

# Study on Effectiveness of Blastocyst Transfer over Cleavage Stage Embryo Transfer in Patients with Failed Conception

## ABSTRACT

**BACKGROUND:** IVF has been proved a blessing for many couples. Embryo selection and transfer are key steps in ART. In-vitro fertilization includes embryo transfer either at cleavage stage (day2/ day3) or at blastocyst stage (day5). Implantation rate can be improved by transferring blastocyst stage embryo. Implantation rate may decrease after repeated unsuccessful implantation. This study aims to assess the effectiveness of Blastocyst Transfer in patients with history of failed Cleavage Stage Embryo Transfer.

**METHODOLOGY:** This will be an observational study conducted at Wardha Test Tube Baby Centre, AVBRH. 40 infertile women with history of previous IVF failure will be included in the study. Embryo Transfer will be done at blastocyst stage and  $\beta$ hcg level will be measured on 14<sup>th</sup> day of embryo transfer.

**EXPECTED RESULTS:** We expect that blastocyst stage embryo transfer may give more positive result compare cleavage transfer. We predict that transferring embryo at blastocyst stage may increase implantation and pregnancy rate.

**CONCLUSION:** Blastocyst stage embryo transfer is effective compared to Cleavage Stage Embryo Transfer.

**KEYWORDS :** Cleavage Stage Embryo Transfer, Blastocyst Transfer, In-vitro Fertilization, Assisted reproduction, B-HCG Level, Conception.

## INTRODUCTION

The couple who unable to conceive a child is known as infertility. Various male and female factors can be a cause of infertility. Various treatments like IUI, IVF, ICSI can be carried out to treat this problem. Combining an egg and sperm (fertilization) artificially in the laboratory and transferring the embryos to the female uterus is refers to (ISCI) or intra-cytoplasmic sperm injection.

Embryo transfer is one of the crucial step in art treatment. Selection of embryo in ART cycle is important for positive pregnancy result. In (IVF) in-vitro fertilization embryo transfer is perform either at cleavage stage (day2/ day3) or at blastocyst stage (day5)<sup>(1)</sup>. The main factor in In-vitro fertilization is implantation, particularly among couples with earlier failed IVF cycle.

Implantation rate may be better by transferring blastocyst stage embryo instead to pick premature stage without knowing their expanding ability and allow better selection of embryos. Thus decreases the number of transferring more embryos<sup>(2)</sup>.

The most capable embryos expand to form blastocyst stage and are chosen for the transfer and the embryos that have stopped their growth are recognized and are not selected for further procedure<sup>(2)</sup>.

Implantation rate decreases after repeated unsuccessful implantation. Transfer of cleavage stage embryo is a reason for unsuccessful implantation<sup>(3)</sup>.

According to studies after many unsuccessful IVF cycles the rate of implantation decreases. The given reason for this unsuccessful implantation is transfer of day 2 / day 3 (cleavage stage) embryo to the uterus<sup>(2)</sup>. There are two options for such a couple who have had previously failed IVF cycle:

- 1) More than two day 2 or day 3 (cleavage stage) embryos can be placed in the uterus, there is a risk of multiple implantation of embryos or
- 2) To transfer day 5 (blastocyst stage) embryo to increase more chances of implantation<sup>(3)</sup>.

Previously human embryos were frequently cultured for just 2/3 days in a simple Earle's balanced salt solution or equivalents, frequently enlarged with serum. These media (simple culture media), however, are not sufficient enough to help the embryos for their metabolic need and therefore only a few number of embryos (competent embryos) grow till blastocyst stage<sup>(4)</sup>.

Now the new sequential media (culture media) has been developed without serum supplement. This culture media (sequential culture media) helps to grow embryos, provide metabolic and physiological needs to developing embryos<sup>(4)</sup>.

There is progress in the practice of transfer of day 5 stage embryo or blastocyst stage embryo after the development in sequential media which enhance embryonic growth through activation of genome, development of blastocoel, and expansion of embryo, this allows selection of capable embryos which have a higher rate of implantation<sup>(3)</sup>.

With blastocyst transfer implantation rate and pregnancy rate are higher compared with transfer of premature cleavage stage embryo<sup>(3)</sup>.

Reasons for blastocyst embryo is more successful than cleavage stage embryos because, cleavage stage embryo is physically premature stage embryo to the uterus environment. Naturally in the female reproductive part fertilized embryos pass through the oviduct and does not enter the uterus before the morula stage. Nutritional milieu is provided by the uterus from the oviduct, early embryo or premature embryo can affect with homeostatic stress, this may result in failure in implantation<sup>(5)</sup>.

