Single versus Double Frozen Embryo Transfer in PCOS Patients

Walid M Elnagar MD, Khaled F Helal MD
Department of Obstetrics & Gynecology, Faculty of Medicine, Zagazig University

Objectives : The study targets to evaluate the outcomes of polycystic ovarian syndrome (PCOS) infertile women undergoing intracytoplasmic sperm injection (ICSI) using single (SBT) versus double blastocyst transfer (DBT) strategy.

Patients & Methods : 271 infertile PCOS women diagnosed according to the Rotterdam criteria, aged 20-35 years, had body mass index (BMI) of <35 kg/m2 and mild hyperandrogenemia were randomly categorized into SBT and DBT groups and underwent elective frozen-thawed autologous day-5 good-quality (5A) BT. Study outcomes included the live birth rate (LBR), and the incidence of pregnancy-related maternal, fetal and neonatal complication.

Results: Chemical and clinical pregnancy rates were non-significantly higher with DBT. However, the incidence of early pregnancy loss and multiple pregnancy was significantly higher, while the incidence of gestational hypertension and diabetes mellitus, ectopic pregnancy, premature preterm rupture of membrane were non-significantly higher with DBT. On contrary, the incidence of preterm labor (PTL) and the need for cesarean section were significantly higher among women of DBT group. Neonatal outcomes were better with SBT with significantly lower incidence of low birth weight (BW), need for NICU admission and hospital neonatal mortality with significantly higher LBR.

Conclusion: DBT even of good quality worsens the outcomes of ICSI for PCOS women. DBT is significantly associated with small BW, high incidence of PTL, need for operative delivery and NICU admission with subsequent reduction of LBRs. Also, DBT non-significantly increased the incidence of maternal complications than SBT.

Keywords: PCOS, ICSI, Blastocyst transfer, Single blastocyst transfer, double blastocyst transfer, Live birth rate

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is condition characterized by being complex, familial and polygenetic metabolic condition [(1)] with heterogeneous involvement of several genes in the hypothalamic-pituitary-gonadal axis [(2)]. PCOS is the most incident endocrine-

Corresponding author:
Walid M Elnager,
Whitewhale1977@gmail.com,
01224252626

metabolic syndrome that is characterized by hyperandrogenemia, menstrual irregularities, and/or small cysts in one or both ovaries [3] and could be considered as the main cause of female infertility [2].

Assisted reproductive technologies (ART) were defined by the American Center for Disease Control as any fertility-related treatments where eggs or embryos are manipulated [4]. In vitro fertilization involves the transfer of fresh embryos, however, the freeze-all strategy that entails cryopreservation of all embryos to be transferred subsequently in un-stimulated cycle to guard against the negative effects of controlled ovarian stimulation on the endometrium and to minimize the risk of ovarian hyper-stimulation syndrome [5]. Intracytroplasmatic sperm injection (ICSI) is a common procedure used to improve reproductive results, even among couples without male factor infertility [6].

However, there are several points of controversy in the fields of ART traditionally, multiple embryo transfer strategy was used to increase the chance for getting a baby [7], however, multiple pregnancy is a common complication for this strategy with subsequent adverse fetal, maternal and neonatal outcomes [8]. Also, the developmental stage of embryos at time of transfer; cleavage versus blastocyst was a matter of debit, but giving the chance for in-vitro maturation to the stage of blastocyst increased the possibility of getting good quality embryo to be transferred [9]. However, the exaggeration of prolongation of the in-vitro duration appeared as a trend, but recent studies found no advantages for transfer of D7 or D6 blastocyst over D5 blastocyst transfer (BT) [10].

Objectives

The study tried to evaluate the outcomes of PCOS infertile women assigned for ICSI using single (SBT) versus double blastocyst transfer (DBT) strategy.

Design

Prospective randomized comparative study.

Setting

Gynecology & Obstetrics Department, Faculty of Medicine, Zagazig University and multiple private centers

Participants

Infertile PCOS women who attended the Infertility clinic from June 2021 till Aug 2022 were evaluated for inclusion criteria

Inclusion criteria

Infertile women fulfilling at least two of the Rotterdam criteria for diagnosis of PCOS (11, 12), aged 20-35 years, had body mass index (BMI) of less than 35 kg/m2 (13, 14), mild hirsutism on Ferriman-Gallwey (FG) visual scoring system (15, 16), their hormonal profile of serum AMH >4.5, FSH <5.85 LH>5.39 and had no previous attempts of ICSI were included in the study.

