Višja stopnja živorojenosti in nižja stopnja spontanih splavov v protokolih z naravnim ciklusom za pripravo endometrija za prenos zamrznjenih blastocist v primerjavi s protokoli z umetnim ciklusom

Higher live birth and lower pregnancy loss rates in natural compared with artificial cycle protocols for endometrial preparation of frozen blastocyst transfer

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Izvleček

Namen: *Cilj raziskave je bila primerjava reproduktivnega izida in stopnje živorojenosti med skupinama pacientk, ki so za pripravo endometrija za postopek prenosa zamrznjenih zarodkov (FET) uporabile protokol z naravnim ciklusom (NC) ali z umetnim ciklusom (AC).*

Metode: *Retrospektivno smo analizirali 1.317 postopkov za pripravo endometrija za FET z NC in 528 postopkov z AC. Analizirani postopki so bili opravljeni v treh letih v terciarni kliniki za postopke IVF. Primerjali smo stopnjo kliničnih spontanih splavov, zanositev in živorojenosti. Z logistično regresijo smo ustvarili modele za identifikacijo neodvisnih dejavnikov, ki so bili pomembno povezani z živorojenostjo in s kliničnimi spontanimi splavi.*

Rezultati: *V postopkih z NC smo opazili pomembno nižjo stopnjo klinič-*

Abstract

Purpose*: The aim of this study was to compare the reproductive outcomes and live birth rates of natural cycle (NC) and artificial cycle (AC) protocols for endometrial preparation of frozen–thawed embryo transfer (FET).*

Methods: *A total of 1317 NC and 528 AC protocols for endometrial preparation of FET performed during a 3-year period at a tertiary in vitro fertilisation (IVF) clinic were analysed. The clinical spontaneous abortion, pregnancy, and live birth rates were compared. Logistic regression models were constructed to identify the factors that are significantly associated with the live birth and clinical spontaneous abortion rates.*

Results: *A significantly lower clinical spontaneous abortion rate (13.68 % vs. 27.32 %; p < 0.001) and a com-*

nih spontanih splavov (13,68 % vs 27,32 %; p < 0,001), primerljivo stopnjo zanositev in pomembno višjo stopnjo živorojenosti (32,82 % vs 26,70 %; p < 0,01) v primerjavi s postopki z AC. Neodvisni napovedni dejavniki za živorojenost so bili starost ženske (OR 0,94; 95 % CI 0,91–0,98), prenos morfološko optimalnih blastocist (OR 1,72; 95 % CI 1,28–2,31) in način priprave endometrija (OR 0,63; 95 % CI 0,45–0,88). Neodvisna napovedna dejavnika za spontani splav sta bila način priprave endometrija (OR 2,26; 95 % CI 1,39–3,68) in starost ženske ob FET (OR 1,07; 95 % CI 1,02–1,13). **Zaključek:** *Pri postopkih z NC smo opazili boljši reproduktivni izid, saj je bila stopnja živorojenosti pomembno višja in stopnja kliničnih spontanih splavov pomembno nižja v primerjavi s postopki z AC. Glede na ugodnejši izid, prijaznost do pacientk in nižjo ceno predlagamo, da je protokol z NC metoda prvega izbora za FET pri pacientkah z rednimi menstruacijami.*

parable pregnancy and a significantly higher live birth rate (32.82 % vs. 26.70 %; p < 0.01) were associated with the NC compared to the AC protocol. Independent predictors for live birth were maternal age (odds ratio (OR), 0.94; 95% confidence interval (CI), 0.91–0.98), transfer of morphologically-optimal blastocysts (OR, 1.72; 95% CI, 1.28–2.31), and the method used to prepare the endometrium (OR, 0.63; 95% CI, 0.45–0.88). Independent predictors of clinical spontaneous abortion were the method used to prepare the endometrium (OR, 2.26; 95% CI, 1.39–3.68) and maternal age at the time of FET (OR, 1.07; 95% CI, 1.02–1.13).
Conclusion: *A better reproductive outcome was observed with the NC protocol; specifically, the clinical spontaneous abortion rate was significantly lower and the live birth rate was significantly higher compared with the AC protocol. Based on better cycle outcomes, patient convenience, and low cost, we suggest using the NC protocol as the first option for FET in patients with regular menses.*

INTRODUCTION

In recent years, cryopreservation of embryos has become an important part of in vitro fertilization programs. In fact, the proportion of frozen–thawed embryo transfer (FET) compared to fresh cycles has reached 34.5%–50.0 % in some centres. This increase is mainly due to a trend towards elective single embryo transfer, freeze-all policy, and the result of improved implantation and pregnancy rates of FET with the development of vitrification techniques and appropriate endometrial preparation (1–4).

