The Influence of Delayed Blastocyst Development on the Outcome of Frozen-Thawed Transfer of Euploid and Untested Embryos

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Objective: The primary objective is to compare live birth rates (LBRs) following frozen embryo transfer (FET) of euploid day 5 with day 6 blastocysts. We also compared LBRs following FET of untested blastocysts vitrified on day 5 and day 6 in self-oocyte and ovum donation (OD) cycles. Design: This was a retrospective observational study. Setting: Nova IVF Fertility, Ahmedabad.

Materials and Methods: Ninety-seven FET using self-oocytes following preimplantation genetic testing A (PGT-A), 464 FET following OD, and 907 FET using self-oocytes without PGT-A testing between January 2016 and December 2017 were included in this study. Main Outcome Measures: LBR following FET in day 5 versus day 6 blastocysts in euploid embryos using self-oocytes and in untested embryos using both self and donor oocytes. Results: In PGT-A cycles, no statistically significant difference was observed in LBRs following transfer of euploid blastocysts developed on day 5 or day 6 (D5: 53%; D6:40%, P = 0.83). However, the LBRs with day 5 blastocysts were higher compared with day 6 group in untested group using both self and donor oocytes (self D5: 52.7%; D6: 38.2%; P = 0.001 and OD D5: 44.7%; D6: 29.8%; P = 0.001). Miscarriage rates were comparable in both the groups. Conclusions: The present study demonstrated comparable pregnancy outcomes following FET of euploid embryos vitrified on day 5 and day 6. However, higher LBRs were reported in day 5 group in untested embryos.

Keywords: Day 5 blastocyst transfer, day 6 blastocyst transfer, euploid blastocyst transfer, frozen embryo transfer, preimplantation genetic testing.

INTRODUCTION

Embryo transfers at blastocyst stage have been on the constant increase over the years worldwide.[1] The rationale behind this increasing trend are the advantages of blastocyst transfers over cleavage stage transfers in terms of higher implantation rate and reduction in number of multiple pregnancies.[10] Transferring embryos at blastocyst stage is considered to be more physiological as the embryo traverses the uterotubal junction on day 3 or early on day 4 as in a natural cycle. Another advantage is that embryo self-selection occurs after activation of embryonic genome on day 3; therefore, blastocysts are thought to have better implantation potential.[5] Third, embryo biopsy for chromosomal screening is considered to be safe when performed at blastocyst stage compared with cleavage stage.[4] According to a recent Cochrane review, fresh blastocyst transfer has higher clinical pregnancy rate than cleavage stage transfer.[5]

Several studies have reported higher pregnancy rates in fresh transfers with day 5 blastocysts compared with day 6 blastocysts.[6-8] Rapid developing embryos outperform their slower counterparts in fresh autologous cycles because controlled ovarian hyperstimulation and the resulting supraphysiological hormones have been...
suggested to have a detrimental effect on oogenesis, implantation of embryos,\(^9\) endometrial development,\(^{10}\) frequency of uterine contraction,\(^{11}\) and perinatal outcomes.\(^{12}\)

Transferring embryos in frozen and thawed cycle negates the effect of ovarian stimulation as the endometrial development is more controlled in frozen cycles. The research findings on frozen-thawed blastocysts are not consistent with the outcome on comparing with the day of vitrification. Several studies have reported that day 6 blastocysts have similar clinical outcomes compared with day 5 blastocysts in frozen- thawed cycles,\(^{13-17}\) while other studies have reported better clinical outcomes with day 5 blastocyst transfers.\(^{18-20}\) A recent meta-analysis reported higher pregnancy rates with day 5 frozen embryo transfers (FETs) as compared to day 6 FET.\(^{21}\) However, when the analysis was restricted to studies that compared day 5 and day 6 embryos with similar morphology, pregnancy outcomes were similar.