By increasing the time of embryo culture till day 5/6 it permits transfer of embryo into synchronized uterine surroundings<sup>(3)</sup>. Thus it allows to select only higher quality embryos that have ability for further development and it also helps to avoid the occurrence of number of multiple pregnancy. Previously it has been observed that day 3 embryos are abnormal at chromosome level thus contribute 80 to 90% in implantation failure<sup>(5)</sup>.

Only good quality embryos will grow till blastocyst stage (day 5 to day 6) and this will allow to choose only those embryos which have capability to grow till blastocyst stage and therefore this would result in higher implantation rate, thereby lowering the multiple birth rate, thereby decreases the multiple birth rate<sup>(3),(5)</sup>.

The Blastocyst transfer increase the pregnancy rate rather than cleavage stage (day 2 and day 3) transfer and it also improves the implantation rate, the aim of my study is a simple comparison cleavage stage vs blastocyst stage transfer for prediction blastocyst stage embryo is more effective than cleavage stage embryo overall pregnancy outcome.

#### **Background/ Rationale :**

1. There is a study in December 2013 by Kaur P et al. They evaluated the efficacy of blastocyst transfer in comparison with cleavage stage transfer for this they performed randomized, prospective study on 300 patients aged 25-40 years undergoing *in vitro* fertilization. When three or more Grade I embryos were observed on Day 2 of culture, patients were divided randomly into two study groups, cleavage stage transfer and blastocyst transfer group having 150 patients each. Primary outcomes evaluated were, clinical pregnancy rate and implantation rate. The results were analyzed using proportions, standard deviation and Chi-square test, they found that Both the groups were similar for age, indication and number of embryos transferred. Clinical pregnancies after blastocyst transfer were significantly higher 66 (44.0%) compared to cleavage stage embryo transfer 44 (29.33%) ( $p < 0.01$ ). Implantation rate for blastocyst transfer group was also significantly higher ( $p < 0.001$ ) from these findings they concluded that Blastocyst transfer having higher implantation rate and clinical pregnancy rate leads to reduction in multiple pregnancies.
2. There is one study in March 2017 by SafakHatirnaz and Mine KanatPektas, they included 190 women who had ICSI after retrieval of more than four oocytes on the day of fertilization check were randomly assigned to undergo embryo transfer either on day 3 or day 5. They found that Day 3 and day 5 transfers were statistically similar with respect to the age of woman ( $p=0.107$ ), duration of infertility ( $p=0.528$ ), cause of infertility ( $p=0.850$ ), number of collected oocytes ( $p=0.119$ ), number of metaphase II oocytes ( $p=0.178$ ), number of fertilized oocytes ( $p=0.092$ ), and number of transferred embryos ( $p=0.556$ ). The number of grade 1 embryos was significantly higher in day 5 transfers than in day 3 transfers ( $p=0.001$ ). Day 3 and day 5 embryo transfers had statistically similar implantation, clinical pregnancy, twinning, and live birth rates ( $p=0.779$ ,  $p=0.771$ ,  $p=0.183$ , and  $p=0.649$ , respectively). The live birth rates in singleton pregnancies conceived after day 3 and day 5 embryo transfers were statistically similar ( $p=0.594$ ) from this result they concluded the efficacy of blastocyst transfer is not inferior to that of embryo transfer on cleavage stage. Performing blastocyst transfer may have benefits because it is associated with

acceptable pregnancy rates and morphologic assessment on day 3 has limited predictive value for subsequent embryonic development.

3. The study in 2002 by Ingrid Van der Auwera et al. they blindly randomized patients for transfer on day 2 (group 1) or day 5/6 (group 2) was performed before stimulation. Oocytes and embryos were cultured in sequential media in 5.5% CO<sub>2</sub>, 5% O<sub>2</sub>, 89% N<sub>2</sub> and 90% humidity. They transferred maximum 2 embryos per patient and they found that the success rate was significantly higher in group 2 than group 1.
4. One more study in April 2017 by W. P. Martins et al. they performed systematic review and meta-analysis of randomized controlled trials (RCTs) comparing the transfer of blastocysts (days 5–6) with the transfer of cleavage-stage embryos (days 2–3) in women undergoing in-vitro fertilization or intracytoplasmic sperm injection they observed low-quality evidence of no significant difference of blastocyst transfer on live birth/ongoing pregnancy (relative risk (RR), 1.11 (95% CI, 0.92–1.35), 10 RCTs, 1940 women, I<sup>2</sup> =54%), clinical pregnancy (RR, 1.10 (95% CI, 0.93–1.31), 12 RCTs, 2418 women, I<sup>2</sup> =64%), cumulative pregnancy (RR, 0.89 (95% CI, 0.67–1.16), four RCTs, 524 women, I<sup>2</sup> =63%) and miscarriage (RR, 1.08 (95% CI, 0.74–1.56), 10 RCTs, 763 pregnancies, I<sup>2</sup> =0%). There was moderate-quality evidence of a decrease in the number of women with surplus embryos after the blastocyst-stage embryo transfer (RR, 0.78 (95% CI, 0.66–0.91).
5. There is one more study in 2000 by Serdarcoskun et al. from Saudi Arabia, they achieved fertilization by regular IVF and intra cytoplasmic sperm injection method. Morphologically best 2 or 3 embryo chosen for transfer for both groups, they found no significant difference between those groups.

