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The Success of True Natural Cycle in Patients Undergoing Frozen Thawed Embryo Transfer

Gerçek Doğal Siklus ile Yapılan Donma Çözme Embriyo Transferinin Başarısı

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ABSTRACT

Objective: To analyze the success rate of true natural cycle in patients undergoing frozen thawed embryo transfers. **Materials and Methods:** This retrospective analysis included ovulatory 23 women undergoing natural frozen embryo transfer cycles between January 2022 and July 2023. In true natural frozen embryo transfer cycles hCG trigger and luteal phase supplementation were not utilized. The mean number of embryos transferred, the mean menstrual day of embryo transfer, mean peak estradiol level on the day of LH surge (>17iu/ml), and mean serum progesterone levels on embryo transfer day were recorded. The main outcome measures included implantation and clinical pregnancy rates. **Results:** Mean number of embryos transferred was 1.7, the mean implantation and clinical pregnancy rates were 55.8% and 69,5% respectively. Among the patients, single embryo transfer was performed in 12 and double embryo transfer was performed in 11 subjects. With regard to preimplantation genetic analysis, 8 patients received single euploid embryo transfer and 7/8 (87.5%) conceived. The mean menstrual day of transfer was 19,38±1.67 days (range; 17-23 days), mean peak estradiol level on the day of LH surge (>17ıu/ml) was 307,5±102,5 pg/ml (range; 178-492 pg/ml), and the mean serum progesterone on the day of ET was 24,33±16,4 ng/ml (range; 14.5-41). **Conclusions:** True natural cycle is a promising physiological alternative for patients undergoing frozen thawed embryo transfer cycles. Despite the numbers are low, the success rate seems to be higher in patients undergoing euploid embryo transfer.

Keywords: In vitro ferrilization, embryo transfer, cryopreservation

ÖZET

Amaç: Donma çözme embriyo transfer sikluslarında gerçek doğal siklusun başarısını araştırmak amaçlanmıştır. **Gereç ve Yöntemler:** Bu retrospektif kohort analizde Ocak 2022 ile Temmuz 2023 tarihleri arasında gerçek doğal siklus ile yapılan donma çözme embriyo transferi yapılan 23 vaka değerlendirilmiştir. Gerçek doğal siklus embriyo transferinde hCG tetikleme ve luteal faz desteği kullanılmamıştır. Çalışmadaki ana amaçlar, implantasyon ve klinik gebelik oranları olarak belirlenmiştir. Ortalama transfer edilen embriyo sayısı, ortalama transfer edilen menstrüel siklus günü, LH pik günü (>17iu/ml) ortalama en yüksek estradiol düzeyi, ve embriyo transferi günü ortalama serum progesteron düzeyleri değerlendirilmiştir. **Bulgular:** Ortalama transfer edilen embriyo sayısı 1.7, ortalana implantasyon ve klinik gebelik oranları sırası ile %55.8 ve % 69,5 olarak saptanmıştır. Hastalardan 12'sine tek embriyo, 11'ine ise 2 embriyo transferi yapılmıştır. Preimplantasyon genetik analiz yapılan 8 hastaya tek euploid embriyo transferi yapılmıştır, ve bu hastalardan 7'si (%87.5) gebe kalmıştır. Ortalama transfer edilen menstrüel siklus günü 19,38±1.67 (17-23), LH pik günü ortalama serum estradiol 307,5±102,5 pg/ml (78-492 pg/ml), ve embriyo transfer günü ortalama serum progesteron düzeyi ise 24,33±16,4 ng/ml (14.5-41) olarak saptanmıştır. **Sonuç:** Gerçek doğal siklus ile yapılan donma çözme embriyo transferi umut vaat eden başarılı bir alternatif olarak karşımıza çıkmaktadır. Vaka sayıları az olmakla birlikte euploid embriyo transferi yapılan hastalarda gebelik oranları artmış gözükmektedir

Anahtar Kelime: İn vitro fertilizasyon, embriyo transfer, kriyoprezervasyon

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Since the first introduction of IVF and birth of Louise Brown in 1978, various developments has been achieved in the field of ART such as; improvements in cryopreservation technologies, integration of preimplantation genetic studies for genetic diseases or aneuploidy screening to improve IVF success, and perfections in recombinant gonadotropin technologies.[1] Besides these paramount improvements, pregnancy success has stabilized during the last decades without a further increase, and various novel treatment modalities and technological innovations have been practiced finding a way around the obstacle.

In modern medicine, one of the integral parts defining the success of a procedure is patient friendly treatment modalities that also incorporates short-term and long-term patient safety. In recent years it has been constantly demonstrated that, cryopreservation of embryos not only is associated with increased IVF success in some selected patients, but also avoids the risk of ovarian hyperstimulation syndrome. Among the other advantages of embryo freezing are providing a more physiological endometrium, lower ectopic pregnancy rates, lower low birth weight infant rates, and lower risk of hypertensive disorders of pregnancy and enabling preimplantation genetic analysis.