One of the reasons for implantation failure is embryo aneuploidy. It is unclear whether the aneuploidy rates are affected by delayed blastulation as some studies have demonstrated higher aneuploidy rates with delayed development,\(^{22-24}\) while others have reported that aneuploidy rates are unaffected by delayed blastulation.\(^{25,26}\) A study conducted by Taylor et al.\(^{27}\) reported similar pregnancy outcomes in euploid embryos irrespective of the day of embryo biopsy. Both endometrial receptivity and quality of embryo have an important role in implantation; hence, we compared the outcomes in both euploid and untested embryos vitrified on day 5 versus day 6 when transferred in a controlled FET cycle using the same hormone replacement therapy (HRT) protocol.

**Materials and Methods**

**Study population**

Overall, 97 FET using self-oocytes with preimplantation genetic testing A (PGT-A), 464 FET using donor oocytes, and 907 FET using self-oocytes without PGT-A from January 2016 to December 2017 at our center were included in this study. Euploid embryos in PGT-A cycles were allocated into two groups based on the day of biopsy and vitrification, i.e. day 5 or day 6. Untested embryos using self-oocytes and donor oocytes were classified into two groups based on the day of vitrification either on day 5 or day 6. In self-oocytes without PGT-A group, 907 patients were analyzed (D5 group: \(n = 700\); D6 group: \(n = 207\)), whereas in ovum donation (OD) group, 464 embryo transfers were analyzed (D5 group: \(n = 360\); D6 group: \(n = 104\)). In this study, only embryos that developed into fully expanded blastocysts were vitrified either on day 5 or on day 6 of fertilization. The grading of embryos was done according to the Association for the Study of Reproductive Biology (ASEBIR) criteria for morphological evaluation of blastocysts. Embryos that underwent PGT-A were biopsied on day 5 or day 6 of development. A cycle in which both day 5 and day 6 blastocysts had been transferred was also excluded from this study. The methodology used for vitrification and warming was consistent during the study period, and no significant protocol or laboratory changes were made during this period.

**Embryo grading and cryopreservation**

The blastocysts formed during Intracytoplasmic sperm injection (ICSI) were assigned grades based on the morphological features of trophectoderm and inner cell mass and also on the degree of blastocoele expansion as described by the ASEBIR criteria. Embryos were vitrified with Vitrification Kit (Kitazato Corporation, Shizuoka, Japan) on a Cryotop as per the manufacturer’s protocol.

**Trophectoderm biopsy**

The blastocysts were biopsied either on day 5 or day 6 whichever day they fulfilled the biopsy criteria. If they did not meet the biopsy criteria on day 6, they were discarded. PGT-A was done by next-generation sequencing method and the embryos were vitrified postbiopsy.

**Thawing**

All embryos were thawed using the thawing kit (Kitazato Corporation, Shizuoka, Japan) and incubated for 2–4 h before embryo transfer. Only viable embryos with good cell survival rate and expansion were transferred.

**Endometrial preparation and evaluation**

The patients were started on an oral dose of 2 mg estradiol valerate (Bayer Zydus, Thane, India) twice daily, beginning from the 2nd day of the menstrual cycle after the baseline ultrasound, which was increased to 4 mg twice a day after 4 days. The patients were assessed for endometrial thickness by endovaginal ultrasound after 10 days. Endometrial thickness was considered adequate when it was \(\geq 7\) mm in thickness with trilaminar echogenicity. If the thickness was inadequate, an additional 2 mg estradiol valerate was added twice a day intravaginally and ultrasound assessment was done after 3 days to reconfirm further endometrial thickness. If endometrial thickness was \(< 7\) mm even after increasing the dose of estradiol valerate for 1 week, the cycle was canceled.
Luteal support

Patients were started on oral dydrogesterone 10 mg (Abbott, Puducherry, India) and vaginal micronized progesterone 400 mg twice a day (Miprogen Bharat serum, Mumbai, India) once the endometrial lining was ≥7 mm. Embryo transfer was done after 5 days of progesterone supplementation. The maximum number of embryos transferred was 2. Pregnancy was detected with serum beta human chorionic gonadotropin (hCG) after 14 days of embryo transfer. Women who had a positive pregnancy test were advised to continue the same support till 12 weeks of pregnancy.

