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DAY 3 VERSUS DAY 5 EMBRYO TRANSFER A PROSPECTIVE RANDOMIZED STUDY

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ABSTRACT

Background: Transfer of embryos at the blastocyst stage has been associated with exceptionally high implantation rates. There are, however, only a prospective randomized studies comparing day 3 versus day 5 embryo transfers. Furthermore, the number of embryos replaced in the day 3 group transfer is often higher than the number of blastocysts replaced, thereby affecting implantation rates-Subjects & Methods: A total of 86 patients undergoing standard Intracytoplasmic sperm injection who had developed at least five 8-cell embryos, symmetrical cells and showing <11% extracellular fragmentation on day 3 were randomized for day 3 or day 5 transfer. In this prospective, randomized study the implantation and pregnancy potential of embryos transferred on day 3 or day 5 were compared-Results: There was statistically significant difference between day 3 and day 5 transfer regarding clinical pregnancy rates (29.5% versus 66.7%), implantation rates (7.9% versus 34.7%), twinning rates (2.3% versus 11.9%)-Conclusion: Higher significant difference in clinical pregnancies,implantation rate in blastocyst transfer group than cleavage transfer group so it is possible to decide on performing blastocyst transfer when having 5 or more fertilized oocytes with 8-cell embryos, symmetrical cells and showing <11% extracellularfragmentation on day 3, without risking for a transfer cancellation.

Key words: blastocyst,culture,embryo,transfer

INTRODUCTION

The aim of an IVF procedure should be to allow couples to take home a single healthy baby per stimulated cycle. However, the question remains regarding which stage is optimal for transfer into the uterus[1].

Given the well-recognized risks and advantages of multiple embryo transfers, current focus in IVF programs has been towards mandating the transfer of fewer embryos to the uterus, thereby reducing the multiple gestation rates while maintaining acceptable pregnancy rates. To achieve this balance between the number of embryos transferred and success rates, it is necessary to identify the most viable embryo in a given cohort[2].

The concept of blastocyst transfer (BT) is not new to the field of assisted reproduction. There have been reports of BT pregnancies in human as early as 1978 and even earlier in animals. However, the ability to consistently produce a high percentage of blastocysts from cultured embryos is a relatively recent development[3].

One of the main arguments for doing day five transfers as suggested by Glujovsky et al. is that only the strongest embryos have survived until this point and therefore are more likely to be chromosomally normal and hence develop into the take home baby[4].Another reason some clinics prefer to complete a day five transfer is that when the transfer is completed and the embryo is the uterus, it is in a location more aligned with where the embryo would be expected to be in natural cycle. It was suggested that when transfers occur on day two or three that the embryo is placed in the uterus too prematurely[5].

Also another arguments for doing day 5 is that, Several studies reported higher pregnancy rate by transferring embryos at blastocyst stage ,the most famous study published 10 years ago comparing transferring embryos on day 3 or day

5 was The meta-analysis by Papanikolaou et al., in 2006 analyzing eight randomized controlled trials (RCTs) concluded that the clinical pregnancy rate and live birth rate after fresh IVF were significantly higher after blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer when equal number of embryos were transferred in the two groups compared[6].

It is important to remember that there are two sides to every coin, a recent Cochrane review found that woman who are waiting for a day five embryo transfer have lower transfer rates than woman who have day three transfers, embryos arrest on day four which means that they would have been able to have a day three transfer, but unfortunately none survived to the blastocyst day five stages [5].

SUBJECTS & METHODS

Patients, hormonal treatment and study design

During the period between March 2015 and April 2018, 86 patients seeking infertility treatment were found to be eligible to participate in the study undergoing first attempt ICSI cycle. Eligibility inclusion criteria were: (i) Patient in age group between 20 and 38 years; (ii) Undergoing first attempt ICSI; (iii) With adequate ovarian reserve ; including FSH ≤ 10 IU/ml and antral follicle count [AFC] ≥ 8FSH; (iv) Has normal uterine cavity as evaluated by 2D U/S; (v) Get ≥ 5 good quality embryos; (vi) Accepted to have frozen cycles. Eligibility exclusion criteria were: (i) Patient has endometriosis; (ii) Has poor ovarian reserve; (iii) With recurrent implantation failure and (iv)Severe male factor (TESE).