With the advent of efficient freezing strategies and reassuring safety data, the use of frozen embryo transfer has progressively increased.[2] Various endometrial replacement protocols were defined for frozen embryo transfer (FET) cycles such as; hormone replacement FET, true natural cycle FET, modified natural cycle FET in which hCG trigger was utilized, modified or true natural cycle FET followed by luteal phase support and stimulated cycle FET. There has been a great effort to find the optimal FET protocol, though individualization is of critical importance. Due to ease of monitoring and having the opportunity to avoid weekend transfer many clinics favor hormone replacement FET cycles. According to a study enrolling 84 IVF clinics in the UK, clinics was asked to complete an online survey regarding their clinic's first-line protocols for FET.[3] Sixty-five clinics responded, accounting for approximately 24,419 FET cycles. In ovulatory women, 69% of clinics preferred hormone replacement FET cycles, 26% natural cycle and 5% modified natural cycle FET cycles. In another multicenter retrospective cohort study conducted in France, the use of artificial cycles represented 56% of all frozen thawed endometrial preparation regimens.[4]

However, recent reports consistently advocate the use of natural cycle FET in ovulatory women especially due to decreased pregnancy related complications, decreased pregnancy loss rates and even increased pregnancy outcomes in some reports.[4] A recent systematic review confirmed that natural cycle FET decreases the risk of adverse obstetric and neonatal outcomes compared with hormone replacement FET.[5] In this retrospective cohort study we aimed to assess the IVF outcome in patients undergoing frozen thawed embryo transfer in a true natural cycle.

MATERIAL AND METHODS

Patient datasheets of 23 ovulatory women who underwent natural frozen embryo transfer cycles between 2022 and 2023 were retrospectively reviewed. For any patient undergoing true natural frozen embryo transfer cycles, neither hCG trigger, nor luteal phase supplementation were utilized. All of the patients were regularly monitored using serum hormone measurements including LH, estradiol and progesterone and ultrasound examination every 3-5 days as required. One or two top quality (≥4AB) blastocysts were transferred according to treating physician's discretion, previous IVF failures, patient age, and number of available surplus embryos. The timing of embryo transfer was determined according to an occurring LH surge (>17 IU/mL) plus 6-7 days. In general, all patients who underwent preimplantation genetic analysis received single embryo transfer.

The study was performed under the principles of Helsinki Declaration, however it was performed by reviewing the patient datasheets retrospectively, no ethical approval was provided. Demographic characteristics included age, infertility etiologies, duration of infertility, previous pregnancies, and miscarriages if any. The mean number of previous failed IVF cycles, mean number of embryos transferred, mean

menstrual day of transfer, mean peak estradiol level on the day of LH surge and the mean serum progesterone levels on the day of embryo transfer were recorded. The primary outcome measures were implantation and clinical pregnancy rates.

RESULTS

The mean age of the patients was 35,1±4,1, and the mean duration of infertility 54,2± 38,9 months (Table 1). The infertility etiologies are summarized in Table 1, endometriosis being the leading cause in 8 subjects. Among the other infertility etiologies are unexplained infertility in 4 patients, decreased ovarian reserve in 1, uterine factor in 1, and male factor in 3. Six patients underwent IVF for preimplantation genetic diagnosis and one patient had undergone IVF and embryo freezing before receiving chemotherapy for breast cancer.

Basal hormonal assessment and AMH values are presented in Table 2. The mean serum AMH of the enrolled patients was 2.82±2.2 ng/ml. The mean menstrual day of transfer was 19,38±1.67 days (range; 17-23 days), mean peak estradiol level on the day of LH surge (>17 iu/ml) was 307,5±102,5 pg/ml (range; 178-492 pg/ml) and mean serum progesterone on the day of ET was 24,33±16,4 ng/ml (range; 14.5-41) (Table 3). None of the patients received a progesterone rescue. Of the patients only 6 underwent their first IVF cycle. The mean number of embryos transferred was 1.7, and the implantation and clinical pregnancy rates were 55.8% and 69,5% respectively. Among the patients, single embryo transfer was performed in 12 and double embryo transfer was performed in 11 subjects. With respect to previous miscarriages, 7 patients had 1, 3 of the subjects has 2, while 13 did not have a history of miscarriages.[3] Among the patients undergoing reimplantation genetic analysis, 8 received single euploid embryo transfer and 7/8 (87.5%) conceived.

TABLE 1: Demographic characteristics of the patients undergoing frozen thawed embryo transfer in true natural cycles.

Variables	Value (n=23)
Female Age (mean±SD)	35,1±4,1
Male Age (mean±SD)	38,2±4,9
Duration of infertility (months±SD)	54,21± 40,9
Infertility etiology female diagnosis (n, %)	
Unexplained	4 (17,4)
Poor ovarian reserve	1 (4.3)
Endometriosis	8 (34.7)
Uterine factor	1 (4,3)
Male factor	3 (13.0)
Other (Genetic factors, recurrent pregnancy loss, breast cancer)	6 (26,1)
Previous failed transfers (n,%)	
0	6 (26,0)
1	4 (17,4)
2	8 (34,7)
>2	5 (21,7)
Gravidity (n, %)	
0	11 (47,8)
1	7 (30,4)
>=2	5 (21.7)
Prior miscarriages (n, %)	
0	13 (56,2)
1	7 (30,4)
>=2	3 (13,0)

TABLE 2: Basal hormonal assessment of the patients undergoing frozen thawed embryo transfer in true natural cycles (day 2 or day 3).

