Day 3 versus day 5 embryo freezing: which is better, A comparative study

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Abstract:

Background: Freezing of embryos and gametes is considered one of the corner stones of ART. Its application increases both IVF safety and efficiency.

Aim and objectives: the aim of the present study is to compare cleaved embryo and blastocyst freezing to determine the optimal time for embryo cryopreservation,

Subjects and methods: A randomized clinical trial, it was involved 300 cases of infertile patients who will undergo ICSI cycles and embryo freezing at the assisted reproduction unit, Qena University hospital, South Valley University, Egypt after complete infertility evaluation. Population was divided into two groups: Group 1 (n =150), underwent embryo freezing at day 3 then will undergo FET (Cleaved embryo). Group 2 (n =150), will underwent embryo freezing at day 5 then will undergo FET (Blastocyst) The duration of the study had been from 6 to 12 months,

Results: the results revealed that there is high significant difference between the studied groups as regard Total number of survival more survival was observed in blastocyst group, while there is no significant difference between the studied groups as regard completed transfer.

Conclusion: The maintenance of embryo culture until day 5 may be a more sensible approach for the correct identification of best quality embryos with the highest probability of success for both transfer and freezing.

Keywords: Embryo, Blastocyst, Transfer, Intra-Cytoplasmic Sperm Injection.

Introduction

Freezing of embryos and gametes is considered one of the cornerstones of ART. Its application increases both IVF safety and efficiency **(Rienzi et al., 2017).** The percentage of frozen transfer cycles compared to fresh transfer cycles is increasing. It represents more than 50% in some countries **(Kupka et al., 2016).** Originally embryo freezing was used for freezing of supernumerary embryos; The percentage of embryo freezing is increasing due the expansion of its indications like freeze all protocols **(Devroey et al., 2011).**

There are two main approaches for embryo freezing: slow freezing and vitrification **(Edgar; Gook, 2012).**Vitrification is associated with higher survival rate in cleavage and blastocyst embryos **(Kolibiankis et al., 2009)** and in some studies higher implantation, clinical and ongoing pregnancy rates **(AbdelHafez et al., 2010).**

Embro transfer is done at cleavage stage or blastocyst stage **(Martins et al., 2017)** either fresh or frozen. Day 5 transfer is supposed to allow more synchrony between females uterus and transferred embryos. This also allow transfer of viable embryos leading to a higher pregnancy rate **(Glujovsky et al., 2012).** On the other hand, theoretically, due to the superiority of in vivo environment to that in vitro, some embryos may be implanted if transferred at day 3 and blocked if extended in vitro **(Racowsky C, 2000).** Also, invitro culture after embryonic genome activation may be harmful to the embryo **(Martins et al., 2017).**

There are multiple studies compared day 3 and day 5 fresh transfer outcome showing that day 5 transfer is associated higher clinical pregnancy and live birth rate **(Glujovsky et al., 2012)** and decrease aneuploidy**.** However, there are few studies compared embryo freezing at day 3 and day 5. There is no evidence to suggest the superiority of any of these two options. Therefore, in our study we will compare day 3 and day 5 embryos cryopreservation as regards the outcome of frozen embryo transfer**.**

Patients and methods:

A randomized clinical trial; Infertile patients who had been undergone ICSI cycles and embryo freezing at the Assisted Reproduction Unit, Qena University hospital, South Valley University, Egypt after complete infertility evaluation. Women were recruited from April 2019 to December 2019.

Inclusion criteria: 1. Age: 18-35 years, 2. Body mass index (BMI): ≤ 30, 3. Anti-mullerian hormone (AMH): 1 – 5, 4. No gynecological problem e.g. fibroid, endometriosis, uterine polyp,hydrosalpinx or adenomysis, 5. Male factor: mild to moderate oligo or asthenospermia and 6. AFC between 7 and 15.

Exclusion criteria: 1. Patient who refused to participate, 2. Recurrent implantation failure, 3.Patient with less than 3 embryos at day 3 and 4.Patient planned for PGD .

