Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2023**; 50(11): 231
https://doi.org/10.31083/j.ceog5011231

Original Research

Retrospective Analysis of Fresh Single Blastocyst Transfer versus Two Cleavage-Stage Fresh Day-3 Embryo Transfer with High-Quality Embryos during Gonadotropin-Releasing Hormone Antagonist Cycles in High Responders

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Academic Editor: Michael H. Dahan
Submitted: 5 May 2023 Revised: 15 July 2023 Accepted: 24 July 2023 Published: 21 November 2023

Abstract

Background: High responders are characterized by a large number of retrieved oocytes and/or a high level of estradiol on the day of administration of human chorionic gonadotropin. There is controversy in the literature regarding live birth rates from fresh day-5 single blastocyst transfer (day-5 SBT) compared to cleavage-stage fresh day-3 embryo transfer (day-3 ET) in high responders. The aim of this study was therefore to compare reproductive outcomes between day-5 SBT and day-3 ET using high-quality embryos and gonadotropin-releasing hormone (GnRH) antagonist protocols in high responders undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). **Methods**: This was a single-center retrospective study of 282 high responders who underwent fresh day-5 SBT (day-5 SBT group, n = 142) or two cleavage-stage fresh day-3 embryo transfer (day-3 ET group, n = 140) between 2015 and 2019. **Results**: No significant differences were observed between the day-5 SBT and day-3 ET groups in terms of clinical pregnancy rate (51.41% *vs.* 59.29%, $p = 0.183$) or live birth rate (41.55% *vs.* 52.86%, $p = 0.057$). The incidence of multiple pregnancy (1.37% *vs.* 36.14%) and of low birth weight (5.00% *vs.* 32.26%) were significantly less frequent in the day-5 SBT group than in the day-3 ET group ($p < 0.001$ and $p < 0.001$, respectively). **Conclusions**: SBT may be the preferred choice for high-quality embryos in high responders undergoing IVF/ICSI during GnRH antagonist cycles with fresh embryo transfers. This is due to the lower incidence of obstetric complications compared to day-3 ET, although the clinical outcomes for the two groups are comparable.

Keywords: high responder; fresh cycle; blastocyst transfer; cleavage-stage embryo; gonadotropin-releasing hormone antagonist

1. Introduction

Since its advent almost 40 years ago, *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) has helped many infertile couples to become parents. Controlled ovarian stimulation (COS) is an essential step in IVF/ICSI treatment, with a subset of patients found to be sensitive to COS. These patients are known as high responders and are characterized by a total follicle count of 15 or more and/or an estradiol (E2) concentration >3000 pg/mL on the day of ovulation induction [1].

Many studies have shown that frozen embryo transfer (FET) is preferable for high responders since it has a higher live birth rate (LBR) and a lower incidence of ovarian hyperstimulation syndrome (OHSS) compared to fresh embryo transfer (ET) [2,3]. However, other studies have reported that FET had no advantage over fresh ET in terms of reducing OHSS in high responders [4]. Moreover, for infertile women with polycystic ovary syndrome (PCOS), it was reported that FET was associated with a higher risk of preeclampsia after the first transfer than fresh ET [2]. Furthermore, fresh ET can result in shorter times to pregnancy and live birth than FET, thus making it more cost-effective [5]. In addition, the use of gonadotropin-releasing hormone (GnRH) antagonists is associated with a reduced incidence of OHSS and does not seem to compromise ongoing pregnancy rates [6]. Therefore, fresh ET is worth considering for some high responders undergoing GnRH antagonist cycles.