Exclusion criteria

Patients who were out of the predetermined age range and BMI, had uterine abnormalities, hydrosaplnix, recurrent miscarriage, medical diseases interfering with or being complicated by occurrence of pregnancy, and cases requiring ICSI with TESE samples, had obesity-inducing endocrinopathy, severe symptomatizing hypovitaminosis or maintained on hormonal contraception within the last 6 months before inclusion in the study, were excluded from the study.

Clinical evaluation

The enrolled patients were clinically evaluated for determination of demographic data, fertility status and obstetric history. Then, patients underwent clinical and ultrasonographic examinations, and gave fasting blood samples for routine

investigations, estimation of serum AMH, FSH, LH, testosterone and estradiol.

Ethical Consideration

The study protocol was discussed with each couple after being preliminary acceptance by the University Ethical Committee and couples accepted to participate in the study according to the randomization sequence were enrolled in the study after signing the written consent. At the end of the study, the final approval was obtained at 27-11-2022; approval number: ZU-IRB#10066/27-11-2022. The study design, protocol and the probable outcomes were registered at ClinicalTrials.gov. by the number: NCT05854810

Randomization and grouping

Patients were randomly divided into two groups (SBT & DBT) using the random block sizes of 2 and 4 by 1:1 allocation computer randomization method (Excel 2007, Microsoft, Redmond, WA, USA) to generate the sequence of patients between both groups.

Study rational

All women were assigned to receive frozen embryo that was frozen at D-5 blastocyst stage using the vitrification ultra-rapid cryopreservation as previously described (17). The blastocysts were staged according to the ASEBIR classification system that included evaluation of the internal cell mass (A-C grades), trophectoderm (A-C grades) and assessment of the degree of blastocele expansion on 2-6 scale; good quality blastocysts must be graded as AA6 on day-5 (18). Only good quality (AA6) blastocysts were selected for transfer.

Patients' preparation and BT

At 1-week before the expected date of menses, gonadotropin-releasing hormone (GnRH)-agonist therapy was started as subcutaneous injection of triptoreline (Decapeptyl, Ferring Pharmaceuticals Ltd., Wittland, Germany) in a dose of 0.1 mg. On the 2nd day of the menstrual cycle, estradiol valerate (Progynova, 2 mg, Bayer Schering Pharma, UK) was given 6-mg daily for 4 days and then dose was adjusted according to the endometrial thickness. Endometrial thickness was judged by TVU (Sonoline Prima 7.5 MHz, Siemens) in the midsagittal plane as the distance between the outer edges of the endometrial/myometrial interface on days 10 to 12. When the endometrial thickness was 8 mm, progesterone therapy 400 mg twice daily as progesterone vaginal supp (Cyclogest; Actavis Co., USA) for 5 days and BT was commenced after rapid thawing on the 6th day of start of progesterone therapy. Progesterone therapy was continued after BT for 14 days at time of chemical diagnosis of pregnancy that was assured clinically depending on detection of viable embryo with pulsating heart by US examination.

Study outcomes

1. The primary outcome is the live birth rate (LBR) after SBT and DBT.
2. The secondary outcomes include

- The chemical (CHPR) and clinical pregnancy rates (CPR) and the incidence of early pregnancy loss (EPL).
- The incidence of pregnancy-related maternal complications especially gestational diabetes mellitus (GDM) and gestation hypertension (GH), and need for cesarean section (CS).
- The incidence of adverse pregnancy outcomes including preterm and very preterm labor, premature preterm rupture of membranes, low and very low-birth weights
- The incidence of adverse neonatal outcomes as low APGAR score, need for NICU admission, duration of NICU stay and neonatal mortality rate.

Statistical analysis

Results were analyzed using paired t-test and Chi-square test (X2 test) using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) with P-value cutoff point for significance is <0.05.

Results

During the study duration 312 PCOS infertile women were evaluated; 22 women were excluded and 290 women had fulfilled the inclusion criteria and were randomly divided into two groups according to number of BT; single (SBT group) and DBT (DBT group). Unfortunately, 19 women were missed after BT and were excluded from the statistical analysis as shown in figure 1. The enrollment data of the studied women are shown in table 1.