Multifollicular ovarian stimulation:

All the patients start treatment with long GNRH agonist protocol by subcutaneous daily injections of GnRH-a on day 21 of the cycle (Triptorelin, 0.1mg Decapeptyl, Ferring, Sweden). Stimulation is performed using human menopausal gonadotropin (Merional, IBSA, Italy) or purified urinary FSH (Fostimon, IBSA, Italy) starting on day 3 of the cycle in a daily dose of 3 ampoules and adjusted according to ovarian response. Ovarian response is monitored by trans-vaginal ultrasonography and E2 level.

Oocytes retrieval:

When there are five or more follicles with a mean diameter of >17 mm and serum estradiol (E2) level of ≥ 400 Pg/ml per mature follicle, 10,000 IU of HCG (Choriomon , IBSA, Italy) is administered.Oocytes are retrieved trans-vaginally under U/S guidance; 34-36 hours after HCG administration.

Culture media and procedure for culturing

On the morning of day 3, patients with five or more 8-cellembryos with <20% extracellular fragments, symmetrical cells were randomly selected to have their embryos cultured for either 3 or 5 days in the sequential media system used in the standard ICSI programme.

Randomization

The randomization was performed on day 3 of embryo culture if the patients had ≥ 5 good quality embryos by the embryologist in the IVF laboratory using a computer-generated randomized list. All the patients gave their signed informed consent. Our primary outcomes were clinical pregnancy rate per patient randomized, expressed as a percentage. The secondary outcome measure was the implantation rate.

Embryo evaluation

All embryos are evaluated by an embryologist at 16-18 hours post ICSI for detection of fertilization and subsequently on day 3 and day 5 using an inverted microscope with a hoff man modulation contrast system under 400 x magnifications.All embryos are evaluated on day 3 according to Society for Assisted Reproductive Technology grading system as follows: Blastomere number and quality, symmetry, fragmentation into good, fair, poor. A top-quality embryo was considered as having at least eight cells on day 3, with ≤11% fragmentation, regular size of the blastomeres.

Embryo morphology classification, embryo transfer

The 1st group (44 patients): Embryo transfer was performed on day 3; vaginally under abdominal ultrasound guidance. Up to four embryos were transferred The 2nd group (42 patients): These embryos were selected for extended blastocyst culture till day 5 by transferring embryos in the morning of day 3 into a new culture dish with a blastocyst medium (Sage, Quinn's advantage protein plus blastocyst medium, coopersurgical, USA) . Blastocyst quality was assessed according to the criteria of Gardner and

Schoolcraft (Gardner and Schoolcraft, 1999)[7]. We evaluate the blastocyst stage morphology by identifying degree of Expansion, Compactness of inner cell mass (ICM) and number of Trophectoderm cells and classify it into good, fair, poor. Blastocyst transfer is performed on day 5; vaginally under abdominal ultrasound guidance.

Luteal phase support and pregnancy test

Luteal phase was supported by vaginal progesterone 600 mg daily (Utrogestan; Besins International, Drogenbos, Belgium). To assess treatment outcome, serum β-HCG was measured 14 days after oocyte pickup and was repeated 3 days later. A rise in serum HCG (>20 IU) on two consecutive blood tests indicated pregnancy. Clinical pregnancy was defined by the ultrasound observation of fetal cardiac activity after 7 weeks of gestation. The implantation rate was detected as the number of gestational sacs divided by the number of embryos.

STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Continuous Quantitative variables e.g. age were expressed as the mean ± SD & median (range), and categorical qualitative variables were expressed as absolute frequencies "number" & relative frequencies (percentage). Continuous data were checked for normality by using Shapiro Walk test. Mann-Whitney U test was used to compare two groups of non-normally distributed data.

Categorical data were compared using Chi-square test or Fisher's exact test when appropriate. All tests were two sided. p-value< 0.05 was considered statistically significant (S), p-value <0.001 was considered highly statistically significant (HS), and p-value ≥ 0.05 was considered statistically insignificant (NS).