To obtain an optimal clinical pregnancy rate (CPR), two cleavage-stage embryos are usually transferred. However, this can have adverse consequences related to multiple pregnancy in women and their new-borns [7]. Evidence shows that single blastocyst transfer (SBT) may be an effective way to lower the risk of multiple pregnancy without affecting the CPR [8–10]. Nevertheless, several retrospective studies on high responders in IVF/ICSI cycles have shown that fresh SBT was inferior to fresh day-3 ET (two embryo transfer) in terms of the CPR [3,11]. Thus, clinicians face a dilemma between the use of day-3 ET or day-5 SBT during fresh cycles, especially when multiple high-quality embryos are available for high responders. Further studies are therefore needed to provide more reliable evidence when faced with this dilemma.

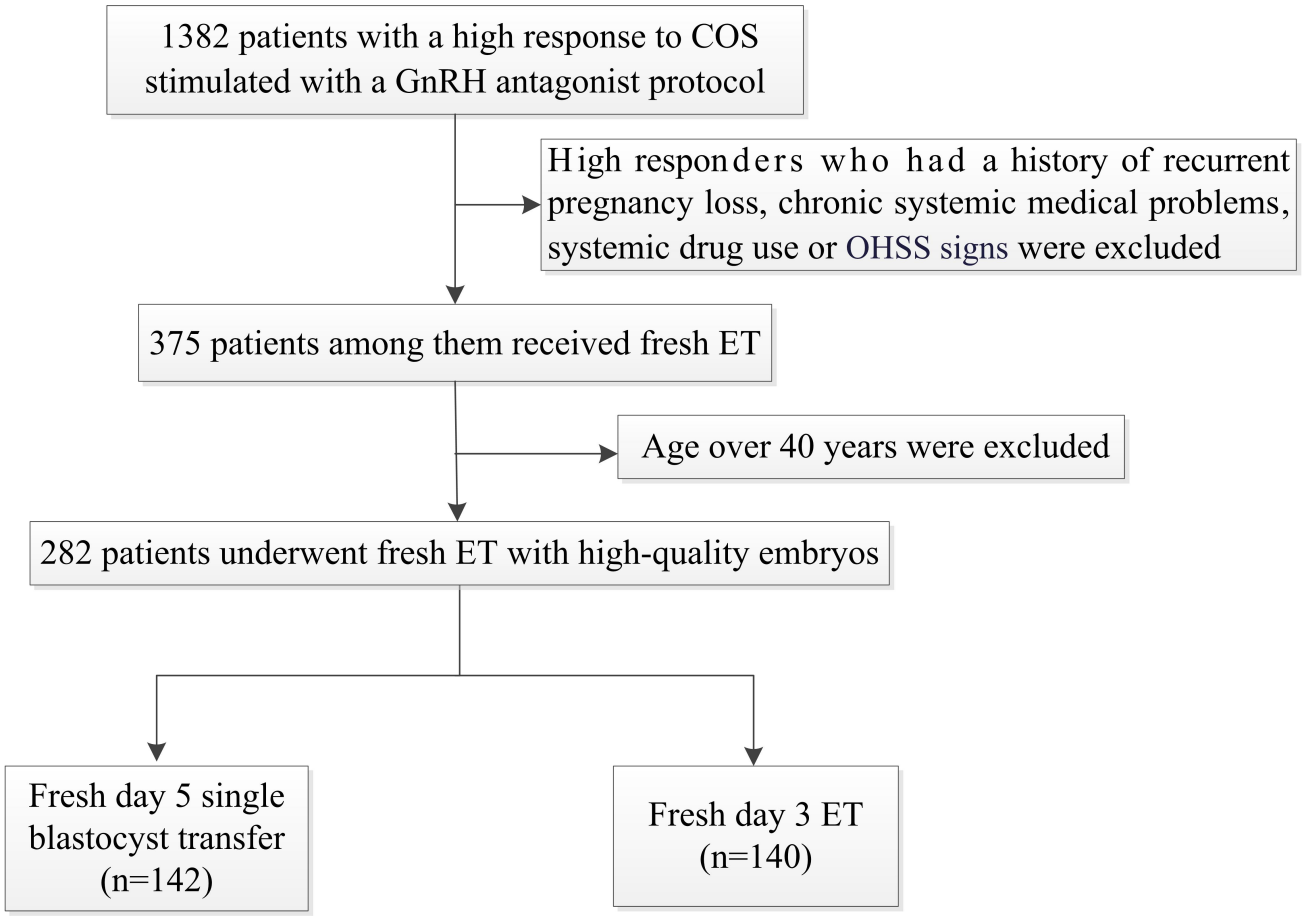

Fig. 1. Study flowchart. COS, controlled ovarian stimulation; OHSS, ovarian hyperstimulation syndrome; GnRH, gonadotropin-releasing hormone; ET, embryo transfer.

To the best of our knowledge, clinical outcomes with day-3 ET or day-5 SBT for high responders stimulated with GnRH antagonists in fresh cycles have not been reported to date. In the present study, we compared the CPR, LBR, OHSS and other IVF/ICSI-associated complications between fresh day-5 SBT and fresh day-3 ET using high-quality embryos in high responders. The European Society of Human Reproduction and Embryology (ESHRE) guidelines recommend the GnRH antagonist protocol for high responders due to its better safety and equivalent efficacy [12]. This comparative analysis therefore included only patients who were stimulated with GnRH antagonists.

2. Materials and Methods

2.1 Patients and Study Design

The patient cohort for this retrospective study consisted of high responders [1] stimulated with a GnRH antagonist protocol. Between September 2015 and December 2019, these patients underwent IVF/ICSI cycles at the Reproductive Medical Center, Third Affiliated Hospital, Sun Yat-sen University.