Table 1: Enrollment data of the studied women categorized according to number of the transferred blastocysts

Data		SBT (n=136)	DBT (n=135)	P-value
Age (years)	<25	5 (3.7%)	12 (8.9%)	**0.209**
	25-30	85 (62.5%)	80 (59.3%)	
	>30	46 (33.8%)	43 (31.8%)	
	Average (±SD)	29.5 (2.9)	29 (3)	**0.163**
BMI (kg/m^2)	Overweight (<30)	23 (16.9%)	36 (26.6%)	**0.052**
	Obese (30-34.9)	113 (83.1%)	99 (73.4%)	
	Average (±SD)	31.5 (1.8)	31.4 (1.9)	**0.634**
Infertility	Primary	80 (58.8%)	86 (63.7%)	**0.409**
	Secondary	56 (41.2%)	49 (36.3%)	
Lab findings	AMH (ng/ml)	4.66 (1.65)	4.52 (1.9)	**0.513**
	FSH (mIU/ml)	5.2 (1.3)	5.15 (1.4)	**0.758**
	LH (IU/ml)	5.5 (1.6)	5.8 (1.7)	**0.133**
	Testosterone (ng/ml)	1.035 (0.15)	1.03 (0.14)	**0.782**

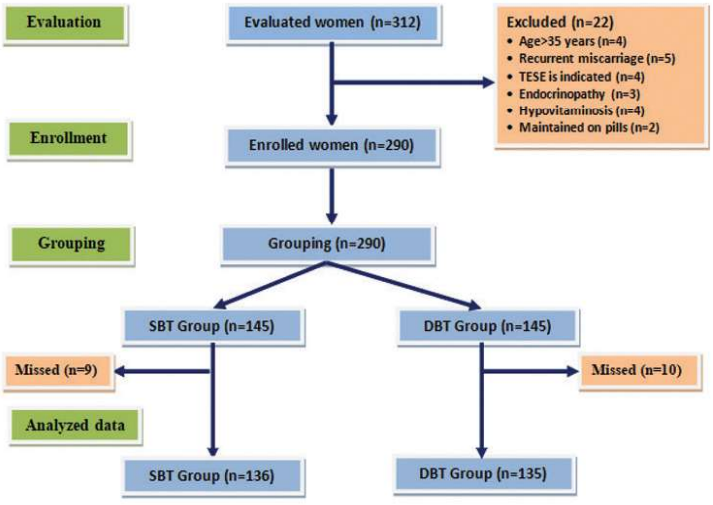

Figure 1: Study Flow Chart

Table 2: Pregnancy data of the studied women categorized according to number of the transferred blastocysts

Data	Group	SBT (n=136)		DBT (n=135)		P-value
		No.	%	No.	%	
Chemical pregnancy (CHP)	Positive	78	57.4	88	65.2	**0.232**
Clinical pregnancy (CP) among	Total women	52	38.2	63	46.7	**0.160**
	Women had +ve CHP		66.7		71.6	**0.422**
Ectopic pregnancy		0	0	1	0.74	**0.315**
Early pregnancy loss among	Total women	14	10.3	27	20	**0.026**
	Women had +ve CP		26.9		42.9	**0.021**
Women completed follow-up	Total women	38	27.9	36	26.7	**0.814**
	Women had +ve CP		73.1		57.1	**0.076**

Chemical pregnancy (CHP) test was positive in 166 women for a chemical pregnancy rate of 61.3% with non-significantly (P=0.232) higher CHP rate for women of DBT (n=88; 65.2%) than for women of SBT (n=78; 57.4%). US examination detected viable embryo in 115 pregnant women for clinical pregnancy (CP) rate of 42.4% with non-significantly (P=0.160) higher CP rate among women of DBT group (n=63; 46.7%) in comparison to women of SBT group (n=52; 38.2%). The CP rate among women had positive CHP rate (n=166) was 69.3% and was non-significantly (P=0.422) higher rate among women of DBT group (71.6%) compared to the rate detected for SBT group (66.7%). Only one woman in DBT group had ectopic pregnancy for an incidence of 0.74% and 0.37% among the total studied women. Unfortunately, 41 women had early pregnancy loss (EPL) for an incidence rate of 35.7% among women had CP, 24.7% among women had CHP and 15.1% among the total enrolled women. Women of DBT group had significantly higher EPL rate (n=27) 42.9% among women had CP (P= 0.026) and 20% among total DBT women (P=0.021) in comparison to EPL rates for women of SBT; 26.9% and 10.3%, respectively. Seventy-four women continued their pregnancy for pregnancy rate of 44.6%, 64.3% and 27.3% among women had CHP, CP and total enrolled women, respectively with non-significantly (P=0.076) high rate among women had SBT (n=38) than women had DBT (n=36) as regards rate among women had CP (73.1% vs. 57.1%) and among total studied women (27.9% vs. 26.7%) as shown in table 2