Study tools:

•Initial evaluation:this had been include:

1. Detailed history and clinical examination.
2. Ovarian reserve testing (serum antimullarian hormone (AMH), basal serum follicular stimulating hormone (FSH) and basal antral follicular count (AFC) by transvaginal ultrasonography (TVUS)**).**

Hormone Analysis

Blood samples were collected on the day of the ovulation trigger, and serum P levels were measured using a chemilumi- nescent immunoassay for quantitative determination of the hormone (Diagnostics Biochem Canada Inc), with a sensitivity of 0.1 ng/mL.

3. Uterine cavity examination (bytrasvaginal 3-dimentional ultrasound or office hysteroscopy)
4. Routine investigations (Complete blood count, blood grouping, liver function tests, kidney function tests, prothrombin time , prothrombin concentration , serum bloog sugar)
5. Evaluation of male factor (husbnad semen analysis).

• **Ovarian stimulation:** All the patients in the study had been undergo stimulation by gonadotrophins and had been used GnRH long agonist protocol during their ICSI cycles.

• **Final oocytes maturation:**Triggering of ovulation had been done by using Human chorionic gonadotropins (HCG) 10000 IU(ovitrelle250 microgram /0.5 ml- 2 ampoules IM) 34 – 36 hours prior to ovum pick-up (OPU).

•Transvaginal ultrasound-guided oocyte retrieval: Egg retrieval was performed by aspiration of follicular fluid by passing a hollow needle through the wall of the vagina into the ovarian follicles under sonographic guidance. The fluid aspirate was then

inspected under the microscope to do egg collection.

•Intracytoplasmic sperm injection (ICSI): After oocytes denudation .a single sperm was injected into the cytoplasm of each mature oocyte using RI micromanibulator, oocytes had been cultured in Global Total media microdroplets under oil in co2 incubator , then fertilzation check had been done 16 hours post injection.

- **Eligible women are divided into two groups:**

1. Group 1 (n =150), has undergone embryo freezing at day 3 then had been undergo FET
2. Group 2 (n =150), has undergone embryo freezing at day 5 then had been undergo FET

Embryos had been frozen by vitrification method using Dimethyl sulphoxide (DMSO) (Global DMSO Vitrification kit,LifeGlobalGroup,Canada) as cryoprotectant.

Ethical consideration:

- Informed consent was obtained from all participants after being informed about the aims and process of the study as well as applicable objectives.
- The study procedures were free from any harmful effects on the participants as well as the service provided.
- The principal investigators have kept individual data as private information safely. There was no extra fee to be paid by the participants and the investigators covered all the costs in this regard.

Data management and analysis:

Data entry, processing and statistical analysis was carried out using SPSS version 26.0. According to the type of data qualitative represent as number and percentage , quantitative data represent by mean ± SD , the following tests were used to test differences for significance.

Results:

Population were divided into two groups:

1. Group 1 (n =150), underwent embryofreezing at day 3 then had been undergo FET(Cleaved embryo)
2. Group 2 (n =150), had been underwent embryofreezing at day 5 then had been undergo FET(Blastocyst).

Table (1)shows that there is no significant difference between the two groups as regard age, BMI, marriage duration, infertility type, infertility cause or cyce of previous treatment.

Table (2) shows that there is no significant difference between the studied groups as regard E2, FSH or AMH

Table (3) shows that there is no significant difference between the studied groups as regards Cycle characteristics

Table (4) shows that the blastocycst group has a significantly higher rate of survival when compared to the cleaved embryo group. However, the transfer rate was non significantly different.

Table (5) shows that blastocyst group has a significantly higher rate of Chemical pregnancy, Clinical pregnancy and FHB/embryo transferred when compared to cleaved embryo group.