To optimize the success of FET, various endometrial preparation strategies have been described. The most frequently used cycle regimens include the natural ovulatory cycle protocol with LH detection in the blood or urine and the modified natural cycle protocol during which ovulation is induced by human chorionic gonadotrophin (hCG) with optional luteal supplementation. An artificial cycle, in which the endometrium is prepared by estrogen and progesterone with or without a gonadotrophin-releasing hormone agonist, is also available. Mild stimulation with exogenous gonadotrophins or oral agents is also a possible option for endometrial preparation (2, 5–8).

The optimal method for endometrium preparation of FET has not been established. According to a Cochrane review and recent meta-analysis, there are no significant differences between protocols according to pregnancy and live birth rates (2,6,7); however, a higher pregnancy loss rate, which results in a lower success rate, has been noted in artificially-prepared cycles. The reasons for the higher pregnancy loss rate have not been completely described and the findings of these studies are inconclusive (9–11).

The purpose of this retrospective study was to compare and evaluate two different protocols for vitrified-warmed blastocyst transfer and to identify predictive factors for pregnancy loss and live births.

MATERIALS AND METHODS

This retrospective study included 1865 vitrified-

warmed blastocyst transfers cycles performed during a 3-year period (2013–2015) in the Department of Reproductive Medicine and Gynaecologic Endocrinology (University Medical Centre Maribor, Slovenia). There were 1317 and 528 FETs performed using natural cycle (NC) and artificial cycle (AC) protocols, respectively.

The NC protocol with luteal hCG supplementation was used in patients with regular menstrual cycles (range, 25–34 days). On days 8–10 of the menstrual cycle, a basal vaginal ultrasound examination was performed. After observing the growth of the leading follicle and thickening of the endometrium, the patients were instructed to perform morning and evening urinary luteinizing hormone LH tests. FET was scheduled on the 6th day after positive morning and evening LH tests. Immediately after FET, patients received a subcutaneous hCG injection (Pregnyl, 2500 IU; MSD, Hertfordshire, Great Britain) for luteal supplementation.

The AC protocol was used mainly in patients with irregular menstrual cycles (> 34 days), as well as patients with unpredictable cycles. The endometrial preparation started with oral estradiol (Estrofem, 2 mg × 3 / day; Novo Nordisk, Dublin, Ireland) from the second day of a natural or gestagen-induced menstrual cycle. A vaginal ultrasound examination was performed after 8–10 days of estrogen therapy. When the thickness of the endometrium was at least 7–8 mm in diameter and there were no dominant follicles, vaginal progesterone was administered (Estima Ge, 200 mg x 3 daily; EFFIK S.A. Meudon la Fôret, France). FET was performed on the 6th day of progesterone therapy, and progesterone was continued until a pregnancy test was performed. In the case of a positive pregnancy test, the patients were instructed to continue with both medications until 10–12 weeks gestation.

Only embryos in the blastocyst stage were cryopreserved on day 5 or 6 after oocyte pick–up and according to the standard vitrification and warming protocols (12). After thawing, only blastocysts having at least 50% intact blastomeres and starting to re-expand were assessed as suitable for transfer.

One or two embryos were transferred using a Labotec catheter (Labotec GmbH, Rosdorf, Germany). The number of embryos transferred in each case depended on the quality of available embryos, the number of previous assisted reproductive technology (ART) treatments, the number of embryos frozen in the same straw, and according to patient-physician agreement.

Pregnancy tests were performed 14 days after FET in all patients. A vaginal ultrasound examination was performed 14 days after a positive pregnancy test to confirm a clinical pregnancy (the presence of an intrauterine gestational sac and a viable embryo).

A biochemical pregnancy was defined as a positive pregnancy test without ultrasound confirmation of an intrauterine pregnancy. A clinical spontaneous abortion was considered a pregnancy loss until 22 weeks gestation after previous confirmation of a clinical pregnancy.

