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Assisted reproduction technology: Comparison of clinical outcomes between day 3 and day 5 embryo transfers

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Abstract:

BACKGROUND: Embryo transfer (ET) is a critical step in *in vitro* fertilization (IVF). Selecting the day of transfer for achieving the desired outcomes has been a great challenge.

AIMS AND OBJECTIVES: The aim of this study was to compare the pregnancy rates of day 3 and day 5 ET in assisted conception.

MATERIALS AND METHODS: This is a longitudinal prospective study of 122 eligible patients that underwent assisted reproduction program in our facility. All patients had controlled ovarian hyperstimulation using antagonist protocol. Patients with four or more zygotes were randomly allocated on day 1 to either day 3 or 5 transfers (58 vs. 64 patients). Fertilization was achieved through conventional IVF. Zygotes were kept in a single-step medium (Global total®) for day 3 and 5 transfers, respectively. The morphologically best two or three embryos or blastocysts were chosen for transfer in both groups.

RESULTS: The overall clinical pregnancy and live birth rates for both groups were 40.2% and 33.6%. There was no statistically significant difference between day 3 and day 5 transfer regarding clinical pregnancy rates (36.2% vs. 43.8% [$P = 0.51$]), live birth rates (27.6% vs. 9.1% [$P = 1.0$]), twinning rates (18.8% vs. 20% [$P = 1.0$]), and rates of early pregnancy loss (8.6% versus 4.7% $P = [0.2]$).

CONCLUSION: In this study, the clinical outcomes of blastocyst transfer are similar to day 3 ET. This underscores the need for patient selection for the choice of days of ET. Further controlled randomized prospective studies with larger sample sizes are recommended.

Keywords:

Blastocyst, cleavage, day 3 transfer, Ilorin, Nigeria

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Introduction

Embryo transfer (ET) is a crucial step in *in vitro* fertilization (IVF). Determining the day of transfer for achieving favorable outcomes has been a challenge for reproductive medicine specialists. In the beginning, ETs were done on day 2 and day 3 at the cleavage stage (4–8 cell stage) with low implantation rates of 10%–20%, due to difficulties in culturing human embryos for a period of 5 days.[1] Hence, the usual practice was to transfer multiple embryos into the uterus to improve the pregnancy rate. Hence, the usual practice was to transfer multiple embryos into the uterus to improve the pregnancy rate, which result in high orders multiple pregnancy complications such as abortions and premature deliveries.[2]

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Embryos that are 2–3 days old are normally found in the fallopian tubes, not in the uterus. The embryo reaches the uterus about 80 h after ovulation. Embryo implantation process begins about 3 days later – after blastocyst formation and hatching out of the embryonic shell have occurred.

Many 2-day-old or 3-day-old embryos do not have the capacity to become high-quality blastocysts and make a viable pregnancy. However, on day 2 or 3 of culture, we do not have methods to determine which embryos will be viable on long-term and which will soon arrest their development.[3]

With the introduction of blastocyst culture and transfer, several studies have reported higher pregnancy rate.[1,4-6] However, there are conflicting reports on the superiority of day 5 ET compared with day 3 ET. Hence, the study aimed at comparing the clinical outcomes of embryos transferred on day 3 to those transferred on day 5.

Materials and Methods

This is a longitudinal prospective study of patients that underwent conventional IVF treatment cycles at the Assisted Reproductive Technology unit of the University of Ilorin Teaching Hospital between January 1, 2012, and December 31, 2016. A total of 122 patients were included in the study having four or more zygotes on day 1 (after confirmation of fertilization as evidenced by the presence of two distinct pronuclei and two polar bodies) and were subsequently allocated to either day 3 or day 5 groups using simple random sampling technique. Inclusion criteria were women's age < 40 years, body mass index of 18–30 kg/m^2, normoresponders, having more than four zygotes on day 1, and male partner with normozoospermia, i.e., (sperm count of at least 15 million cells per milliliter of semen and progressive sperm motility [Grade A + B] of 50% or greater).