As shown in Fig. 1, 1382 patients with high response to COS using the GnRH antagonist protocol were initially evaluated. Of these, 375 received fresh ET. The final study cohort consisted of 282 patients who underwent transfer with high-quality embryos. Of these, 142 received one fresh day-5 blastocyst ET and were assigned to the day-5 SBT group. The remaining 140 patients received fresh day-3 ET with two cleavage-stage embryos and were assigned to the day-3 ET group.

The inclusion criteria were as follows: (1) infertility caused by tubal factors, endometriosis, anovulation, male factors, or unexplained reasons; (2) use of GnRH antagonist protocol; (3) high responders [1], defined as patients with 15 or more retrieved oocytes and/or an E2 concentration $>$3000 pg/mL on the day of human chorionic gonadotropin (hCG) administration; and (4) fresh ET with high-quality embryos. The exclusion criteria were as follows: (1) age $>$40 years; or (2) a history of recurrent pregnancy loss, chronic systemic medical problems, or systemic drug use.

2.2 Ovarian Stimulation

All patients in this study received the GnRH antagonist protocol. The starting dose was 50–300 IU of gonadotrophin (Merck Serono, Modugno, Italy; MSD, Ravensburg, Germany; Lizhu Pharmaceutical Trading,

Table 1. Comparison of demographic characteristics and baseline hormone levels between day-5 SBT and day-3 ET groups.

	Day-5 SBT (n = 142)	Day-3 ET (n = 140)	*p* value
Age (years)	30.50 (27.00–33.00)	31.00 (28.00–34.00)	0.624
BMI (kg/m^2)	20.30 (19.25–23.27)	20.58 (19.09–22.85)	0.744
Duration of infertility (years)	3.00 (2.00–4.75)	3.00 (2.00–5.00)	0.442
Primary cause of infertility			0.701
Male	40 (28.17)	35 (25.00)	
Female	91 (64.08)	93 (66.63)	
Both	6 (4.23)	9 (6.43)	
Unexplained	5 (3.52)	3 (2.14)	
AMH (ng/mL)	5.01 (3.46–7.12)	5.44 (2.75–8.59)	0.889
Baseline FSH (IU/L)	6.19 ± 1.64	6.42 (5.75–7.59)	0.002
Baseline LH (IU/L)	5.49 (4.43–8.44)	5.18 (4.31–7.49)	0.479
Baseline E2 (pg/mL)	35.87 (27.11–50.16)	37.17 (27.06–50.23)	0.514

SBT, single blastocyst transfer; ET, embryo transfer; BMI, body mass index; AMH, anti-Mullerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol.