RESULTS

Table (1): Comparison between 3 day embryo transfer group and 5 day blastocyst transfer group regarding basic characteristics

Item	GroupA (N=44)		Group B (N=42)		Test	P-value
	No.	%	No.	%		
Age (years)						
Mean ± SD	31 ± 5.47		28.81 ± 4.86		1.959*	0.053
Median (Range)	32 (20 – 38)		28.5 (19 – 38)			
Duration of infertility(years)						
Mean ± SD	3.07 ± 1.64		3.43 ± 2.20		-0.863*	0.390
Median (Range)	3 (1 – 10)		3 (1 – 9)			
Type of infertility						
Primary	30	68.2%	32	76.2%	0.685#	0.407
Secondary	14	31.8%	10	23.8%		
Causes of infertility						
Male	19	43.1%	25	59.5%	2.54#	0.280
Tubal	7	15.9%	6	14.3%		
Unexplained	18	40.9%	11	26.2%		
Count of sperms in millions						
Mean ± SD	41.09± 21.22		39.48 ± 26.12		909.00	0.896
Median (Range)	45 (3 – 85)		42.5(2 – 75)			
Motility						
Mean ± SD	31.11 ± 16.09		31.71 ± 17.04		888.5	0.758
Median (Range)	30 (1 – 70)		32 (2 – 60)			
Abnormal forms						

Item	GroupA (N=44)		Group B (N=42)		Test	P-value
	No.	%	No.	%		
Mean ± SD	93.18± 5.97		93.30± 7.46		701.0	0.050
Median (Range)	96 (65 – 98)		96 (60 – 99)			

Table (2): Comparison between 2 groups regarding embryos evaluation

Item	GroupA (N=44)	Group B (N=42)	Test	P-value
Number of oocyte retrieved				
Mean ± SD	15.84± 5.95	16.97 ± 5.91	759.5	0.266
Median (Range)	15 (8 – 34)	18 (7 – 30)		
Number of oocyte injected				
Mean ± SD	11.75 ± 4.08	13.45 ± 4.8	686.5	0.081
Median (Range)	11 (6 – 27)	12.5 (6 – 26)		
No of embryos fertilized				
Mean ± SD	8.55± 3.316	9.8± 3.32	622.0	0.081
Median (Range)	8 (5 – 23)	9 (5 – 18)		
No of embryos cleaved				
Mean ± SD	8.32± 3.21	9.56± 3.11	611.0	0.07
Median (Range)	8 (5-23)	8 (5-16)		

* Mann Whitney U test. # Chi-square test. P < 0.05 is significant. NS: Not significant.

Table 3: No of Embryo transferred, Clinical pregnancy, Implantation rate

Item	Group A (N=44)		Group B (N=42)		Test	P-value
No. of embryo transferred						
Mean ± SD	3.11 ± 0.38		2.26 ± 0.44		-6.813•	<0.001
Median (Range)	3 (2 – 4)		2 (2 – 3)			
Two embryos	1	2.3%	31	73.8%	48.188‡	<0.001
Three embryos	37	84.1%	11	26.2%		
Four embryos	6	13.6%	0	0%		
Remaining embryos for cryopreservation	3.98 ± 1.74		3.24 ± 1.67		2.667	0.008
Clinical pregnancy	13	(29.5%)	28	(66.7%)	10.87	0.000
Implantation rate (%)						
Mean ± SD	9.95±16.47		36.45±30.83		-4.507	<0.001
Median (Range)	0 (0-66)	7.9 %	50(0-100)	34.7 %	Chi squ	<0.001
No. of gestational sacs						
Mean ± SD	0.31 ± 0.51		0.78 ± 0.64		3.516	<0.001
Median (Range)	0 (0 – 2)		1 (0 – 2)			
One sac	12	(27.3%)	23	(54.8%)	Fisher	0.001
Two sacs	1	(2.3%)	5	(11.9%)	exact	