Variables	Value (n=23)
Estradiol (pg/mL)	55,2±13,8
FSH (IU/mL)	7,54±2,5
LH (IU/mL)	7,7±3,1
TSH (IU/mL)	2,53±2,1
PRL (pg/mL)	19,44±13,87
AMH (pg/mL)	2,82±2,2

TABLE 3: IVF outcome measures of the patients undergoing frozen thawed embryo transfer in true natural cycles.

Variables	Value (n=23)
Transferred embryos (n, mean±SD)	1.7±0.7
Peak serum E2 level on the day of LH peak* (pg/ml, mean±SD)	307,5±102,5
Serum P on the day of ET (ng/ml, mean±SD)	24,33±16,4
Mean day of ET (mean±SD)	19,38±1.67
Implantation rate (%)	55.8
Clinical pregnancy rate (%)	69,5

E2: estradiol P: progesterone ET: embryo transfer
*LH peak is defined LH>17IU/ml

DISCUSSION

In this small cohort study, even though not comparative, we demonstrated that frozen thawed embryo re-

placement in a true natural cycle was associated with quite favorable implantation and pregnancy rates. The increased pregnancy rates in this cohort may not only be due to the protocol used per se, but also as a result of increased utilization of preimplantation genetic studies in the group and transferring only top-quality blastocysts.

In the modern era of ART thanks to the more liberal use of cryopreservation technologies and improved success rates, FET cycles have been increasingly utilized by many IVF clinics worldwide. From a physiological standpoint, it appears that natural cycle is superior to programmed cycles due to providing a more suitable environment which is free from the detrimental effects of supraphysiological hormone levels leading to embryo-endometrium asynchrony. In a recent well designed systematic review, the risk of hypertensive disorders of pregnancy (OR;1.9), placenta previa (OR;1.27), placenta accrete (OR: 6.29) preterm birth (OR; 1.63), preterm premature rupture of membranes (OR;1.84), postpartum hemorrhage (OR 2.53), and macrosomia (OR 1.18) were all found higher in hormone replacement cycles compared to natural FET cycles FET.[6] Furthermore, in some reports the rate of early pregnancy loss in artificially prepared cycles is also reported higher compared to natural cycle FET.[6-8]

Among one of the unclarified issues is whether progesterone supplementation is beneficial in natural cycle FET. In a recent prospective randomized study, supplementation with progesterone significantly increased live birth rates in natural cycle frozen-thawed embryo transfer.[9] In the progesterone supplemented group, 34.2% had a live birth, compared to 24.1% in the control group (OR: 1.635). Some studies argued that the pregnancy rates are decreased when serum progesterone levels are found lower on the day of ET. In a systematic review enrolling 2927 patients the salvage effect of rescue progesterone implementation was analyzed in patients with low serum progesterone.[10] There was no difference between patients with low serum progesterone receiving the rescue dose and those with adequate serum progesterone levels in terms of ongoing pregnancy (OR; 0.98), live birth and (OR; 0.92) and miscarriage rates (OR; 0.98).[10] In our cohort, luteal phase supplementation was not utilized in any of the patients, since serum progesterone on ET day was >10ng/ml in all patients. Although there is no consensus regarding the optimal luteal phase progesterone values in FET cycles, a range between 22 and 31 ng/ml has been proposed by some authors.[11] The mean serum progesterone on the day of ET was 24,3 in our study which fell within the desired values without luteal phase supplementation.

Another questioned issue in performing natural cycle FET is whether there is a difference in pregnancy rates between spontaneous and triggered ovulation. Some retrospective studies failed to demonstrate a difference in pregnancy rates between those having spontaneous ovulation and hCG triggered ovulation.[12,13] On the contrary, a large retrospective study demonstrated a significant difference in clinical pregnancy rates in favor of the true natural cycle FET without luteal phase support compared to those undergoing modified natural cycle FET with luteal phase support.[14] As an important remark, subjects receiving hCG trigger despite an occurring LH surge was excluded from the analysis. None of our patients received hCG trigger or luteal phase supplementation in our current cohort. Further studies are definitely required to find the optimal regimen for increased success in natural cycle FET. Another highly debated and still unresolved issue is the definition of LH cut-off which ranges between 15 and 20IU/ml in the studies for determining the timing of embryo transfer.[15] Even though not confirmed in later studies, some authors reported decreased pregnancy rates following hCG trigger in patients with already occurred an LH surge.[16,17]

CONCLUSION

Performing frozen thawed embryo transfer in a true natural cycle is a promising alternative with favorable pregnancy rates. Whether it is out of the scope of the present study, decreased pregnancy complications with increased safety profile makes FET a superior alternative to artificial cycles in some selected group of patients.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

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