### **AIM AND OBJECTIVES**

#### **AIM:**

To study the Effectiveness Blastocyst transfer over cleavage stage embryo transfer in patients who failed to conceive with cleavage stage embryo transfer.

#### **OBJECTIVE**

- To study serum  $\beta$ HCG level on day 14<sup>th</sup> in post embryo transfer.
- To observe Cleavage vs Blastocyst transfer and correlate it's  $\beta$ HCG level. Evaluation of outcome.

### **INCLUSION CRITERIA**

- Female who failed to conceive in previous IVF cycle with cleavage stage transfer.

### **EXCLUSION CRITERIA**

- Patients not giving consent for IVF.
- Patients having uterine agenesis.
- Patients not fit for IVF having viral infections like HIV, HbsAg etc.
- Patient who's embryo does not reach till blastocyst stage.

## **MATERIALS AND METHODS**

**Study Design** – observational Study

**Study duration** – September 2019- July 2021

**Study population** – Rural infertile patients attending WARDHA TEST TUBE BABY CENTRE AT AVBRH

**Place of study** – WARDHA TEST TUBE BABY CENTRE AT AVBRH

**Sample size** – 40 infertile women for sequential embryo transfer, having history of previous IVF failure.

**Sample size formula** –

$$N = \frac{\chi^2 * N * p(1-p)}{C^2(N-1) + \chi^2 p(1-p)}$$

Total population = N=15 during 6 months

$\chi^2$  = Chi square value for 1 degrees at some desired probability level. This is 3.84 at 5%

level of significance.

P=50% proportion

Q=100-p

=50

C= Confidence interval of the one choice(95% CI)

=0.05

$$N = \frac{3.84 * 40 * 0.5 * 0.5}{(0.05)^2 * 40 + 3.84 * (0.5 * 0.5)}$$

=40

## **METHODOLOGY**

This study is done in Wardha test tube baby centre AVBRH (SAWANGI) WARDHA Relevant data on the demographics and treatment history as well as the indications for IVF treatment will be recorded. Couples undergoing research work their counselling will be done. Both verbal and written consent will be taken from the couples. Patient history will be taken.

Suitable patients undergo screening taking into account parameters such as indication, previous treatment, age, BMI, etc. Both verbal and written consent will be taken properly for research work from the couples registered for research work.

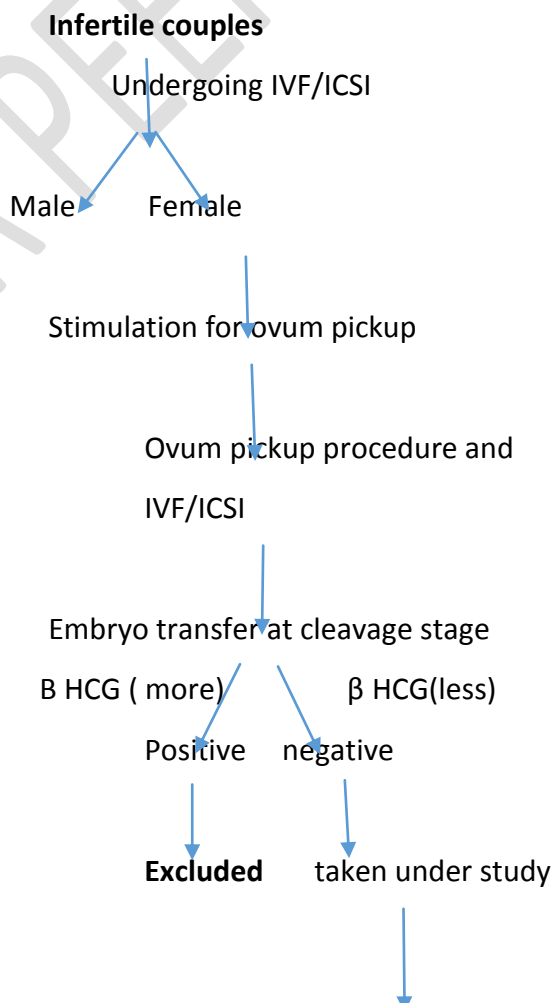
For ovum pickup procedure standard stimulation protocol is followed, from day 2 of menstruation ovarian stimulation will be given to the patient.