Study outcomes

The primary outcome of the study was live birth rates (LBRs) and the secondary outcome measures included clinical pregnancy rates and miscarriage rates. Pregnancy was defined as serum beta hCG >10 mIU/ml after 14 days of embryo transfer. All pregnancies with beta hCG <100 which subsequently did not show a sac were considered as biochemical pregnancies. Clinical pregnancy was defined as the presence of gestational sac with fetal cardiac pulsations on ultrasound. The miscarriage rates included both biochemical and clinical miscarriages. Live birth was defined as delivery of a viable infant ≥28 weeks of gestation. Clinical pregnancy rate is defined as the number of gestational sacs observed with ultrasound at 6 weeks of pregnancy/100 embryos transferred. LBR is defined as the number of deliveries that resulted in a live-born neonate, expressed/100 embryo transfers.

Statistical analysis

Comparison of continuous variables between the two groups was conducted using Student’s t-test. Chi-square was used for comparison of categorical variables. Logistic regression analysis was used for multivariate analysis. Statistical significance was defined as $P < 0.05$.

RESULTS

Patient characteristics

Table 1 shows the baseline characteristics of the two groups (day 5 and day 6 blastocysts) in PGT-A cycles. No statistically significant difference was observed in terms of age distribution, type of infertility, and duration of infertility between the two groups. Body mass index (BMI) was comparable in both the groups (D5: 27.2 ± 3.22; D6: 28.1 ± 5.23; $P = 0.31$). A higher proportion of Grade A and B embryos were biopsied in day 5 group (63%) compared with day 6 group (28%; $P ≤ 0.01$). The average number of embryos transferred in day 5 group was 1.54 ± 0.5, whereas in day 6 group, it was 1.24 ± 0.43. Tables 2 and 3 depict the baseline characteristics of day 5 and day 6 blastocysts in untested embryos using self-oocytes and donor oocytes, respectively. No statistically significant difference was observed in terms of age distribution, type of infertility, and duration of infertility between the two groups. The donors recruited in OD cycles were <30 years. In self-oocyte cycles, patients in day 6 group had higher BMI compared with day 5 group (D5: 26.1 ± 4.96; D6: 27.3 ± 5.4; $P = 0.002$), whereas it was comparable in OD group (D5: 27.1 ± 4.95; D6: 26.9 ± 6.1; $P = 0.73$). A higher proportion of Grade A and B embryos were transferred in day 5 group (77.1%) compared with day 6 group (51.2%; $P ≤ 0.001$) in self-oocyte cycles, whereas this difference was not significant in OD group. The mean number of embryos transferred in self-oocytes in day 5 group was 1.75 ± 0.4, whereas in day 6, it was 1.67 ± 0.46. In the OD group, and the average number of embryos transferred in day 5 group was 1.65 ± 0.4, whereas in day 6 group, it was 1.74± 0.4.

Embryo transfer outcomes

In patients undergoing FET with self-oocytes following PGT-A [Table 4], no statistically significant difference was observed in terms of LBRs (D5: 53%; D6:40%, $P = 0.83$), though the clinical pregnancy rates were

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Table 1: General characteristics in preimplantation genetic testing for aneuploidy cycles

| Category            | Subcategory | D5 blastocyst group (n=72) | D6 blastocyst group (n=25) | $P$ |
|---------------------|-------------|-----------------------------|-----------------------------|-----|
| Age group (years)   | <35         | 68% (49)                    | 52% (13)                    | 0.32*|
|                     | 35–40       | 28% (20)                    | 44% (11)                    |     |
|                     | ≥40         | 4% (3)                      | 4% (1)                      |     |
| Infertility         | PI          | 51% (37)                    | 40% (10)                    | 0.32*|
|                     | SI          | 49% (35)                    | 60% (15)                    |     |
| Duration of infertility (years) | 4.7±4.02 | 3.4±3.22                    | 0.14*|
| BMI (kg/m²)         | 27.2±3.22   | 28.1±5.23                   | 0.31*|
| Number of embryos transferred per cycle | 1.54±0.5 | 1.24±0.43                   | 0.008**|
| Grade               | A and B     | 63% (45)                    | 28% (7)                     | 0.01**|
|                     | C           | 36% (26)                    | 64% (16)                    |     |
|                     | D           | 1% (1)                      | 8% (2)                      |     |