Forty-three women; 38 had SBT and 5 had DBT developed single gestational sac containing singleton fetus, while 31 women had DBT developed two gestational sacs for a multiple pregnancy rate of 23% among total women of DBT group and 86.1% among women who completed their pregnancy. The incidence of singleton fetus was significantly (P<0.001) higher in women had SBT as shown in figure 2.

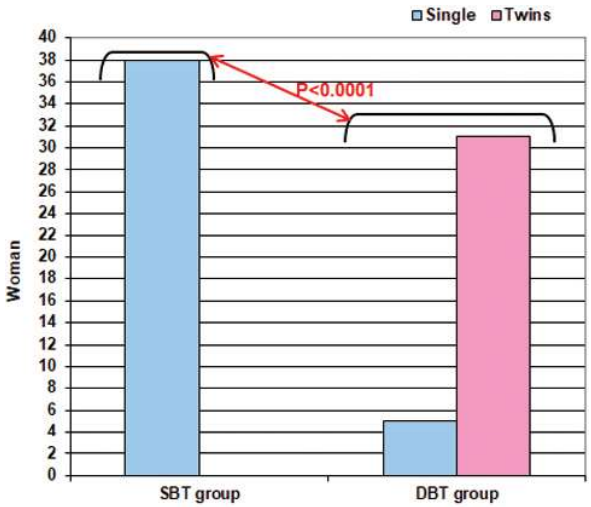

Fig. (2): Incidence of multiple pregnancy among women of both groups

Among women continued their pregnancy 22 women (29.7%) developed hypertensive manifestation; 5 had early preeclampsia (6.7%), 8 women developed late preeclampsia (10.8%) and 9 women (12.2%) had gestational hypertension without proteinuria with non-significantly (P=0.093) higher hypertensive complication rates among women had DBT than women had SBT. Further, 10 pregnant women (13.5%) developed gestational diabetes mellitus with non-significantly (P=0.440) higher incidence of gestational diabetes mellitus among women had DBT. Seven women (9.5%) had preterm premature rupture of membranes with non-significantly (P=0.205) higher incidence among DBT women. Fifteen women (20.3%) had preterm labor with significantly (P=0.0065) higher incidence among women of DBT (33.3%) than women of SBT group (7.9%). Twenty-six women (35.1%) required cesarean section with significantly (P=0.034) higher cesarean section rate among women of DBT group (47.2%) than among women of SBT (23.7%) group (Table 3, Fig. 3).

Table 3: Maternal complications of the studied women categorized according to number of the transferred blastocysts

Outcomes			SBT (n=38)	DBT (n=36)	P-value
Gestational hypertension (GH)	Preeclampsia	Early-onset	2 (5.3%)	3 (8.3%)	**0.093**
		Late-onset	3 (7.9%)	5 (13.9%)	
	GH		3 (7.9%)	6 (16.7%)	
	No hypertension		30 (78.9%)	22 (61.1%)	
Gestational diabetes mellitus		Yes	4 (10.5%)	6 (16.7%)	**0.440**
		No	34 (89.5%)	30 (83.3%)	
Preterm premature rupture of membrane		Yes	2 (5.3%)	5 (13.9%)	**0.205**
		No	36 (94.7%)	31 (86.1%)	
Preterm labor	<37 gestational weeks		3 (7.9%)	12 (33.3%)	**0.0065**
	≥37 gestational weeks		35 (92.1%)	24 (66.7%)	
Cesarean section	Yes		9 (23.7%)	17 (47.2%)	**0.034**
	No		29 (76.3%)	19 (52.8%)	