Stimulation protocol

All patients were treated with the gonadotropin-releasing hormone (GnRH) antagonist protocol. They were commenced on 150 IU (2 vials) of recombinant follicle-stimulating hormone (FSH) Gonal F (Gonal F[R]; Merck Serono, Germany) and 75 IU (1 vial) highly purified FSH (Folliculin®; Barrat pharmaceutical, India) on day 3 of menstrual cycle for 11–14 days. Transvaginal ultrasonographic scan was done at interval from day 5/6 of stimulation to determine the numbers, size of follicles, and endometrial thickness. Subcutaneous 2.5 mg daily GnRH antagonist (Cetrotide®; Merck Serono, Germany) was administered whenever the follicles have grown to 14 mm size usually around day 6/7 of stimulation and was continued till the day of trigger to prevent premature luteinizing hormone surge. 83 μg (2000 IU) of recombinant human Chorionic gonadotrophin (hCG: Ovitrelle; Merck Serono, Germany) and 0.25 mg of buserelin (Supricur®; Aventis Pharm, West Malling, UK) were administered subcutaneously for trigger whenever two or more follicles have grown to 18 mm or more.

Oocyte retrieval

Oocyte retrieval was done at 35.5 h of hCG injection by transvaginal needle aspiration under ultrasound guidance with the aid of paracervical block. The aspirate in conical test tubes each containing 1 ml of Global Collect® was then transferred immediately to the laboratory for oocyte screening and pickup. Oocytes were rinsed in oocyte handling medium (Global collect®; LifeGlobal, Europe) and also rinsed and cultured in a center well dish (Oosafe, Denmark) of 1 ml fertilization media (Global total for fertilization, LifeGlobal, Europe) overlaid with 1 ml paraffin oil (LifeGlobal, Europe) which is then incubated for 4–6 h prior insemination.

Sperm preparation

All semen samples were allowed to liquefy at room temperature for 30 min. Semen analysis was performed according to the World Health Organization (WHO) guidelines (WHO, 2010).[7] The density gradient centrifugation method of semen preparation was used. AllGrad 90% and 45% (LifeGlobal, Europe) were overlayed with a maximum of 2 ml raw semen and centrifuged at 300 g for 20 min. The pellets were resuspended into Falcon tube containing 5 ml AllGrad wash (LifeGlobal, Europe) and centrifuged again at 300 g for 10 min.

Insemination procedure

Postwash spermatozoa at a final concentration of 150,000 × 10^6/ml were added to the oocytes and incubated for 16–18 h. A maximum of eight oocytes were inseminated in a center well dish (Oosafe, Denmark) of global total for fertilization (LifeGlobal, Europe) overlaid with paraffin oil (LifeGlobal, Europe).

Assessment of fertilization

Denudation of cumulus cells was performed by the use of glass denuding pipettes (Vitromed GmbH, Germany). The oocytes were then rinsed four times in a single-step medium (Global total, LifeGlobal, Europe) and assessed for fertilization before further culture in global total (Life Global, Europe). The oocytes were considered fertilized when two distinct pronuclei and two polar bodies were visible.

Simple random sampling was used to randomly allocate patients with four or more zygotes on day 1 (post retrieval) to either day 3 or day 5 transfers. Randomization was done by drawing lots in sealed envelopes.

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Embryo culture

A maximum of six fertilized oocytes were cultured in a center well dish with 1 ml single-step media (Global total, Life Global, Europe) under oil (Paraffin oil; Life Global, Europe) at 37°C in a humidified atmosphere of 5% O_2 in air for day 3 or day 5.

Embryo grading

Assessment of cleavage stage embryos and grading was done on postretrieval day 3, based on the number of blastomeres, symmetry (evenness of blastomere size), and the degree of fragmentation using the Society for Assisted Reproductive Technology grading system.[8] Assessment of blastocyst and grading was also done postretrieval day 5, based on the expansion, inner cell mass, and Trophectoderm according to Gardner *et al*.[9]

Embryo transfer

Best cleavage stage embryos with ≥8 cells, symmetrical, and <20% extracellular fragments were transferred for the day 3 group, while the best quality blastocyst and expanded blastocyst were transferred for the day 5 group. ET was done under transabdominal ultrasound guidance, and the transfer catheters were checked to ensure that all the embryos were transferred. In case of retained embryo (s), the embryo (s) were reloaded in a new transfer catheter and transferred immediately. The number of embryo transferred was individualized, 2 or 3 in most cases.

All ET were performed with a soft catheter (Kitazato, Spain).

Luteal phase support

The luteal phase support was conducted with progesterone (800 mg twice daily [Cyclogest pessaries®; Cox, Barnstaple, UK] and intramuscular 100 mg twice weekly [Gestone®; Ferring, Pharmaceutical, Mumbai, India]).

Pregnancy test

Serum pregnancy test was carried out 2 weeks after ET and subsequently transvaginal ultrasound at 6th week for detection of gestational sac and/or viability of the fetus.