BMI=Body mass index, PI=Primary infertility, SI=Secondary infertility, k=Chi square test, t=T-test, *=Statistically significant
higher in day 5 group. Multiple pregnancy rates were comparable in both the groups (D5: 14%; D6: 9.09%; \( P = 0.6 \)). Birth weight was found to be higher in day 6 as compared to day 5 group (2.9 ± 0.38 vs. 2.2 ± 1.25 kg; \( P = 0.004 \)). The mean gestational age was higher in day 6 group (D5: 35.6 ± 4; D6: 37.7 ± 1.73; \( P = 0.01 \)). Preterm birth rate was 31.5% in day 5 group, whereas it was 20% in day 6 group.

The clinical pregnancy rates (D5: 61.9%, D6: 44.2%; \( P < 0.001 \)) and LBRs (D5:52.7%; D6:38.2%; \( P = 0.001 \)) were significantly higher in day 5 group compared with day 6 group in OD cycles, as depicted in Table 5. Multiple pregnancy rates were similar in both the groups (D5: 21.5%, D6: 26%; \( P = 0.5 \)). Miscarriage rates were similar in both the groups (D5: 34.4%; D6:40.3%; \( P = 0.46 \)). Birth weight was found to be higher in day 5 as compared to day 6 group (2.3 ± 0.56 kg vs. 2 ± 0.52 kg; \( P < 0.001 \)). A more number of preterm births were observed in day 6 as compared to day 5 group (D5: 39.1%, D6: 64.5%; \( P = 0.009 \)).

Table 6 presents the embryo transfer outcomes in cycles using self-oocytes without PGT-A in both the groups. The clinical pregnancy rates were significantly higher following transfer of embryos vitrified on day 5 as compared to day 6 (D5: 65.7%; D6: 51.7%; \( P \leq 0.001 \)). Similarly, LBRs in day 5 group were significantly higher than day 6 group (D5:52.7%; D6:38.2%; \( P = 0.001 \)). Multiple pregnancy rates were significantly higher in day 5 as compared to day 6 group (D5:25.6%; D6:15.8%; \( P = 0.03 \)). Miscarriage rates were similar in both the groups (16.9% vs. 13%; \( P = 0.3 \)). The mean birth weight of infants was 2.4 ± 0.59 kg in day 5 group and 2.4 ± 0.59 kg in day 6 group. A more number of preterm births were observed in day 6 as compared to day 5 group, though the difference was not statistically significant (D5: 36.3%, D6: 44.3%; \( P = 0.18 \)).

**Discussion**

The present study compared LBRs following the transfer of euploid embryos vitrified on day 5 and day 6. We also compared the outcomes after FET of embryos cryopreserved on day 5 versus day 6 in untested embryos using self-oocytes and donor oocytes. We found comparable pregnancy outcomes in euploid embryos in day 5 and day 6 groups. However, day 5 embryos had better pregnancy outcomes than day 6 embryos in untested using both self-oocytes and donor oocytes.