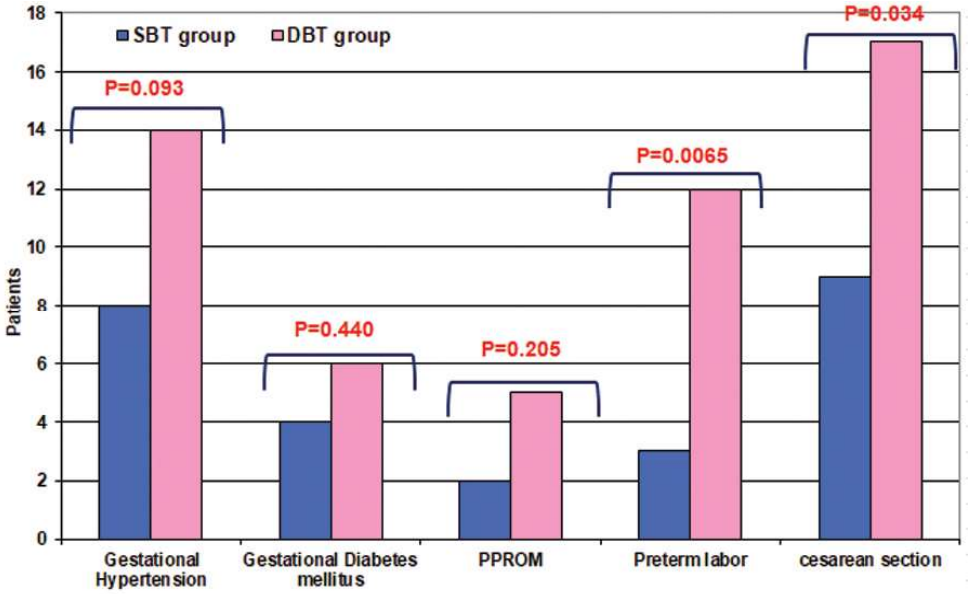

Fig. (3): Patients' distribution according to maternal complications

The applied study protocol resulted in 105 neonates; 64 neonates (61%) had normal average birth weight and 41 neonates (39%) had birth weight of <2.5 kg with significantly (P=0.031) higher incidence of low birth weight among neonates of DBT women (53.7%) than SBT women (73.7%). Further, 49 neonates (46.7%) required admission to NICU; 11 neonates of women of SBT group (28.9%) and 38 neonates of women of DBT (56.7%) with significantly (P=0.006) higher incidence of NICU admission among neonates of DBT group. Thirty-five neonates (33.3%) died; 8 neonates of SBT and 27 neonates of DBT (21.1% vs. 40.3%) groups with significantly (P=0.044) higher mortality rate among neonates of DBT group (Table 4, Fig. 4).

Table 4: Neonatal outcomes

Outcomes		SBT (n=38)	DBT (n=67)	P-value
Birth weight (kg)	<2.5	10 (26.3%)	31 (46.3%)	**0.031**
	Normal weight	28(73.7%)	36(53.7%)	
NICU admission	Yes	11 (28.9%)	38 (56.7%)	**0.006**
	No	27 (71.1%)	29 (43.3%)	
Live births	Yes	30 (78.9%)	40 (59.7%)	**0.044**
	No	8 (21.1%)	27 (40.3%)	

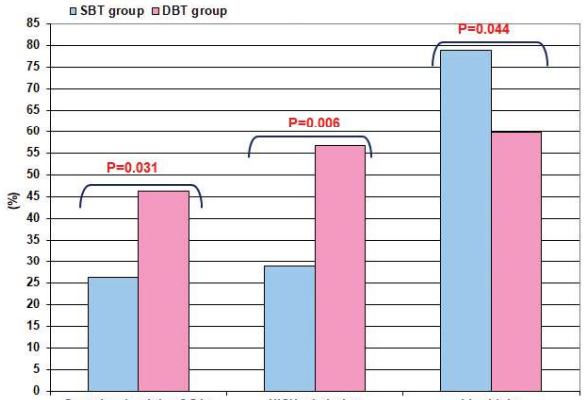

Fig. (4): Neonatal outcomes of pregnant women of both groups

Discussion

Considering the live birth rate (LBR) not the pregnancy rate as the target for any ART trial, the current study reported significantly higher LBR after single blastocyst transfer (SBT) than after double BT (DBT) despite of the significantly higher rate of multiple pregnancy after DBT. Additionally, the frequency of preterm premature rupture of membrane and preterm labor was higher women had DBT, this deleteriously affected neonatal outcome as evidenced by higher percentages of neonates with birth weight (BW) of <2.5 kg, required NICU admission and hospital neonatal mortality rate. Further, DBT was associated with higher incidence of gestational hypertensive disorders especially early-onset preeclampsia.