Table (1): Demographic and baseline characteristics in between the two studied groups

	Group1 (n=150)	**Group2 (n=150)**	**P value**
Age (y) Mean±SD(Range)	37.21±1.74 (35-42)	37.13±1.94 (33-42)	0.787
BMI (kg/m^2) Mean±SD (Range)	30.69±3.10 (24-37)	30.38±3.28 (24-37)	0.517
Healthy weight (18.5-24.9 kg/m^2)	12 (8.0%)	16 (10.7%)	0.638
Overweight	53	60	

(25-29.9 kg/m^2)	(35.3%)	(40.0%)	
Obese (≥30 kg/m^2)	85 (56.7%)	74 (49.3%)	
Marriage duration (y) Mean±SD(Range)	6.04±3.24 (2-18)	6.14±3.60 (1.5-18)	0.849
Infertility type			0.644
1ry	79 (52.7%)	85 (56.7%)	
2ry	71 (47.3%)	65 (43.3%)	
Infertility cause			
Male factor	28 (18.7%)	28(18.7%)	1.000
Endometriosis	4 (2.7%)	2 (1.3%)	1.000
Tubal Factor	37 (24.6%)	25 (16.7%)	0.184
Combined male & female factors	7(4.7%)	0 (0.0%)	0.121
Unexplained	74 (49.3%)	95(63.3%)	0.063
cycles of previous treatment Mean±SD (Range)	5.69±1.77 (2-10)	5.69±1.82 (0-10)	1.000

Table (2): Hormonal profile in between the two studued groups:

	Group1 (n=150)	**Group2 (n=150)**	**P value**
E2 (PG/ML) Mean±SD(Range)	86.13±18.59 (23-124)	87.32±14.79 (36-119)	0.644
FSH Mean±SD(Range)	13.67±1.87 (6.7-19.1)	14.01±2.15 (6.40-19)	0.271
AMH Mean±SD(Range)	0.66±0.22	(0.70-0.24)	0.284
	(0.25-1.1)	(0.36-1.1)	

Table (3): Cycle characteristics in between the two studied groups:

	Group1 (n=150)	**Group2 (n=150)**	**P value**
length of stimulation (days) Mean±SD(Range)	10.71±2.30 (8-14)	10.49±2.27 (8-14)	0.547
Dosage of GN used Mean±SD(Range)	406.76±29.48 (375-450)	403.53±29.92 (375-450)	0.479
Picked up follicles Median(Range)	3 (1-5)	3 (1-6)	0.948
Picked up oocytes Median(Range)	2 (1-4)	2 (1-4)	0.123
Metaphase II Median(Range)	2 (1-3)	2 (1-3)	0.134

Table (4): Survival outcomes in between the two studied groups:

	Group1 (n=150)	**Group2 (n=150)**	**OR (95% CI)**	**P value**
Total number of survival	105 (70.0%)	130 (86.7%)	2.78(1.5-5)	<0.001*
Transfer completed	26(17.3%)	28(18.7%)	0.913(0.51-1.6)	0.654

Table (5): Pregnancy outcome in between the two studied groups:

	Group1 (n=150)	Group2 (n=150)	OR (95% CI)	P value
Chemical pregnancy	75 (50.0%)	100 (66.7%)	2(1.25-3.1)	0.003*
Clinical pregnancy	71 (47.3%)	97 (64.7%)	2(1.28-3.2)	0.002*
FHB/embryo transfered	55 (36.7%)	74 (49.3%)	1.68(1.06-2.66)	0.02*

DISCUSSION

As many as one in six couples will experience difficulty conceiving and may seek assisted reproduction to achieve a pregnancy. One of the most important steps during an assisted reproduction cycle is the transfer of the embryo from the laboratory to the uterus. Traditionally, cleavage-stage embryos were transferred on day 3, but over the past decade there has been a move to transferring blastocysts on day 5 or 6. Transfer at this stage is considered to be a more physiologically appropriate time as it more closely mimics the time of natural implantation and may improve synchrony between the endometrium and embryo development (**Maheshwari et al.,2015**). An extraporation of this physiological advantage of day 5 transfer of fresh transfer is the theoretical advantage of day 5 freezing.

Direct comparisons between the two stages of embryo development appear to support the use of blastocyst transfers in clinical practice. Women who undergo fresh blastocyst transfers achieve higher live-birth rates compared with those who receive fresh cleavage-stage transfers . However, the results are not quite so conclusive when the transfers of frozen embryos are considered (**NICE., 2013**).