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**Table 2: General characteristics table - self without preimplantation genetic testing for aneuploidy**

| Category                  | Subcategory | D5 blastocyst group (\( n =700 \)) | D6 blastocyst group (\( n =207 \)) | \( P \) |
|---------------------------|-------------|------------------------------------|------------------------------------|-------|
| Age group (years)         | <35         | 85.9% (601)                        | 85.5% (177)                        | 0.84* |
|                           | 35–40       | 12.7% (89)                         | 13.5% (28)                         |       |
|                           | ≥40         | 1.4% (10)                          | 1% (2)                             |       |
| Infertility               | PI          | 59.1% (414)                        | 58.9% (122)                        | 0.95* |
|                           | SI          | 40.9% (286)                        | 41.1% (85)                         |       |
| Duration of infertility (years) | 4.4±3.42    | 4.5±3.1                            | 0.7*                              |       |
| BMI (kg/m²)               |             | 26.1±4.96                          | 27.3±5.46                         | 0.002* |
| Number of embryos transferred per cycle | 1.75±0.43   | 1.67±0.46                          | 0.02*                             |       |
| Grade                     | A and B     | 77.1% (540)                        | 51.2% (106)                        |       |
|                           | C           | 22.6% (158)                        | 46.9% (97)                         |       |
|                           | D           | (0.3%) 2                           | 1.9% (4)                           |       |

BMI=Body mass index, PI=Primary infertility, SI=Secondary infertility, k=Chi square test, t=T-test, *=Statistically significant

**Table 3: General characteristics table - ovum donation**

| Category                  | Subcategory | D5 blastocyst group (\( n =360 \)) | D6 blastocyst group (\( n =104 \)) | \( P \) |
|---------------------------|-------------|------------------------------------|------------------------------------|-------|
| Age group (years)         | <35         | 32.5% (117)                        | 26% (27)                           | 0.32* |
|                           | 35–40       | 32.5% (117)                        | 39.4% (41)                         |       |
|                           | ≥40         | 35% (126)                          | 34.6% (36)                         |       |
| Infertility               | PI          | 53.9% (194)                        | 60.6% (63)                         | 0.23* |
|                           | SI          | 46.1% (166)                        | 39.4% (41)                         |       |
| Duration of infertility (years) | 8.3±6.68    | 9.1±6.62                           | 0.28*                             |       |
| BMI (kg/m²)               |             | 27.1±4.95                          | 26.9±6.18                         | 0.73* |
| Number of embryos transferred per cycle | 1.65±0.47   | 1.74±0.44                          | 0.08*                             |       |
| Grade                     | A and B     | 77.8% (280)                        | 69.2% (72)                         | 0.7*  |
|                           | C           | 22.2% (80)                         | 30.8% (32)                         |       |
|                           | D           | 0% (0)                             | 0% (0)                             |       |

BMI=Body mass index, PI=Primary infertility, SI=Secondary infertility, k=Chi square test, t=T-test, *=Statistically significant
Table 4: Embryo transfer outcomes of euploid embryos in preimplantation genetic testing for aneuploidy cycles

| Category          | D5 blastocyst group (n=72) | D6 blastocyst group (n=25) | P     |
|-------------------|----------------------------|-----------------------------|-------|
| PR                | 76% (55/72)                | 52% (13/25)                 | 0.02* |
| Clinical PR       | 69% (50/72)                | 44% (11/25)                 | 0.02* |
| Multiple PR       | 14% (7/50)                 | 9.09% (1/11)                | 0.66  |
| Abortion rate     | 27.2% (15/55)              | 23% (3/13)                  | 0.75  |
| Live birth rate   | 53% (38/72)                | 40% (10/25)                 | 0.83  |
| Birth weight (kg) | 2.2±1.25                  | 2.9±0.38                    | 0.004* |
| Preterm birth rate| 31.5% (12/38)              | 20% (2/10)                  | 0.48  |
| Gestational age   | 35.6±4                     | 37.7±1.73                   | 0.01* |

