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Full Length Research Paper

Comparative Study between Natural and Artificial Cycles in Frozen Thawed Embryos

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Abstract
The first successful pregnancy following frozen-thawed embryo transfer (FET) was reported in 1983 by Trounson and Mohr. Subsequently freezing of embryos has become an integral part of assisted reproductive technology (ART) programmes. Pregnancy rates following FET treatment have always been found to be less than following embryo transfer using fresh embryos. Nevertheless, FET increases the cumulative pregnancy rate, reduces the cost, is simple to undertake and can be done in a shorter time period compared to repeated fresh cycles. Different protocols for preparation of the endometrium for FET have been described: spontaneous ovulatory cycles (natural cycle), cycles in which the endometrium is artificially prepared by estrogen and progesterone hormones (artificial cycle), and cycles in which ovulation is induced by drugs (ovulation induction cycle).The window of implantation starts shortly after ovulation, If an embryo is transferred within this window, chances of pregnancy are greater. A recent Cochrane systematic review on treatment regimes in FET concluded that current evidence does not demonstrate a significant difference in pregnancy rates between these methods of endometrial preparation in regular ovulatory cycles. However, it should be individualized, and many substances which improve the endometrial receptivity may be promising. One hundred and sixty women underwent frozen thawed embryo transfer at Al Ahram fertility center, in the period from April 2014 till May 2017. ***Results:*** *Both natural and artificial preparation of endometrium yielded comparable outcome regarding implantation rates, pregnancy rates and live birth rates in frozen-thawed embryo transfer. Thus, the decision for preparing endometrium naturally or artificially must be individualized according to other factors such as menses regularity, and the suitable protocol must be used.*

Key words: *ART – ICSI – FET – Endometrial preparation – Natural cycle – Artificial cycle – Endometrial receptivity – Window of implantation.*

Introduction

The first successful pregnancy following frozen-thawed embryo transfer (FET) was reported in 1983 by Trounson and Mohr. Subsequently freezing of embryos has become an integral part of assisted reproductive technology (ART) programmes. Pregnancy rates following FET treatment have always been found to be less than following embryo transfer using fresh embryos. Nevertheless, FET increases the cumulative pregnancy rate, reduces the cost, is simple to undertake and can be done in a shorter time period compared to repeated 'fresh' cycles.In vitro fertilization treatment cycles often produce more embryos than can be transferred during the fresh treatment cycle. Other causes favor postponing the embryo transfer such as ovarian hyperstimulation syndrome (Basile N, Garcia-Velasco JA.2016).FET has been carried out through different cycle regimens; spontaneous ovulatory cycles (natural cycle), cycles in which the endometrium is artificially prepared by estrogen and progesterone hormones (artificial cycle), and cycles in which ovulation is induced by drugs (ovulation induction cycle) (GhobaraTet al., 2017,Cochrane 2017).

Endometrial Receptivity

Endometrial receptivity is defined as the window of time when the uterine environment is conductive to blastocyst acceptance and subsequent implantation. Successful implantation needs a properly developed blastocyst, a receptive endometrium, and series of molecular interactions. In humans, 75% of failed pregnancies are secondary to implantation failure, therefore it is essential to understand basic molecular interactions involved in the process. Under the influence of estradiol, endometrium proliferates and reaches a critical thickness to support implantation. After ovulation, the endometrium differentiates in response to progesterone and becomes receptive to the newly hatched blastocyst. Implantation occurs around 6 days post ovulation, ranging between 6 and 12 days.Assessment of endometrial receptivity includes *(1) Biophysical (ultrasound) parameters*like *endometrial thickness*, which is the most common easy noninvasive method. It has been proposed that an endometrial thickness below a minimum value of 7–8 mm is of *negative predictive value* when estimating endometrial receptivity and likeliness for implantation and pregnancy to occur. However, the endometrial thickness more than this minimum is not of *positive predictive value* for endometrial receptivity

and consequently cannot predict implantation. *Endometrial pattern (echogenicity),endometrial volume and endometrial blood flow* are another parameter.*(2) Morphological (histological) features*like presence of pinopodes in the site of implantation.*(3)Functional parameters*: like hormones and cytokines(Integrins – LIF – Interleukins – CSF- IGF).*(5) Endometrial ReceptivityArray,* which classify the endometrium into receptive and non- receptive (Lessey BA., 2011).