These data are in line with previous studies evaluating a similar topic, wherein, Peng et al. (19) in a meta-analysis detected a significant increase in cumulative LBR, higher gestational age at birth and BW after two consecutive cycles of single embryo transfer (SET) than one cycle of double ET (DET) with a lower risk of CS, antepartum hemorrhage, preterm birth, low BW, and NICU admission, and concluded that the 2 SETs strategy is beneficial especially for women younger than 35 years and BT provides more favorable outcomes than cleavage stage ET. Huang et al. (20) found an elective single cleavage ET can maintain the relatively high LBR with an acceptable low twin birth rate than double cleavage ET.

Further, Williams et al. (21) found DET significantly increased the multiple pregnancy LBR, with 43% twins and 0.9% triplets and BT had higher LBR than cleavage stage embryos (52.5% vs. 39.5%). Chen et al. (22) investigated the risk factors for twin pregnancy in IVF and found transfer of one good-quality embryo (GQE) was associated

with significantly reduced rate of twin pregnancy, low BW and preterm birth than transfer of two GQE.

Recently, Ozmen et al. (23) detected higher incidence of multiple pregnancy, CS and NICU admission and duration of NICU stay per neonate after DBT versus SBT. Also, Rodriguez-Wallberg et al. (24) revised the 10-y outcomes after SET versus DET or BT in the Sweden registry and detected higher risk of neonatal deaths in singletons that were born after DET than SET, and in case of frozen embryo, DET was associated with higher risk of low BW, and in case of BT, DBT was associated with higher risk of very preterm births and low BW and concluded that DET or DBT is associated with higher risk of adverse outcomes even if it resulted in singleton fetus in comparison to singletons that were born after SET or SBT.

Fortunately, one woman (0.74%) in DBT group, but none in SBT group developed ectopic pregnancy (EP). This low rate of EP among the studied population (0.37%) could be attributed to the selection of good blastocyst to be transferred, irrespective of being SBT or DBT. In line with this attribution, Anzhel et al. (25) retrospectively reported significantly lower EP rate after top-quality than poor-quality ET and significantly higher risk of EP after DET than SET. Further, Xue et al. (26) retrospectively investigated the impact of previous EP on outcomes of subsequent IVF and found the odds of EP were lower by 82.2% lower after BT than after cleavage embryo and were 6-times higher after DET than SET.

The current study tried to avoid bias of the reported LBR due to multiple effectors, so the study looked-like selective study including only PCOS as an underlying pathogenesis for infertility and all women were free of other etiological factors for infertility, women were in age range of 20-35 years and had BMI of <35 with average BMI of about 31.4 kg/m2 and mild PCOS-induced hormonal disturbances. Also, to equalize the chance for all women to get a live born baby, the study rational was to transfer frozen not fresh, blastocyst not cleaved embryo and of good quality of 5A grade

In line with the selective rational of the current study, Williams et al. (21) reported progressive reduction in the LBR with increasing the recipient age (OR, 0.8 for 40-44, 0.77 for age of 45-49 and 0.65 for age >49 years), a steady decline in the LBR with increases in recipient BMI above normal and with cleavage stage embryos than BT. Also, Yang et al. (27) tried to determine the outcomes of SET versus DET according to the recipient's age and concluded that for women younger than 35 years SET is the appropriate, while for older women individualized choices are required because the outcomes for women aged 35-39 depends on the number of oocytes retrieved and blastocyst quality, while for women older than 39 years the outcomes were low in both single and double ET with non-significant differences

Unfortunately, review of literature could not provide an explanation the reported low LBR, irrespective of the number of the transferred blastocyst and despite of the strict inclusion criteria. Some attributions were provided depending on the effect of uterine receptivity (28) intrauterine inflammatory milieu (29), intrauterine and systemic oxidative stress (30). Also, endometrial dysbiosis was found to affect embryo implantation through inflammation-related endometrial changes (31).