Zhuhai, Guangdong, China) on days 2–3 of the menstrual cycle. Follicular development was assessed by both transvaginal ultrasonography and analysis of the serum hormone profile. Cetrorelix (Merck Serono, Halle, Germany) or ganirelix (Organon, Ravensburg, Germany; Zhengdatianqing Pharmaceutical Group, Nanjing, Jiangsu, China) was added from day-5 of stimulation, or when the dominant follicle was $\geq$14 mm. hCG (6000–10,000 IU) (Merck Serono, Modugno, Italy; Lizhu Pharmaceutical Trading, Zhuhai, Guangdong, China) or 0.2 mg of GnRH agonist (triptorelin, Ipsen, Boulogne-Billancourt, France; Ferring GmbH, Kile, Germany) plus hCG (2000 IU) (Lizhu Pharmaceutical Trading, Zhuhai, Guangdong, China) was administered after at least two follicles had reached $\geq$18 mm diameter. Transvaginal oocyte retrieval, along with IVF/ICSI and embryo culture, was performed 32–36 hours after hCG injection.

2.3 Embryo Transfer Strategies

After informing patients of the advantages and disadvantages of blastocyst culture, they were given the choice of either day-3 ET or day-5 SBT. In the SBT group, one blastocyst was selected for transfer. High-quality blastocysts on day-5 after oocyte retrieval were defined as 4BB grade or better according to the Gardner and Lane criteria [13]. In the day-3 ET group, two high-quality embryos were selected for transfer. These were defined as $\geq$7 cells that were better than grade 3 according to Puissant's criteria [14]. The luteal phase was supported using 40 mg of progesterone (intramuscular injection) (Xianju Pharmaceutical Trading, Xianju, Zhejiang, China) or 90 mg of progesterone vaginal gel (Crinone 8%; Merck Serono, Geneva, Switzerland) once daily, together with 10 mg of oral dydrogesterone (Abbott Biologicals, OLST, Netherlands) taken twice daily and starting from the day of oocyte retrieval.

2.4 Outcome Parameters

All baseline and clinical data were collected from the hospital records. Serum levels of anti-Müllerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), E2 and progesterone (P) were measured using chemiluminescence methods. The primary outcome parameters were CPR and LBR. Secondary outcome parameters were OHSS development, implantation rate (IR), and multiple pregnancy status. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound approximately four to five weeks after ET. IR was defined as the number of observed gestational sacs divided by the number of embryos transferred. LBR was defined as the delivery of any viable infant at $\geq$28 weeks of gestation. Preterm birth was defined as gestational age $<$37 weeks at delivery [15]. Low birth weight (LBW) was defined as a birth weight $<$2500 g [16], and macrosomia as a birth weight $>$4000 g [17].

2.5 Statistical Analysis

All data were analyzed using SPSS (version 20.0, IBM, Armonk, NY, USA). Numerical data are presented as the mean ± standard deviation (SD) or the median (range), and categorical data are presented as numbers and percentages. The Kolmogorov-Smirnov test was used to evaluate the distribution of data. In both groups, serum P levels on the hCG trigger day were normally distributed, and differences between the two groups were analyzed by the continuous *t* test. Other continuous but non-normally distributed variables were analyzed using Mann-Whitney U tests. Univariate analyses of categorical data were performed using the chi-square test or Fisher's exact test. p-values < 0.05 were considered statistically significant.

Table 2. Comparison of cycle characteristics between day-5 SBT and day-3 ET groups.