Endometrial preparation in frozen-thawed embryo transfer

Although the increased success in FET and personalized approaches in reproductive medicine, the best- approach for endometrial preparation for FET is still debatable.(Casper RF, Yanushpolsky EH., 2016). Various cycle protocols are used for the preparation of the endometrium in an FET cycle. The first is spontaneous ovulatory cycle (*natural cycle* - NC-FET), either with or without triggering of ovulation with HCG (modified natural cycle "mNC" and true natural cycle "tNC", respectively).The second procedure involves the artificial preparation of the endometrium through the administration of exogenous estrogen and progesterone (*artificial cycle* - *AC*-FET), which can be performed with or without the association of a gonadotropin-releasing hormone agonist. In the third procedure, the cycle is stimulated (*ovulation induction cycle*) either by gonadotropins or aromatase inhibitors and ovulation is induced by recombinant-human chorionic gonadotropin (r-Hcg) or hCG(GhobaraTet al., 2017 ,Cochrane 2017).

Subjects and methods

One hundred and sixty women underwent frozen thawed embryo transfer at Al Ahram fertility center, in the period from April 2014 till May 2017. They were selected according to the following:

Inclusion criteria: Age of < 40 years - Primary or secondary infertility - Undergoing FET irrespective of the indication of embryo freezing (supernumerary embryos obtained following fresh embryo transfer, elective embryo freezing as a result of ovarian hyper stimulation syndrome or for other reasons) - Regular menstrual cycles.

Exclusion criteria were: Age > 40 years- Irregularan ovulatory cycle - Associated uterine anomalies or fibroids.The selected women were classified into two comparable groups undergoing ICSI and freezing of embryos for subsequent transfer: Group A (had FET in a natural cycle - NC-FET) and Group B (had FET in an artificial cycle - AC-FET).

Group A (NC-FET)

Folliculometry started on day 10 or 11 of the cycle, the endometrial thickness and mean diameter of the dominant follicle were evaluated. When the endometrium was 8 mm or more and the diameter of the dominant follicle was 16-20 mm, a blood sample was taken measuring LH level and ovulation was induced using human chorionic gonadotropin (HCG) injection. Thawing and transferring was commenced 3 to 5 days after HCG according to the stage of cryopreserved embryos. Number of transferred embryos was from two to four. If during ultrasonic evaluation no follicle was visible ovulation mostly have occurred, so, no thawing or transferring take place, this cycle is regarded a drop-out.

Group B (AC-FET)

From day two of the cycle, patients commenced oral estradiol valerate 2 mg three times daily. A transvaginal ultrasound was performed on day 10 or 11 of the cycle. If no leading follicle (≥ 14 mm) was present and the endometrial thickness is ≥ 8 mm, progesterone 400 mg vaginal pessaries are added. Thawing and transferring is commenced 5 to 7 days later according to the stage of cryopreserved embryos.If the endometrial thickness is less than 8 mm, the estradiol valerate dose was raised to 2 mg 4 times daily and the endometrium was checked once again. When the endometrial thickness is > 8 mm, progesterone is added.If a follicle is visible during ultrasound scan, serum LH and progesterone levels were determined. If these were raised, luteinization of the follicle was considered to have taken place and because of the associated diminished conception rates, thawing and transferring was not performed. If serum levels were below the above mentioned levels thawing and transferring was performed. A maximum of three embryos were transferred.

Statistical methodology

The collected data were organized tabulated and statistically analyzed, graphically represented by Microsoft office excel 2010 and statistical package for social sciences (SPSS) version 18; running on personal computer with windows 7 operating system. The numerical (quantitative) data was presented as arithmetic mean and standard deviation (SD), while categorical (qualitative) data were presented as frequency (count) and percentage distribution. For comparison between two means, the independent samples student (t) test was used, while for comparison between categorical variables, Chi square test was used. The difference was considered significant if P value< 0.05.

Results

The comparison between the two groups, NC-FET and AC-FET, had shown the following results:

Regarding female age, ranged from 25 to 36k years, and there was no statistically significant difference between Natural and artificial cycle groups as regard female age (it was 31.46±2.14 and 31.10±2.38 years in natural and artificial groups respectively).