Statistical analysis

Statistical analysis was done using Epi Info version 7.1.3.0 (Centers for Disease Control and Prevention, Atlanta, USA). Categorical data were expressed as numbers and percentages, while numerical data were expressed as a mean and standard deviation. Associations of categorical variables were tested using Chi-square test, while statistical significance was set at $P \leq 0.05$. Results were presented in tables.

Results

A total of 122 patients were enrolled for the study. The patients aged 27–40 years with a mean age of 32 ± 3.3 years, while their spouse aged 31–50 with a mean age of 38 ± 4.5 years. The duration of infertility ranged from 1 to 18 years (4.4 ± 2.2 years) with majority (73%) presenting with 1–5 years of infertility. More than half (55.7%) had primary infertility, while female factor alone (40.2%) is the leading cause of infertility [Table 1].

The mean number of oocyte retrieved and fertilized for day 3 and day 5 groups was 10.33 ± 4.4, 7.14 ± 2.9 and 11.77 ± 5.3, 7.95 ± 3.3 respectively [Table 2].

Table 1: Demographic data (*n*=122)

Variables	Frequency (%)
Age (years)	
25-29	23 (18.85)
30-34	62 (50.82)
35-39	31 (25.41)
40-44	6 (4.92)
Range	27-40
Mean	32.63±3.3
Age of spouse (years)	
30-34	19 (15.57)
35-39	54 (44.26)
40-44	32 (26.22)
45-49	14 (11.48)
50-54	3 (3.45)
Range	31-50
Mean	38.72±4.5
Duration of infertility (years)	
1-5	89 (72.95)
6-10	29 (23.77)
11-15	3 (2.46)
16-20	1 (0.82)
Range	1-18
Mean	4.46±2.7
Type of infertility	
Primary	68 (55.74)
Secondary	54 (44.26)
Causes of infertility	
Male factor alone	25 (20.49)
Female factor alone	49 (40.16)
Male/female factor	31 (25.41)
Unexplained	17 (13.93)
Day of embryo transfer	
Day 3	58 (47.54)
Day 5	64 (52.46)

Table 2: Oocytes retrieved and fertilized

Variables	Group 1 (day 3)	Group 2 (day 5)
Mean number of oocytes retrieved	10.43±4.4	11.77±5.3
Mean number of oocytes fertilized	7.14±2.9	7.95±3.3

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Table 3: Clinical outcome

Variables	Group 1 (day 3) (*n*=58)	Group 2 (day 5) (*n*=64)	χ^2	df	*P*
Clinical pregnancy per ET (%)	21 (36.2)	28 (43.8)	1.3466	2	0.51
Miscarriage rate (%)	5 (8.6)	3 (4.7)	1.5064		0.2009
Live birth rate (%)	16 (27.6)	25 (39.1)	1.1924	0	1.0000
Multiple birth rate (%)	3 (18.8)	5 (20.0)	0.3234	0	1.0000

ET: Embryo transfer

The overall clinical pregnancy and live birth rates for both groups were 40.2% and 33.6%, respectively. The clinical pregnancy rate per embryo transferred was higher for the day 5 transfer than the day 3 transfer (43.8% vs. 36.2%), while the miscarriage rate was higher for the day 3 transfer than the day 5 transfer (8.6% vs. 4.7%). However, the live birth rate was higher for the embryos transferred day 5 than those transferred day 3 (39.1% vs. 27.6%) and the multiple birth rates were also higher for the day 5 transfer group than the day 3 transfer group (5 [20%] vs. 3 [18.8%]). The differences were not statistically significant [Table 3].

Discussion

ET is a pivotal step in IVF. Assigning the day of transfer for attaining the desired outcomes remains a global enigma. This study compared the clinical outcomes of embryos transferred on day 3 to those transferred on day 5. The outcome measures were clinical pregnancy per ET, miscarriage rate, live birth rate, and multiple birth rates.

In this study, the embryos transferred on day 5 had higher clinical pregnancy per embryo transferred than those transferred on day 3, although the difference was not statistically significant. This is similar with the findings of previous studies.[3,9,10] This could be due to the fact that prolonged cell culture allows for improved selection of good grade embryos and decreases the rate of aneuploidy[11] and also, day 5 ET allows for better synchrony between the day 5 embryo, or "blastocyst," and the uterine lining, which could theoretically result in an improvement in pregnancy rates.[9] Conversely, Scholtes and Zeilmaker reported comparable overall results of pregnancy and implantation rates after day 3 and day 5 in their prospective randomized study.[12]

The miscarriage rate was higher for the embryos transferred on day 3 than those transferred on day 5, although the difference was not statistically significant. This is similar with the findings of Levron *et al*.[13] and Wilson *et al*.,[14] who reported decreasing miscarriage rate with blastocyst transfer, i.e., day 5 transfer. This may be related to the higher probability of transferring genetically and developmentally competent embryos at day 5.