PR=Pregnancy rate, k=Chi square test, t=T-test, *=Statistically significant

Table 5: Embryo transfer outcomes- ovum donation

| Category          | D5 blastocyst group (n=360) | D6 blastocyst group (n=104) | P     |
|-------------------|----------------------------|-----------------------------|-------|
| PR                | 69.2% (249/360)            | 51.9% (54/104)              | <0.001* |
| Clinical PR       | 61.9% (223/360)            | 44.2% (46/104)              | <0.001* |
| Multiple PR       | 21.5% (48/223)             | 26% (12/46)                 | 0.5   |
| Abortion rate     | 34.4% (85/249)             | 40.3% (21/54)               | 0.46  |
| Live birth rate   | 44.7% (161/360)            | 29.8% (31/104)              | 0.001* |
| Birth weight (kg) | 2.3±0.56                  | 2.6±0.52                    | <0.001* |
| Preterm birth rate| 39.1% (63/161)             | 64.5% (20/31)               | 0.009* |
| Gestational age   | 35.7±2.67                  | 35.2±3.12                   | 0.106 |

PR=Pregnancy rate, k=Chi square test, t=T-test, *=Statistically significant

Table 6: Embryo transfer outcomes- self without preimplantation genetic testing for aneuploidy

| Category          | D5 blastocyst group (n=700) | D6 blastocyst group (n=207) | P     |
|-------------------|----------------------------|-----------------------------|-------|
| PR                | 70.7% (495/700)            | 54.1% (112/207)             | <0.001* |
| Clinical PR       | 65.7% (460/700)            | 51.7% (107/207)             | <0.001* |
| Multiple PR       | 25.6% (118/460)            | 15.8% (17/107)              | 0.03* |
| Abortion rate     | 16.9% (118/495)            | 13% (27/112)                | 0.3  |
| Live birth rate   | 52.7% (369/700)            | 38.2% (79/207)              | 0.001* |
| Birth weight (kg) | 2.4±0.59                  | 2.4±0.59                    | 1    |
| Preterm birth rate| 36.3% (134/369)            | 44.3% (35/79)               | 0.18  |
| Gestational age   | 36±2.85                   | 36.1±3                      | 0.66  |

PR=Pregnancy rate, k=Chi square test, t=T-test, *=Statistically significant

Whether slow-growing embryos have higher rates of aneuploidy than fast-growing embryos is still questionable. There are conflicting reports in the literature regarding the influence of delayed development on aneuploidy. Magli et al.[28] analyzed embryo morphology in relation to chromosomal status in 662 patients with a poor prognosis who underwent 916 cycles of PGT-A. They concluded that the incidence of chromosomal abnormalities was significantly higher in slow-cleaving, very fast-cleaving, or arrested embryos. The presence of fragmentation or uneven number of blastomeres was also associated with aneuploidy. The lower pregnancy rates in day 6 blastocysts when compared with day 5 could be attributed to spindle abnormalities, mitochondrial deficiencies, or gene expression.[22,23] However, when we compared euploid embryos, the LBRs were similar in both the groups.

Alfarawati et al. demonstrated higher aneuploidy rates in slow-growing blastocysts.[24] Capalbo et al.[25] demonstrated that complex aneuploidy was associated with blastocyst morphology, but the fast- and slow-growing embryos had a similar aneuploidy rate. Similarly, Kroener et al. showed that delayed blastulation does not affect aneuploidy rates, but the absence of blastulation is associated with increased aneuploidy.[26]

Taylor et al. demonstrated that day 5 embryos have better euploidy rates as compared to day 6 embryos. However, they concluded that when euploid embryos were transferred, similar outcomes were observed irrespective of the day of biopsy.[27] Our study also demonstrated that live births were similar after transferring euploid embryos regardless of their day of development which could be due to improved embryo–endometrial synchrony in HRT cycles.

Our results concluded that day 5 blastocysts had significantly higher LBRs compared with day 6 in untested embryos when they are transferred in programmed hormone replacement cycles, which suggests that the developmental delay seen in day 6 blastocysts may have an impact on implantation potential when compared with day 5 blastocysts, as we possibly had improved synchronization between the endometrium and embryos in HRT cycles as all the embryo transfers were performed after 5 days of progesterone supplementation.