	Day-5 SBT (n = 142)	Day-3 ET (n = 140)	*p* value
FSH on hCG trigger day/(IU/L)	10.94 (8.99–13.54)	11.99 (9.95–14.13)	0.288
LH on hCG trigger day/(IU/L)	3.09 (2.11–4.68)	2.51 (1.80–3.86)	0.129
E2 on hCG trigger day/(pg/mL)	3353.35 ± 645.48	3318.0 (3002.0–3720.5)	0.981
P on hCG trigger day/(ng/mL)	0.78 ± 0.31	0.79 ± 0.32	0.736
GnRHa + hCG trigger rate	39 (27.46)	36 (25.71)	0.739
Oocytes	15.50 (11.00–17.75)	15.00 (11.00–17.00)	0.258
Moderate/Severe OHSS	3 (2.12)	3 (2.14)	0.986

SBT, single blastocyst transfer; ET, embryo transfer; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol. P, progesterone; hCG, human chorionic gonadotrophin; GnRHa, gonadotropin-releasing hormone agonist; OHSS, ovarian hyperstimulation syndrome.

3. Results

As shown in Table 1, no significant differences in age, body mass index (BMI), duration of infertility, primary cause of infertility, AMH, and baseline LH or E2 level were detected between the day-5 SBT and day-3 ET groups ($p > 0.05$). The SBT group had significantly lower baseline FSH levels than the day-3 ET group ($p = 0.002$).

As shown in Table 2, no significant differences were observed between the two groups in terms of the FSH, LH, E2 and P levels on the hCG trigger day, the trigger rate for GnRH agonist plus hCG, the number of oocytes, or the incidence of moderate/severe OHSS ($p > 0.05$).

Table 3 compares the outcomes between the day-5 SBT and day-3 ET groups. No significant differences were found with regard to the incidence of CPR, LBR, early pregnancy loss, ectopic pregnancy, prematurity, macrosomia, congenital anomalies, or neonatal gender. The day-5 SBT group had a significantly higher IR than the day-3 ET group, but lower incidence of multiple pregnancy and LBW.

Table 4 shows the comparison of outcomes between day-5 SBT and day-3 ET groups according to patient age. For both age groups, no significant differences were observed between day-5 SBT and day-3 ET groups with regard to CPR, LBR, early pregnancy loss, ectopic pregnancy, prematurity or macrosomia, congenital anomalies or neonatal gender. For patients aged $\leq$30 years, the day-5 SBT group had a significantly lower incidence of multiple pregnancy and LBW than the day-3 ET group. For patients aged >30 years, the day-5 SBT group had a significantly higher IR, but lower incidence of multiple pregnancy and LBW than the day-3 ET group.

Table 3. Comparison of pregnancy outcomes between day-5 SBT and day-3 ET groups.

	Day-5 SBT (n = 142)	Day-3 ET (n = 140)	*p* value
CPR	73 (51.41)	83 (59.29)	0.183
IR	71 (50.00)	111 (39.64)	0.042
Early pregnancy loss	11 (15.07)	6 (7.22)	0.117
Ectopic pregnancy	2 (2.74)	1 (1.20)	0.911
LBR	59 (41.55)	74 (52.86)	0.057
Multiple pregnancy	1 (1.37)	30 (36.14)	<0.001
Prematurity	8 (13.56)	14 (18.92)	0.409
LBW	3 (5.00)	30 (32.26)	<0.001
Macrosomia	0 (0.00)	3 (3.23)	0.280
Congenital anomalies	0 (0.00)	0 (0.00)	
Neonatal gender			0.187
Boy	34 (56.67)	42 (46.16)	
Girl	26 (43.33)	51 (54.84)	

SBT, single blastocyst transfer; ET, embryo transfer; CPR, clinical pregnancy rate; IR, implantation rate; LBR, live birth rate; LBW, low birth weight.

4. Discussion

This study found that day-5 SBT and day-3 ET showed similar CPR and LBR following fresh transfer of high-quality embryos in high responders stimulated with a GnRH antagonist protocol. Moreover, day-5 SBT showed significantly higher IR and lower incidence of multiple pregnancy and LBW than day-3 ET.