The obtained results after adjustment of inclusion criteria and the lack of explanation for the high pregnancy loss rate indicated the necessity for optimization between the benefits and risks before taking the decision for the elective frozen-thawed autologous blastocyst transfer as single or double BT for PCOS women undergoing ICSI.

Conclusion

Double BT even of good quality worsens the outcomes of ICSI for PCOS women. DBT is significantly associated with small BW, high incidence of preterm labor, need for operative delivery and NICU admission with subsequent reduction of LBRs. Also, DBT non-significantly increased the incidence of maternal complications than SBT.

Recommendation

Single good quality BT is the appropriate policy for PCOS women fulfilling the inclusion criteria of the current study and no need to take the risk of DBT with preservation of the second blastocyst for another session of ICSI to increase the chance for getting more offspring.

Acknowledgement

The author provides great thanks to the Staff Members of Clinical Pathology Department, Zagazig University Hospital for performing the required investigations. Also, great thanks are provided for Star Center, Benha, for carrying out the statistical analyses, proofreading and plagiarism check

References

1. Zanjirband M, Baharlooie M, Safaeinejad Z, Nasr-EsfahanI M: Transcriptomic screening to identify hub genes and drug signatures for PCOS based on RNA-Seq data in granulosa cells. Comput Biol Med. 2023; 154:106601.
2. Kaur M, Singh S, Kaur R, Beri A, Kaur A: Analyzing the Impact of FSHR Variants on Polycystic Ovary Syndrome-a Case-Control Study in Punjab. Reprod Sci. 2023.
3. Shrivastava S, Conigliaro RL: Polycystic Ovarian Syndrome. Med Clin North Am. 2023; 107(2):227-234. .
4. Chambers G, Dyer S, Zegers-Hochschild F, De Mouzon J, Ishihara O, Banker M, et al.: International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology, 2014†. Hum Reprod. 2021; 36(11):2921-2934. .
5. Vuong LN: Con: freeze-all for all? One size does not fit all. Hum Reprod. 2022; 37(7):1388-1393. .
6. Bosch E, Espinós J, Fabregues F, Fontes J, García-Velasco J, Llácer J, et al.: Spanish Infertility SWOT Group (SISG): ALWAYS ICSI? A SWOT analysis. J Assist Reprod Genet. 2020; 37(9):2081-2092. .
7. Jurado-García E, Botello-Hermosa A, Fernández-Carrasco F, Gómez-Salgado J, Navas-Rojano N, Casado-Mejía R: Multiple Gestations and Assisted Reproductive Technologies: Qualitative Study of the Discourse of Health Professionals in Spain. Int J Environ Res Public Health. 2021; 18(11):6031. .
8. Graham ME, Jelin A, Hoon Jr A, Floet A, Levey E, Graham E: Assisted reproductive technology: Short- and long-term outcomes. Dev Med Child Neurol. 2023; 65(1):38-49. .
9. An BGL, Chapman M, Tilia L, Venetis C: Is there an optimal window of time for transferring single frozen-thawed euploid blastocysts? A cohort study of 1170 embryo transfers. Hum Reprod. 2022; 37(12):2797-2807. .
10. He Y, Tang Y, Liu H, Liu J, Mao Y: No advantage of single day 6 good-quality blastocyst transfer versus single day 5 poor-quality blastocyst transfer in frozen-thawed cycles stratified by age: a retrospective study. BMC Pregnancy Childbirth. 2023; 23: 79
11. Chen MJ, Yang WS, Yang JH, Hsiao CK, Yang YS, Ho HN: Low sex hormone-binding globulin is associated with low

high-density lipoprotein cholesterol and metabolic syndrome in women with PCOS. Hum Reprod 2006; 21:2266–71.