This is why the study was selected to be conducted to compare cleaved embryo and blastocyst freezing to determine the optimal time for embryo cryopreservation.

This randomized clinical trial included 300 cases of infertile patients who will undergo ICSI cycles and embryo freezing at the assisted reproduction unit, Qena University hospital, South Valley University, Egypt.Population were divided into two groups: Group 1 (n =150), underwent embryo freezing at day 3 then underwent FET(Cleaved embryo). Group 2 (n =150), underwent embryo freezing at day 5 then underwent FET(Blastocyst).

There is no significant difference between the two groups as regard age, BMI, marriage duration, infertility type, infertility cause or cycle of previous treatment. This finding negates the selection bias.

Various factors affect blastocyst formation and quality, including culture conditions, number of oocytes, maternal age, and male factor infertility (**Jones and Trounson,1999**). In addition, the quality of early-stage embryos can substantially influence rates of blastocyst formation rates (**Miller et al.,1999**). However, the data do not totally support the idea that the number of eight-cell embryos on day 3 and the potential for blastocyst formation are directly correlated (**Racowsky et al.,2000**); in particular, they do not support the assumption that the blastocysts that form do so from the day-3 eight-cell embryos. Confirmation of this assumption is only possible if all embryos are individually cultured.

The present study shows that there is no significant difference between the studied groups as regard E2, FSH or AMH.There is no significant difference between the studied groups as regard Picked Cycle characteristics. Our results are in line with study of **Thuyet al.,2018** as they reported that there were no statistically significant differences in the total

FSH dose and total days of stimulation.The number of retrieved oocytes, the numbers of mature oocytes using to performance of ICSI, the number of fertilized oocytes, the number of day-5 embryos and day-5 frozen embryos were recorded and analyzed. There were no statistically significant differences between the morphokineticgroup versus morphologic group. They found that there were similarities between the two groups in terms of patient characteristics and laboratory outcomes (**Thuyet al.,2018**).

The rationale of blastocyst transfer is based on increasing the probability of obtaining advanced embryos with the highest chance for survival, i.e., implantation. The prolongation of embryo culture to day 5 requires a relatively high number of top quality blastocysts. Good quality cleavage-stage embryos increases the likelihood of good quality blastocyst embryos. Therefore, it would be prudent to expect no advantage if only a few good quality blastocysts exist in the culture (**Zech et al.,2007).**

In our study we found that there is high significant difference between the studied groups as regards the total number of survivals. More survival was observed in blastocyst group, while there is no significant difference between the studied groups as regard completed transfer.There is significant difference between the studied groups as regard Chemical pregnancy, Clinical pregnancy and FHB/embryo transferred.

Previous study by **Papanikolaou et al.,2008** indicated that recruitment at the blastocyst stage yields better results than selection at day 3, which merely depends on the morphologic evaluation of embryos. These studies also claim that pregnancy rates of up to 50% can be acquired by the transfer of blastocysts when compared with embryo transfer at the cleavage stage which supports our results.

This agree with the study conducted by **Glujovsky et al.,2012** comparing day 3 and day 5 fresh transfer outcome showing that day 5 transfer is associated higher clinical pregnancy and live birth rate and decrease aneuploidy (**Alder et al,2013)..**

In comparison, A randomized study conducted by **Coskun et al.,2000** reported that day 3 and day 5 transfers yielded statistically similar overall implantation (21% vs. 23%), pregnancy (39% vs. 39%) and twinning (11.9% vs. 15%) rates in a cohort of 201 infertile women.

Conclusion

Which embryo to be transferred has represented a great dilemma in the field of ART. Along decades of trials, embryo morphology assessment is considered to be a cornerstone in choosing embryos for higher implantation and pregnancy rates. Cleavage stage has limited value for morphology assessment. Maintenance of embryo culture until day 5 has better clarifation on embryo morphology, so embryos are best evaluated during blastocyst stagefor both transfer and freezing.

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