Regarding weight ranged from 57 to 70kg, while height ranged from 160-169 cm and BMI ranged from 22.27 to 26.35 kg/m^2; and there was statistically non-significant difference between studied groups. Majority (83.1%) of females had primary infertility, while secondary infertility was reported in 16.9%; and there was no significant difference between natural and artificial groups (primary infertility was reported in 78.8% vs87.5% respectively, p >0.05). Regarding infertility duration, it ranged from 2 to 12

year, and there was non-significant difference between both groups (it was 5.26±1.90 and 5.48±1.68 in natural and artificial groups respectively). Regarding the cause of infertility, it was male factor in 54.4%, female factor in 20.0%, male and female factor in 14.4% and unexplained in 11.3%; and there was no significant difference between both groups.

Regarding endometrial thickness, it ranged from 8 to 15 mm and there was statistically significant increase of endometrial thickness in natural when compared to artificial group (11.82±1.16 vs 10.92±0.82 mm respectively).

	Mean	S D	Minimum	Maximum	T	p
Natural	11.12	1.16	8.00	15.00	***5.63***	***<0.001****
Artificial	10.22	0.82	8.00	12.00		
Total	11.37	1.10	8.00	15.00		

The mean follicle size ranged from 17 to 22; and there was no significant difference between natural and artificial groups (19.58±1.33 vs 19.27±1.35 respectively). Regarding the number of previous failed IVF cycles ranged from 1 to 6 cycles and there was statistically non-significant difference between natural and artificial groups (1.88±0.94 vs 2.02±0.72 respectively). Regarding percentage of implantation rate in relation to number of cycles, it ranged from 8.33% to 50.0% and there was statistically non-significant decrease in natural when compared to artificial group (27.36±7.49 vs 29.18±9.63 respectively). As regard to percentage of live birth rate in relation to number of cycles, it ranged from 0 to 50.0%; the total mean percentage of live birth rate was 23.31%; and there was non-significant increase in natural when compared to artificial group (24.38±13.68 vs 22.25±10.76 respectively). The clinical pregnancy rate in relation to transferred embryo ranged from 14.29 to 50.0% and there was non-significant increase in natural when compared to artificial group (27.69±9.12 vs 25.45±7.74 respectively).

	Mean	S. D	Min.	Max.	T	P
Natural	27.69	9.12	14.29	50.00	1.66	0.09
Artificial	25.45	7.74	16.67	50.00		
Total	26.57	8.51	14.29	50.00		

Discussion

In the present study, the researcher intended to evaluate the outcome of natural and artificial preparation cycles for frozen thawed embryo transfer. The study included 160 females scheduled for frozen thawed embryo transfer. They were divided randomly into two equal groups; the first included females with natural preparation of endometrium and the second included females with artificial preparation. In natural cycle, the researcher of the present work as well as Weissman et al. (2011) used human chronic gonadotropin for triggering of ovulation. Weissman et al. (2011) suggested that triggering of ovulation with HCG is superior in terms of implantation, pregnancy and live birth rates in patient preparation for NC-FET. Since ovulation triggering by HCG significantly reduces the number of monitoring that are necessary to determine the day of FET, this approach may be superior in terms of patient convenience and cost-effectiveness of the cycle. For many years, frozen thawed embryo transfer has been successfully performed in natural cycles following spontaneous ovulation. As the NC protocol is relatively simple and avoids the need for prolonged hormonal supplementation in early pregnancy, it is preferred by many patients and clinics. However, problems often occur when this protocol is used, timing of transfer requires accurate determination of ovulation, Daily hormone determinations and ultrasonographic monitoring are required, which make it inconvenient for patients. Natural cycle transfers have a 5% cancellation rate because of the inability to determine the exact time of ovulation (Weissman et al., 2011). The present work used oral estrogen in artificial group that could be supported or not by vaginal pessaries according to ultrasonography examination. Some clinicians perform programmed cycles with the use of oral, transdermal or intravaginal estrogen as opposed to intramuscular administration (Ghobara and Vandekerckhove, 2008). If GnRH agonists are not used, significant concentrations of estradiol must be administered to block the endogenous follicular recruitment.