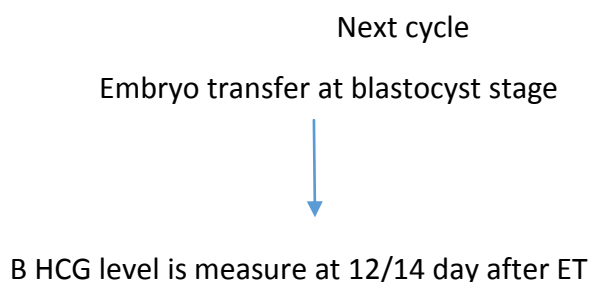
Ovum pickup procedure (ovum aspiration) will be conducted on day 10 or 12 of menstrual cycle and screening of retrieved oocyte will be done. Prior to ICSI or IVF oocytes are analysed morphologically by keeping in account characteristics like oocyte shape, size, perivitelline space, polar body, granulation, zona pellucida, cytoplasmic parameters like refractile bodies, vacuoles, smooth endoplasmic reticulum etc. The oocyte with great morphology will be chosen for procedure like IVF or ICSI.

After IVF or ICSI undergo embryo transfer procedure. Embryo transfer can be performed either at day 2/3 or at day 5 stage of embryo. Patient having history of previously failed IVF cycle with cleavage stage or day 3 stage embryo are taken under study. For this time transfer morphologically best embryo of blastocyst stage.

At last measure  $\beta$ hcg at day 14 of patient to analyse the result.

#### FLOW CHART





### **SCOPE**

By observing the embryos for longer in an advanced culture system, we are more likely to identify the embryo(s) with greater implantation potential and therefore improve pregnancy rate.

### **LIMITATION**

- If embryo doesn't reach blastocyst stage, there is risk of cancellation of procedure.
- Blastocyst culture is costlier in comparison with cleavage stage transfer.

### **EXPECTED OUTCOME**

40 women will be included. Embryo transfer is performed either at cleavage stage or blastocyst stage. Embryo can be graded by Gardner system according to day of transfer. Blastocyst stage embryo transfer to patient who had history of previously failed cleavage stage embryo transfer. We expect that blastocyst stage embryo transfer may give more positive result compared to cleavage transfer. We predict that transferring embryo at blastocyst stage may increase implantation and pregnancy rate.

### **DISCUSSION**

Patient failed with previously failed IVF cycle is a challenging couple to IVF members. After repeated failed IVF cycle rate of implantation decreases<sup>(2)</sup>.

Standard media does not support the embryo long term development thus the percentage or chances of growth of embryo till blastocyst is low and therefore this can be the initial reason for transfer of day 3 or cleavage stage embryo to the uterus<sup>(2)</sup>.

Co-culture was initially introduced to successfully obtain more number of good quality embryos. Afterwards sequential media (culture media) are introduced or designed especially for early embryo (day 2 embryo) and also for 3<sup>rd</sup> and 4<sup>th</sup> day of embryo growth (blastocyst

and morula stage embryo). The result of sequential culture media are similarly as good as with co culture media<sup>(2)</sup>.

The successful outcome of embryo transfer on day5/day6 depends on the level of blastocyst growth (development) at the period of transfer<sup>(2)</sup>.

By observing the embryos for longer in an advanced culture system, we are more likely to identify the embryo(s) with greater implantation potential and therefore improve pregnancy rates<sup>(2)</sup>. Few of the related articles have been reported<sup>8-18</sup>.

It is observed that there is highest number of pregnancy and implantation rates by transferring day5 embryos instead of day3, indicate that the viability can be more for speedy growing embryos. By transferring blastocyst stage embryo there is selection of good quality growing embryos. By increasing the timing of culture may allow the selection of healthiest embryos. There is limitation of waiting development of embryo till day5 i.e, if there is limited number of embryos are available, by increasing exposure of embryo will be risky which may cancel the embryo transfer procedure or poor quality embryo transfer<sup>(2)</sup>.

Good quality growing embryos need to allow to transfer to achieve pregnancy, therefore multiple pregnancies can be avoided.<sup>19-20</sup>

Limitation of extending culture are if embryo doesn't reach blastocyst stage, there is risk of cancellation of procedure also blastocyst culture is costlier in comparison with cleavage stage transfer.

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UNDER PEER REVIEW