Our results were consistent with Haas et al. who reported a significant lower pregnancy rate following frozen-thawed transfer with D6 vitrified blastocysts, even when the embryos were of good quality by morphological assessment.[20] However, the limitation of the above study was that they thawed the vitrified day 5 blastocyst 20–24 h prior to embryo transfer, whereas day 6 embryos were thawed 2–4 h prior to embryo transfer. Embryos in both the groups were transferred on day 6 of the progesterone, and therefore, the different pregnancy rates could not be explained by the different transfer dates. Although the long culture of embryos in vitro may affect the implantation potential, we saw an opposite trend. This demonstrates that day 5 embryos have a better pregnancy rate compared with day 6 blastocysts.

Similarly, Yang et al. reported a higher clinical pregnancy rate in day 5 group as compared to day 6 group. However, pregnancy rates and euploidy rates...
were similar in both the groups when only high-quality embryos were transferred.[20] Meta-analysis by Sunkara et al. which included 15 studies together including 2502 frozen-thawed transfers showed significantly higher clinical pregnancy rates and LBRs with day 5 compared with day 6 frozen-thawed blastocyst transfers. However, sensitivity analysis of the studies including blastocysts at the same stage of development showed no significant difference in clinical pregnancy rates and LBRs.[21]

On the contrary, Kaye et al. analyzed frozen-thawed single embryo transfer of blastocysts cryopreserved either on day 5 and day 6 and found comparable pregnancy rates in both the groups.[30] El-Toukhy et al. reported similar LBRs following frozen-thawed transfers of day 5 and day 6 high-grade blastocysts.[14]

Wide variations in laboratory protocols, culture techniques, criteria for blastocyst freezing, and the method chosen for cryopreservation are some of the factors which may explain the current contradictions in the literature regarding the outcomes of day 5 versus day 6 blastocysts.[21] The reason for delayed blastulation could be suboptimal conditions of in vitro environment or due to factors intrinsic to embryo which negatively influences embryo development. Another possible reason for lower pregnancy rates in day 6 could be associated with nutrient depletion from the culture media.[19]

Many studies have suggested that extended culture leads to heavier birth weight of newborns.[31] Ferreux et al.[32] reported higher birth weight in day 6 group compared to day 5 group following frozen-thawed blastocyst transfer. On the contrary, Du T et al.[33] reported no statistically significant difference in neonatal outcomes among day 5, 6, and 7 cryopreserved embryos. In our study, newborns from day 5 group weighed less than day 6 group in PGT-A cycles, whereas in the OD group, day 5 neonates had higher birth weight compared to day 6 group. No statistically significant difference was reported in the untested self-oocyte group. The heterogeneity among various studies could be explained by the difference in culture media composition, endometrial preparation, and embryo cryopreservation methods.

The method used for cryopreservation also has a role in determining the clinical outcomes in FET cycles. Although vitrification is being used for embryo cryopreservation, the technology is far from standardized. Type of cryoprotectant used, concentrations, vitrification devices, and their cooling rates are some of the factors that vary among laboratories which may influence clinical outcomes. In this study, all the procedures were done in one single laboratory with the same team of embryologists, reducing the variable factors.

To our knowledge, this is the first study that compared pregnancy rates of blastocysts vitrified on day 5 and day 6 using self-oocytes with PGT-A, self-oocytes without PGT-A, and OD cycles. Although untested day 6 blastocysts may have lower implantation potential when compared with untested day 5 blastocysts, euploid embryos have similar pregnancy outcomes regardless of the day of development. The results of the study have practical implications as they could affect cryopreservation policies of the clinic and strategy for better utilization of embryos with delayed development. The main limitation of the study apart from being retrospective in nature is its’ sample size. Thus, a study with a larger sample size will be of immense help in achieving a high pregnancy rate with embryos having delayed development.

Conclusions

The present study demonstrated comparable pregnancy outcomes following FET of euploid embryos vitrified on day 5 and day 6. However, higher LBRs were reported in day 5 group in untested embryos using self-oocytes and donor oocytes.

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Conflicts of interest

There are no conflicts of interest.

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