Similarly, there was insignificant difference between live birth rates on embryos transferred on day 5 and day 3, although day 5 birth rate was higher than day 3. This agrees with the findings of Schwärzler *et al*.[10] and Papanikolaou *et al*.[15] where blastocyst transfer had a higher pregnancy rate and higher total take home baby rate (THBR), although a significant difference was obtained in both groups. However, limited sample size in this study could account for the difference. On the contrary, prolongation of embryo culture did not improve clinical outcomes in terms of THBR.[16]

Multiple birth rates were also higher for the day 5 transfer than the day 3 transfers, although the difference was not statistically significant. This is in consonance with the findings of Schwärzler *et al*.[10] and Papanikolaou *et al*.[15] that reported a higher rate of multiple gestations in the blastocyst group than the cleavage stage group. A possible explanation is that those embryos were chosen that have progressed past embryonic genome activation and thus believed to be more developmentally competent.

Conclusion

Although there appears to be theoretical advantages in favor of ET at the blastocyst stage, i.e. day 5 over day 3 transfer as regards improved clinical outcomes. Our findings revealed similar pregnancy, live birth, miscarriage, and twinning rates in both groups. Hence, day 5 transfer is not superior to day 3 transfer. Therefore, the choice of days of ET should be patient centered. Further controlled randomized prospective studies with larger sample sizes are recommended.

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Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Bungum M, Bungum L, Humaidan P, Yding Andersen C. Day 3 versus day 5 embryo transfer: A prospective randomized study. Reprod Biomed Online 2003;7:98-104.
2. Coskun S, Hollanders J, Al-Hassan S, Al-Sufyan H, Al-Mayman H, Jaroudi K, *et al*. Day 5 versus day 3 embryo transfer: A controlled randomized trial. Hum Reprod 2000;15:1947-52.
3. Hatırnaz Ş, Kanat Pektaş M. Day 3 embryo transfer versus day 5 blastocyst transfers: A prospective randomized controlled trial.

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Turk J Obstet Gynecol 2017;14:82-8.
4. Tobias T, Sharara FI, Franasiak JM, Heiser PW, Pinckyney-Clark E. Promoting the use of selective single embryo transfer in clinical practice. Fertil Res Pract 2016;2:1.
5. Faduola P. Selecting a single embryo for transfer after *in vitro* fertilization: A Translational medicine perspective. Transl Biomed 2015;6:1-19.
6. Gerris J, De Neubourg D. Single embryo transfer after IVF/ICSI: Present possibilities and limits. J Obstet Gynecol India 2005;55:26-47.
7. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva, Switzerland: WHO Press; 2010.
8. Racowsky C, Vernon M, Mayer J, Ball GD, Behr B, Pomeroy KO, *et al.* Standardization of grading embryo morphology. J Assist Reprod Genet 2010;27:437-9.
9. Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB, *et al.* Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertil Steril 1998;69:84-8.
10. Schwärzler P, Zech H, Auer M, Pfau K, Göbel G, Vanderzwalmen P, *et al.* Pregnancy outcome after blastocyst transfer as compared to early cleavage stage embryo transfer. Hum Reprod 2004;19:2097-102.
11. Maxwell SM, Melzer-Ross K, McCulloh DH, Grifo JA. A comparison of pregnancy outcomes between day 3 and day 5/6 embryo transfer: Does day of embryo transfer really make a difference? J Assist Reprod Genet 2015;32:249-59.
12. Scholtes MW, Zeilmaker GH. A prospective, randomized study of embryo transfer results after 3 or 5 days embryo culture in *in vitro* fertilization. Fertil Steril 1998;65:1245-8.
13. Levron J, Shulman A, Bider D, Seidman D, Levin T, Dor J, *et al*. A prospective randomized study comparing day 3 with blastocyst-stage embryo transfer. Fertil Steril 2002;77:1300-1.
14. Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, Lyles R, *et al.* Integration of blastocyst transfer for all patients. Fertil Steril 2002;77:693-6.
15. Papanikolaou EG, D'haeseleer E, Verheyen G, Van de Velde H, Camus M, Van Steirteghem A, *et al.* Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study. Hum Reprod 2005;20:3198-203.
16. Lundqvist M, Rova K, Simberg N, Lundkvist O. Embryo transfer after 2 or 5 days of IVF culture: A retrospective comparison. Acta Obstet Gynecol Scand 2002;81:126-32.