Extended culture to the blastocyst stage is considered a useful tool for embryo selection, since it allows only high-quality embryos to develop into blastocysts [18]. A meta-analysis of 5 randomized clinical trials (RCTs) found that SBT was associated with a higher rate of ongoing pregnancy and LBR than single cleavage-stage transfer [19]. A recent publication involving 15 studies and 2219 women also found the LBR was higher in blastocyst transfer patients than in cleavage-stage ET patients (odds ratio (OR) 1.27, 95% confidence interval (CI) 1.05 to 1.51) following fresh ET [10]. However, in the present study with high-quality embryos we observed a trend ($p = 0.057$) for day-3 ET to have higher LBR than day-5 SBT. This trend was observed in both the younger and older patient groups. Moreover, the CPR with day-3 ET was reported to be significantly higher than SBT in high responders with the GnRH agonist protocol and fresh cycles [3]. A previous study reported no significant difference in the LBR between

Table 4. Comparison of pregnancy outcomes for day-5 SBT and day-3 ET groups according to patient age.

	≤30 years			>30 years		
	Day-5 SBT (n = 66)	Day-3 ET (n = 73)	*p* value	Day-5 SBT (n = 76)	Day-3 ET (n = 67)	*p* value
CPR	29 (43.94)	40 (54.79)	0.201	44 (57.89)	43 (64.18)	0.442
IR	29 (43.94)	55 (37.67)	0.388	44 (57.89)	56 (41.79)	0.025
Early pregnancy loss	5 (17.24)	2 (5.00)	0.122	6 (13.64)	4 (9.30)	0.739
Ectopic pregnancy	0 (0.00)	1 (2.50)	1.000	2 (4.55)	0 (0.00)	0.494
LBR	23 (34.85)	35 (47.95)	0.118	36 (47.37)	39 (58.21)	0.195
Multiple pregnancy	0 (0.00)	17 (42.50)	<0.001	1 (2.27)	13 (30.23)	<0.001
Prematurity	3 (13.04)	8 (22.86)	0.499	5 (13.89)	6 (15.38)	0.855
LBW	0 (0.00)	16 (36.36)	0.001	3 (8.11)	14 (28.57)	0.014
Macrosomia	0 (0.00)	1 (2.27)	1.000	0 (0.00)	2 (4.08)	0.504
Congenital anomalies	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Neonatal gender			0.494			0.202
Boy	13 (56.52)	21 (47.73)		21 (56.76)	21 (42.86)	
Girl	10 (43.48)	23 (52.27)		16 (43.24)	28 (57.14)	

SBT, single blastocyst transfer; ET, embryo transfer; CPR, clinical pregnancy rate; IR, implantation rate; LBR, live birth rate; LBW, low birth weight.

SBT and two cleavage-stage ET [20]. Because blastocysts have good developmental potential, blastocyst transfer with high-quality embryos should result in better clinical outcomes than cleavage-stage ET. However, in the present study blastocyst transfer did not result in significantly better LBR compared to cleavage-stage ET (41.55% *vs*. 52.86%, $p = 0.057$). This may be due to the fact that two embryos were transferred in the cleavage-stage ET group, and a high serum E2 level (>3000 pg/mL) on the hCG trigger day is thought to reduce uterine receptivity [21]. Joo *et al*. [22] also found the serum E2 level negatively affects the CPR and IR in a concentration-dependent manner after it exceeds a certain level during COS. COS has consistently been found to cause histological endometrial maturation and changes in endometrial gene expression, which can adversely affect embryo implantation [23]. In the present study cohort, all participants were high responders with very high levels of serum E2. Therefore, excessive serum E2 levels during COS may advance the window for implantation and thus decrease endometrial receptivity, thereby affecting the CPR, LBR and IR in fresh cycles. Blastocysts are cultured for two days longer than day-3 embryos and this may have a greater effect on endometrial receptivity for SBT following fresh transfer than for day-3 ET.