Mann Whitney U test. P < 0.05 is significant

A total of 86 patients were included in the study. Embryo transfers were performed in 44 patients in the day 3 group (group A) and in 42 in the day 5 group (group B). Demographic data are given in Table 1. No statistical differences were seen between the two groups concerning age, duration of infertility, type of infertility and causes of infertility.Table 2 shows that the two groups were comparable regarding the number of oocytes retrieved, no of embryo fertilized and cleaved, In table 3group A,1 patient had two embryos transferred, 37 patients had three embryos transferred, 6 patients had four embryos transferred, whereas in group B: 31 patients had twoblastocyst transferred, 11 patients had three blastocysts with significant difference between the two groups ($p<0.001$), significantly more patients had embryos cryopreserved on day 3 compared with day 5 ($P = 0.008$). Also the clinical pregnancy was much higher in group B than that in group A (66.7% and 31.8% respectively. Implantation rate was much higher in group B than in group A (34.7%, 7.9 % respectively), 2 gestational sacs were reported in 5 patients (11.9%) in group B compared to 1patient (2.3%) in group A.

DISCUSSION

The primary outcome was the clinical pregnancy rate and implantation rate. The clinical pregnancy rate in day 3 and day 5 embryo transfer in fresh trial was (31.8%, 66.7%) respectively with a highly significant difference between them. As regards to multiple pregnancy, 1 patient in group A had 2 sacs (2.3%) and 5 patients in group B had 2 sacs (11.9 %), while the mean of implantation rate was 9.95±16.47 (7.9%) in group A and 36.45±30.83 (34.7%) in group B with also a significant difference between the 2 groups. The result of this study will give us the confidence to limit transfer to two or lesser blastocyst.

The results of the present study agreed with a meta-analysis published by Papanikolaou et al (2006), where they reanalyzed eight randomized controlled trials (RCTs) and concluded that the clinical pregnancy rate and live birth rate after fresh IVF were significantly higher after blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer when equal number of embryos were transferred in the two groups compared[6].Similar results were reported by Ann et al. (2009) who concluded that there was significantly higher implantation and clinical pregnancy rates in the blastocyst transfer group than cleavage stage group (40.16% vs. 11.43%, P = 0.00; 62% vs. 29.8%, P = 0.00)[8]and by Prabheen- et al. (2014) who states that clinical pregnancy from blastocyst transfer was significantly higher (44%) as compared to cleavage stage embryo transfer which was (30%)[9].Milki et al., (2000) had also shown that blastocyst transfer resulted in a higher pregnancy rate compared to day-3 group in women under age 40 when more than three 8-cell embryos were present on day 3 of culture [10].The same conclusion was made by Hatrnaz et al. (2017) where blastocyst stage resulted in significantly higher pregnancy rate compared to the transfer at the cleavage stage (51.3% vs 27.4%)[11].Subsequently 27 RCTs summarized in the Cochrane review made by Glujovsky et al., 2016 demonstrated that live birth rates is slightly higher by performing fresh blastocyst transfer compared to cleavage stage embryo transfers, but no differences were observed in the analysis of 23 RCTs in either clinical pregnancy rates or miscarriage rates. However, they included trials using a different number of embryos transferred in the groups compared which might have conditioned their results[12].

On the contrary to the present study, Hreinsson et al.,(2004) reported that no significant difference was observed in implantation rate (21.1% vs 20.9% and clinical pregnancy rates (36.7% vs. 32.5%)after blastocyst and cleavage stage transfers for the 2 groups[13].Bungum et al. (2003) also found that in patients who had developed at least three 8-cell embryos showing less than 20% fragmentation, the implantation and pregnancy potential of an equal number of embryos transferred on day 3 or day 5 were comparable and there was no statically difference between the 2 groups regarding clinical pregnancy rate (61% vs. 51%),or implantation rate (44 % vs. 37%)[14].Remaining embryos available for cryopreservation was 3.98 ± 1.74 in group A and 3.24 ± 1.67 in group B with a statically significant difference between the 2 groups.This gave a greater advantage for day 3 transfer as more embryos could be

cryopreserved with more trials of transfer after one retrieval. All the patients in group A had embryos to freeze, thus leading to more trials for thawing and transfer in group A.

CONCLUSION

Higher significant difference in clinical pregnancy ,implantation rate in blastocyst transfer group than cleavage transfer group especially in fresh trials so it is possible to decide on performing blastocyst transfer when having 5 or more fertilized oocytes, without risking for a transfer cancellation, or decreasing the percentage of patients with vitrified embryos and number of embryos vitrified.

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