Patient characteristics and clinical data were collected from our software database. Reproductive outcomes between the AC and NC protocols were compared using t–tests and the Pearson's chi-squared test. Univariate and multivariate logistic regression models were constructed to identify the factors that were significantly associated with live births and clinical spontaneous abortions.

Approval from the Ethics Committee was not required owing to the retrospective nature of the study and the fact that the study data completely excluded the identification of patients. All patients gave consent at the time of treatment for the future use of their clinical data.

RESULTS

In this retrospective analysis, 1865 FET cycles were included. The NC protocol with luteal hCG supplementation was used in 1317 cycles (71.38%) and the AC cycle protocol was used in 528 (28.61%) cycles.

The patient characteristic data between the AC and NC groups were not significantly different with respect to age and number of previous in vitro fertilisation/intracytoplasmic sperm injection (IVF/ICSI) cycles. There was a significantly higher percentage of couples with male (50.78 % vs. 42.52 %; $p = 0.01$) or idiopathic infertility (44.00 % vs. 21.92 %; $p < 0.001$) in the NC group. A significantly higher percentage of couples with ovulation disorders was observed in

the AC group (37.77 % vs. 5.50 %; p < 0.001). There was also a significantly higher percentage of couples without fresh embryo transfers in previous IVF/ICSI cycles in the AC group (22.33 % vs. 10.27 %; p < 0.001; Table 1).

There was a significantly higher mean number of blastocyst transfers in the AC group (1.37 ± 0.50 vs. 1.29 ± 0.45; p < 0.001). Endometrial thickness at the time of FET was significantly higher in the NC group (10.15 ± 2.18 vs. 9.85 ± 2.15; p = 0.02).

There were no significant differences in the percentage of FETs of optimal blastocysts and the percentage of FETs of blastocysts frozen on day 6 between the two groups (Table 1). There were no significant differences in the positive pregnancy test, implantation, and clinical pregnancy rates between the groups. There was a significantly higher rate of biochemical pregnancies, significantly higher clinical spontaneous abortion rate, and significantly lower live birth rate in the NC than in the AC group (19.83% vs. 10.34% [p < 0.001], 27.32% vs. 13.69% [p < 0.001], and 26.70% vs. 32.82% [p = 0.01], respectively; Table 1).

Table 1: *Patient's and cycle's characteristics and cycle's outcomes between NC and AC protocols.*

	Natural cycle protocol N =1317	Artificial cycle protocol N=538	p value
Age (years)	33.92±4.01	33.49±4.73	NS
No. of previous IVF cycles	1.94±2.16	1.91±1.59	NS
Prior birth after fresh IVF (%)	19.5	16.48	NS
No fresh ET in IVF cycle (%)	10.27	22.33	**<0.001**
Male infertility (%)	50.78	42.52	**0.01**
Idiopathic female infertility (%)	44.0	21.92	**<0.001**
Ovulation disorders (%)	5.50	37.77	**<0.001**
Tubal infertility (%)	27.10	23.68	NS
No. of blastocysts transferred	1.29±0.45	1.27±0.50	**<0.001**
ET of optimal blastocysts (%)	34.62	37.31	NS
Endometrial thickness (mm)	10.15±2.18	9.85±2.15	**<0.02**
Difficult ET (%)	5.67	5.21	NS
Positive ß- hCG rate (%)	42.66	45.83	NS
Biochemical pregnancy (%)	**10.34**	**19.83**	**<0.001**
Ectopic pregnancy rate (%)	1.42	1.24	NS
Implantation rate (%)	33.00	31.85	NS
Clinical pregnancy rate (%)	37.00	36.62	NS
Clinical abortion rate (%)	**13.69**	**27.32**	**<0.001**
Live birth rate (%)	**32.82**	**26.70**	**0.01**

IVF – in vitro fertilization, ET – embryotransfer

A logistic regression model was used to identify factors that were significantly correlated with the live birth and clinical spontaneous abortion rates. The method used to prepare the endometrium (OR, 2.26; 95% CI, 1.39–3.68) and maternal age (OR, 1.07; 95% CI, 1.02–1.13) correlated with the clinical spontaneous abortion rate independent of other factors. Endometrial thickness, number of blastocysts transferred, cause of infertility, cycle irregularity, assisted hatching and previous birth after fresh transfer were not associated with the clinical spontaneous abortion rate after a positive pregnancy test (Table 2).

