value
Ongoing pregnancy rate (per cycle)	21 (21.6)	3 (6.4)	0.030
Implantation rate (per ET)	22 (22.7)	4 (8.5)	0.040
Delivery outcome			0.068
Live births	20 (20.6)	3 (6.4)	
Stillborn	1 (0.7)	0 (0.0)	

Although a subgroup analysis of our data in SET revealed statistically higher implantation and ongoing pregnancy rate in Natural group, the number of patients are too low to draw a conclusion.

The major drawback of this analyze is its retrospective design. We have attempted to adjust for parameters that were significantly different between the two study groups, but our conclusions are influenced by the lack of true randomization. In addition, it is restricted to the population of patients who had frozen embryo transfer on day 3 and thus it may not be applicable to blastocyst recipients. Despite these limitations, our findings have been the subject of thorough statistic analysis that adds strength to our conclusions.

Conclusion

Based on our results, women with a regular cycle maybe offered hCG –induced natural or CC induced cycle in FET. The use of clomiphene in FET results in live birth and pregnancy rates as good as in hCG-natural cycles. The major advantage of the hCG –induced natural protocol is that it reduces the inconvenience of an extra drug exposure and its possible risks that CC administration has.

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