In the present work, endometrial thickness ranged from 8 to 15 mm and there was statistically significant increase of endometrial thickness in natural when compared to artificial group (11.82±1.16 vs 10.92±0.82 mm respectively). Although differences reached statistical significance, they are probably of no clinical significance, because for both groups, endometrial thickness is in the range considered favorable for the achievement of implantation and pregnancy. Indeed, pregnancy and implantation were found to be comparable for both groups, and so, these differences most probably arise from the relatively small size of this study. Results of the present work revealed that, both natural and artificial preparation were comparable as regard to implantation, pregnancy and live birth rates when related to number of cycles or number of transferred embryos. Results of the present study are comparable to

those reported by Mounce et al. (2015), who could not find any significant difference in FET cycle outcomes between patients undergoing the natural cycle FET as compared to artificial hormone replacement FET. In addition, Wright et al. (2006) reported that, their results demonstrate comparable implantation and pregnancy rates between artificial and natural cycles for endometrial preparation prior to transfer of frozen–thawed embryos. Furthermore, Gelbaya et al. (2006) evaluated the outcome of cryopreserved-thawed embryo transfer in natural or artificial cycles in women with regular menstrual cycles. The study results show no significant clinical difference in pregnancy rates and live birth rates between patients undergoing FET during natural cycles or during artificial cycles. In line with the present study,Givens (2009) concluded that, there is no significant difference in live birth rates for FET in natural and artificial cycles, whether using own-egg-derived embryos or donor-egg-derived embryos. So, facilities that are able to go far natural cycles will benefit their patients by offering the option of natural-cycle FET.

On the other hand, results of the present work disagreed with Levron et al. (2014) who found a better outcome using the natural cycle FET compared to artificial cycle. FET, and that may be explained by the improvement in laboratory methodology in the later years and the use of vitrified embryos only compared to slow freezing. Thus, their results are in contradiction to results of the present work as regard to clinical pregnancy rate and in agreement with the present work as regard to live birth rate. In the recent study by Orvieto et al. (2016), different luteal phase support strategies have been used during two-time periods; in the tNC arm, during 2012–2014, micronized progesterone soft gel vaginal capsule at a dose of 3 × 200 mg or vaginal bio adhesive gel at 90 mg (8 %) was employed whereas during 2014–2015, in addition to vaginal progesterone, rhCG 250 mcg and 0.1 mg triptorelin were administered in luteal phase. Hence, the time periods employing different LPS strategies have been enrolled as two separate data in the forest plot analysis.

In two studies, no luteal phase support was administered in the tNC arm; there was a statistically significant difference in clinical pregnancy rate favoring tNC against AC without suppression (OR 1.63, 95 % CI 1.24–2.14)(Levron et al., 2014). Luteal phase support was given in other studies; the ORs for clinical pregnancy rates and live birth rates were not significantly different (OR 1.32 (95 % CI 0.90–1.93) and 1.47 (95 % CI 0.72–3.03) (Orvieto et al., 2016). The same authors compared modified natural cycles with artificial cycles and reported that, there was no statistically significant difference between these two protocols of endometrial preparation regarding clinical pregnancy rates (OR 1.11, 95 % CI 0.88–1.41; six studies) and live birth rates (OR 1.14, 95 % CI 0.96–1.37; four studies). They finally reported that, they failed to observe a superiority of a particular protocol to prepare endometrium for frozen thawed embryo transfer cycles when different protocols were compared (Yarali et al., 2016). In summary, comparable outcome (implantation rates, pregnancy rates and live birth rates) in natural and artificial cycle for endometrial preparation prior to implantation of frozen–thawed embryos have been demonstrated in the present study. Thus, researcher could said that, patients eligible to undergo artificial-cycle FET included females with irregular or absent cycles; however, women with regular cycles may be considered for the natural-cycle protocol or artificial cycle protocol. However, each patient must be individualized and the suitable protocol must be used.

Summary & Conclusion

Synchronization between the endometrial development and the embryo is critical for a successful FET program. So, good preparation is required for success of FET (Basile N, Garcia-Velasco JA.2016). FET has been carried out through different cycle regimens; spontaneous ovulatory cycles (natural cycle), cycles in which ovulation is induced by drugs (ovulation induction cycle) and cycles in which the endometrium is artificially prepared by estrogen and progesterone hormones (artificial cycle).Endometrial thickness was significantly increased in natural when compared to artificial groups, while the difference regarding follicular size was statistically non-significant (Cochrane, 2017). Both natural and artificial preparation of endometrium yielded comparable outcome regarding implantation rates, pregnancy rates and live birth rates in Frozen-thawed embryo transfer. Thus, the decision for preparing endometrium naturally or artificially must be individualized according to other factors such as menses regularity, and the suitable protocol must be used.

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