Maternal age (OR, 0.94; 95% CI, 0.91 –0.98), transfer of morphologically-optimal blastocysts (OR, 1.72; 95% CI, 1.28 –2.31), and the method used to prepare the endometrium (OR, 0.63; 95% CI, 0.45 –0.88) were important independent prognostic factors of live births. Male infertility, a previous birth after fresh embryo transfer, number of blastocysts transferred, FET of blastocysts frozen on day 6, and endometrial thickness were not associated with live births (Table 3).

Table 2: *Logistic regression model analysis with factors associated with clinical spontaneous abortion after FET.*

Independent variable	Univariate regression		Multivariate regression	
	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)
Age	0.009	1.06 (1.01-1.12)	**0.004**	**1.07 (1.02–1.13)**
Cycles with ET optimal blastocysts	0.047	1.50 (1.00-2.25)	0.21	0.76 (0.49–1.17)
ET of blastocysts frozen on day 6	0.019	1.66 (1.09-2.53)	0.054	1.55 (0.99–2.43)
Artificial cycle	<0.001	2.37 (1.58-3.56)	0.001	**2.26 (1.39–3.68)**
Ovulation disorders (%)	0.005	1.95 (1.23-3.08)	**0.35**	**2.26 (1.39–3.68)**

Coefficient = 5.15, Final loss: 304,20 Chi2(5)=33,84 p<0.001

Table 3: *Logistic regression model analysis with factors associated with live birth rate after FET.*

Independent variable	Univariate regression		Multivariate regression	
	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)
Age (years)	<0.001	0.95 (0.93-0.97)	**< 0.001**	**0.94 (0.91–0.99)**
Male infertility	0.027	1.25 (1.03-1.53)	0.25	1.18 (0.89–1.54)
Prior birth after fresh ET	0.010	1.42 (1.08-1.87)	0.15	1.28 (0.92–1.7)
No. of blastocysts tranferred	0.021	1.28 (1.04-1.57)	0.06	1.33 (0.99–1.78)
ET of optimal blastocysts	<0.001	1.79 (1.47-2.20)	**< 0.001**	**1.72 (1.28–2.31)**
ET of blastocysts frotzen on day 6	<0.001	0.67 (0.54-0.85)	0.06	0.75 (0.55–1.02)
Endometrial thickness	0.007	1.07 (1.02-1.13)	0.25	1.04 (0.97–1.1)
Artificial cycle	0.009	0.74 (0.59-0.93)	**0.006**	**0.63 (0.45–0.88)**

Coefficient = 2.13, Final loss: 612,40 Chi2(8)=49,93 p<0.001

DISCUSSION

The NC and AC protocols and variants of the NC and AC protocols are frequently used for endometrial preparation of FET. The main advantages of NC protocols are the avoidance of multiple medications, patient convenience, low cost, and simplicity; however, NC protocols are less predictable and only suitable for patients with regular menstrual cycles. While AC protocols offer greater control and flexibility with respect to the timing of transfer, have a low cancellation rate, and are appropriate for patients with irregular menstrual cycles, AC protocols are more intensive and expensive (2,13).

We showed that the NC and AC protocols had comparable positive pregnancy test, implantation, and clinical pregnancy rates; however, the NC protocol group had a significantly higher live birth rate, a significantly lower biochemical pregnancy rate, and a significantly lower clinical spontaneous abortion rate. The present findings are in agreement with several studies in which better reproductive outcomes were reported for the NC protocol compared to the AC protocols for FET.

Givens et al. retrospectively analysed 1677 FET cycles and found not only a significantly higher clinical pregnancy rate, but also a significantly higher pregnancy loss rate in programmed FET cycles. PCOS was the most common diagnosis in this group of patients, which was attributed to higher pregnancy loss (14).

It is known that patients with PCOS are at risk of first trimester loss. Indeed, there are many underlying mechanisms which have been proposed for the increased risk, although some authors have not confirmed a pivotal role for PCOS in spontaneous abortion after FET. In a retrospective analysis of > 4000 FET cycles, Tomas et al. observed a significantly higher pregnancy loss rate in the AC group and could not confirm that irregular cycles were a significant risk factor (9). Although there was a significantly higher rate of patients with irregular cycles in the AC group, Tomas et al. reported that the protocol used for FET was the only factor influencing pregnancy loss, independent of other factors (9, 15–17).