The day-5 SBT group was observed here to have a higher IR than the day-3 ET group, indicating the rate of successful implantation between the two groups was different. The IR was not significantly different when the patients were aged ≤30 years, although this may be related to the small sample size. The high-responder subjects in the present study who underwent ET on day-3 received two high-quality embryos. However, not all of the cleavage-stage embryos transferred on day-3 would have developed into blastocysts. Blastocysts have a higher potential for implantation than cleavage-stage embryos and are likely to be more representative of the totipotency of embryo development [3,10]. Therefore, considering the efficacy of ET, SBT is recommended for high responders in fresh ET cycles.

It has been proposed that GnRH antagonists have the advantage of reducing the risk of OHSS in women with PCOS [6]. Moreover, a GnRH agonist for oocyte triggering resulted in a lower incidence of OHSS in normal patients [24,25]. In the present study, the number of oocytes induced by GnRH agonist plus hCG was almost identical in the day-5 SBT and day-3 ET groups. Since all patients in the current study were stimulated with the GnRH antagonist protocol, the incidence of OHSS was already rather low in high responders due to the beneficial effect of this protocol.

Numerous studies have recommended that SBT be considered first in routine practice when high-quality blastocysts are available, as this reduces the risk of multiple pregnancy and has no effect on the LBR [7,26]. In accordance with these studies, our analysis also found the multiple pregnancy rate with day-5 SBT was markedly lower than with day-3 ET. Multiple pregnancy is considered an important risk factor for LBW and is associated with increased medical, psychological, economic and social costs [27,28]. As expected, the incidence of LBW in the current study was found to be markedly higher in the day-3 ET group than in the day-5 SBT group. Advanced age is an important risk factor for female infertility, pregnancy loss, fetal anomalies, stillbirth, and obstetric complications [29]. Patients who have a good response to COS and undergo fresh ET are usually young [1]. The median age of patients in the day-5 SBT and day-3 ET groups in the current study was very similar at around 30 years. There was no evidence of any differences between the two groups in terms of the

incidence of early pregnancy loss, ectopic pregnancy, prematurity, macrosomia, congenital anomalies, or in terms of neonatal gender. These results concur with those of previous studies [30].

This study was limited by its retrospective design and the use of data from a single-center. The inclusion and exclusion criteria were strictly obeyed in order to minimize the risk of bias in the selection of patients. In view of its limitations, this was a preliminary study to examine the outcome of two fresh ET strategies, day-5 SBT and day-3 ET, for high responders in GnRH antagonist cycles.

5. Conclusions

In conclusion, when considering the CPR, IR, LBR and various complications such as LBW and multiple pregnancy, day-5 SBT appears to be preferable over day-3 ET for high responders undergoing IVF/ICSI cycles with GnRH antagonist in fresh ET cycles with high-quality embryos. However, further RCTs are needed to determine more accurately which fresh ET strategy is best for high responders with high-quality embryos.

Availability of Data and Materials

The raw data generated in this study are available upon reasonable request from the corresponding author.

Author Contributions

Conceptualization: TX, YZ, JO, WX. Data curation: FY, XL, WX. Formal analysis and investigation: TX, FY, JO. Methodology: WX, FY, XL. Resources: FY, XL. Supervision: JO. Writing – original draft: WX, JO. Writing – review & editing: WX, JO, TX. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board of the Third Affiliated Hospital of Sun Yat-sen University ([2021]02-143-01). All patients in this study signed informed consent forms to undergo IVF/ICSI treatments in our center, and the anonymity of the patients has been strictly protected.

Acknowledgment

Not applicable.

Funding

This project was supported by the Medical Scientific Research Foundation of Guangdong Province of China (Grant No.: A2020575).

Conflict of Interest

The authors declare no conflict of interest.

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