12. Chen MJ, Yang WS, Yang JH, Chen CL, Ho HN, Yang YS: Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. Hypertension 2007; 49:1442–7.
13. WHO: Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. 1995;854:1-452..
14. WHO expert consultation: Appropriate body-mass index for Asian population and its implications for policy and intervention strategies. The Lancet, 2004; 363(9403): 157-63.
15. Ferriman D, Gallwey JD: Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961; 21:1440–7.
16. Brodell LA, Mercurio MG: Hirsutism diagnosis and management. Gend Med. 2010; 7:79–87.
17. Balaban B, Urman B, Ata B, Isiklar A, Larman M, Hamilton R, et al.: A randomized controlled study of human day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation. Hum Reprod, 2008; 23: 1976-82
18. Peña F, Dávalos R, Rechkemmer A, Ascenzo A,Gonzales M: Embryo development until blastocyst stage with and without renewal of single medium on day 3. JBRA Assist Reprod. 2018; 22(1): 49–51.
19. Peng Y, Ma S, Hu L, Wang X, Xiong Y, Yao M, et al.: Effectiveness and Safety of Two Consecutive Cycles of Single Embryo Transfer Compared With One Cycle of Double Embryo Transfer: A Systematic Review and Meta-Analysis. Front Endocrinol (Lausanne). 2022; 13:920973. .
20. Huang X, Liu R, Shen W, Cai Y, Ding M, Sun H, et al.: An elective single cleavage embryo transfer strategy to minimize twin live birth rate based on a prediction model from double cleavage embryos transfer patients. J Matern Fetal Neonatal Med. 2022; 35(9):1775-1782.
21. Williams RS, Ellis D, Wilkinson E, Kramer J, Datta S, Guzick D: 4Factors affecting live birth rates in donor oocytes from commercial egg banks vs. program egg donors: an analysis of 40,485 cycles from the Society for Assisted Reproductive Technology registry in 2016-2018. Fertil Steril. 2022; 117(2):339-348.
22. Chen P, Hu K, Jin J, Chen R, Xu Q, Zhao W, et al.: Risk factors for twin pregnancy in women undergoing double cleavage embryo transfer. BMC Pregnancy Childbirth. 2022; 22(1):264. .
23. Ozmen S, Tola E, Karahasanoğlu A: Obstetrics and perinatal outcomes between elective single versus double blastocyst transfer in women younger than 35 years: A cross-sectional study. J Gynecol Obstet Hum Reprod. 2023; 52(2):102527.
24. Rodriguez-Wallberg KA, Palomares A, Nilsson H, Oberg A, Lundberg F: Obstetric and Perinatal Outcomes of Singleton Births Following Single- vs Double-Embryo Transfer in Sweden. JAMA Pediatr. 2023; 177(2):149-159. .
25. Anzhel S, Mäkinen S, Tinkanen H, Mikkilä T, Haltia A, Perheentupa A, et al.: Top-quality embryo transfer is associated with lower odds of ectopic pregnancy. Acta Obstet Gynecol Scand. 2022; 101(7):779-786.
26. Xue Y, Tong X, Zhang H, Zhang S: Pregnancy outcomes following in vitro fertilization treatment in women with previous recurrent ectopic pregnancy. PLoS One. 2022; 17(8): e0272949.

27. Yang D, Chai M, Yang N, Yang H, Wen X, Wang J, et al.: Blastocyst Transplantation Strategies in Women of Different Ages. J Clin Med. 2023; 12(4):1618. .

28. Doyle N, Jahandideh S, Hill M, Widra E, Levy M, Devine K: Effect of Timing by Endometrial Receptivity Testing vs Standard Timing of Frozen Embryo Transfer on Live Birth in Patients Undergoing In Vitro Fertilization: A Randomized Clinical Trial. JAMA. 2022; 328(21):2117-2125. .

29. Lee I, Ahn S, Kim H, Baek H, Park Y, Kim H, et al.: Cytokines in culture media of preimplantation embryos during in vitro fertilization: Impact on embryo quality. Cytokine. 2021; 148:155714.

30. Artini PG, Scarfò G, Marzi I, Fusi J, Obino M, Franzoni F, et al.: Oxidative Stress-Related Signaling Pathways Predict Oocytes' Fertilization In Vitro and Embryo Quality. Int J Mol Sci. 2022; 23(21):13442. .

31. Cela V, Daniele S, Obino M, Ruggiero M, Zappelli E, Ceccarelli L, et al.: Endometrial Dysbiosis Is Related to Inflammatory Factors in Women with Repeated Implantation Failure: A Pilot Study. J Clin Med. 2022; 11(9):2481.