In the current study, logistic regression analysis revealed that the protocol used for FET and maternal age at the time of ET were the main factors influencing the clinical spontaneous abortion rate, independent of other factors. Thus, irregular cycles were not confirmed to be an independent factor responsible for pregnancy loss after FET using the AC protocol.

Similar results regarding the pregnancy loss rate in patients with regular cycles using the AC protocol for FET were observed by Cerrillo et al. (8). In a prospective, observational cohort study that compared the AC, NC, and modified NC with hCG ovulation triggering protocols, significantly higher pregnancy loss and insignificantly lower live birth rates in AC cycles occurred in patients with normal menstrual cycles (8). A possible explanation for a higher pregnancy loss in AC cycles could be a suboptimal estrogen environment or a suboptimal ratio between progesterone and estradiol, as described by Morozov et al. (10). Hormonal substitution for endometrial preparation of FET could increase the risk of pregnancy loss 1.7-fold in comparison to FET in NC protocols and fresh IVF/ICSI cycles, and this effect is independent of the maternal BMI (18). Hormonal preparation for FET was also shown to affect the probability of live births. The increased risk for pregnancy loss with impaired endometrial function was thought to be associated with the hormonal environment in fresh IVF/ICSI cycles and spontaneous ovulatory cycle protocols for FET were thought to have better endometrial function than hormonally-substituted cycles for FET (11, 18).

In our study endometrial thickness was not correlated with pregnancy loss, as shown by logistic regression analysis. We found a significantly thicker endometrium in patients undergoing the NC protocol, but the absolute mean difference in the thickness of endometrium was small (0.3 mm) and we believe that this finding was not one of the reasons for the lower pregnancy loss rate in the NC protocol.

Another possible reason for the higher pregnancy loss in AC cycles was described in the meta-analysis of Yarali et al. (6). In an analysis of seven retrospective studies, a significantly lower pregnancy rate occurred in the AC protocol without gonadotrophin-releasing hormone (GnRH) suppression compared to true NC protocols for FET. This inferior pregnancy outcome in AC cycles might be due to the escape from pituitary suppression, which is encountered in 1.9%–7.4% of

such cycles. In only two of seven studies endocrine and ultrasound monitoring was performed, which might have contributed to the inferior pregnancy outcome (6).

In our study, possible escapes from pituitary suppression were controlled with ultrasound examination. In cases involving follicle growth during hormonal therapy, the patients were excluded and therapy was discontinued. Therefore, we believe that the absence of GnRH agonist use did not contribute to a poor outcome in the AC protocol.

In our patients who underwent the AC protocol, there was a significantly higher percentage of woman without embryotransfer in fresh previous IVF/ICSI cycles (mainly due to a freeze-all strategy after GnRH agonist triggering). These were women with a good prognosis for pregnancy, all of whom had a good ovarian reserve. Nevertheless, we transferred significantly more thawed blastocysts in the AC group, and observed higher pregnancy loss and lower live birth rates with the AC protocol for FET. The method used for endometrium preparation was also shown to be an independent predictor for the live birth rate in our study. It appears that preparation of the endometrium with exogenous estrogen and gestagen could lead to impaired endometrial function and create suboptimal conditions for placentation.

Our study is one of the few studies reporting a live birth rate after FET. In a meta-analysis conducted by Yarali et al., only three studies reported differences in the live birth rate between true NC and AC protocols without GnRH suppression, which were non-significant (6). A meta-analysis conducted by Groenewoud et al. included five such studies and reported similar results with no significant differences in the live birth rate between the true NC and AC protocols for FET (7). The latest Cochrane review, which analysed RCTs only, did not include any studies which compared the live birth rate between true NC with AC protocols without pituitary suppression (19).

A limitation of our study was the retrospective nature of the study and disproportionate number of NC and AC protocols, which is a result of every day clinical practice at our center.

CONCLUSION

In conclusion, our analysis of 1865 FET cycles performed in 3 years at our center revealed significantly lower clinical spontaneous abortion, comparable pregnancy, and significantly higher live birth rates in the NC protocol compared to the AC protocol for FET. Our results indicated better cycle outcomes and many advantages, thus we suggest using the NC cycle protocol for FET as the first option for patients with regular menstrual cycles; however, further research should focus on an optimization of appropriate endometrial preparation for FET.

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