

## RESEARCH ARTICLE

# Blastocyst Culture and Embryo Transfer on Day 5 following Fertilization is a Viable Strategy for Acceptable Results in *in vitro* Fertilization: Intracytoplasmic Sperm Injection Program

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## ABSTRACT

Despite considerable advances in the field of *in vitro* fertilization (IVF), embryo implantation and pregnancy rates have plateaued globally. Thus, much of current research focus is on embryo and endometrial assessment. Day 3 embryo transfers (ETs) have been the standard of practice for long. However, development of blastocyst culture media has led to recent switch toward blastocyst transfers, although this is associated with greater technical skill and know-how in order to optimize the culture process. We analyzed our blastocyst transfer results to see whether this strategy appeared to be a viable intervention in terms of acceptable pregnancy outcome. Our study found a pregnancy rate of 37.5% with blastocyst transfers, with the complete absence of any multiple pregnancies. Thus, we feel blastocyst transfers represent a viable intervention in an IVF program to ensure acceptable pregnancy rates and simultaneously reduce the incidence of multiple births.

**Keywords:** Blastocyst transfer, *In vitro* fertilization, Intracytoplasmic sperm injection.

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## INTRODUCTION

Despite tremendous advances in the field of IVF and assisted reproductive technology, embryo implantation

rates remain poor globally.<sup>1</sup> The embryo and the endometrium are the two principal players involved in the process of embryo implantation. It has thus been a recent research focus to refine and develop more sophisticated techniques for embryo culture and assessment, as well as to assess endometrial receptivity.

Transfer of embryos at the cleavage stage on days 2 or 3 following fertilization has been the standard of practice for long. With the development of advanced blastocyst culture media and sequential media, it has become possible to culture embryos to the blastocyst stage.<sup>2</sup>

Survival of extended *in vitro* culture up to day 5 is in itself a marker of the developmental competence of embryos.<sup>3</sup> Thus, embryos that survive to develop to the blastocyst stage are thought to be the best quality embryos. This process of “self-selection” implies that any embryos which grow to become blastocysts have greater odds to implant into the endometrium compared with day 3 embryos.

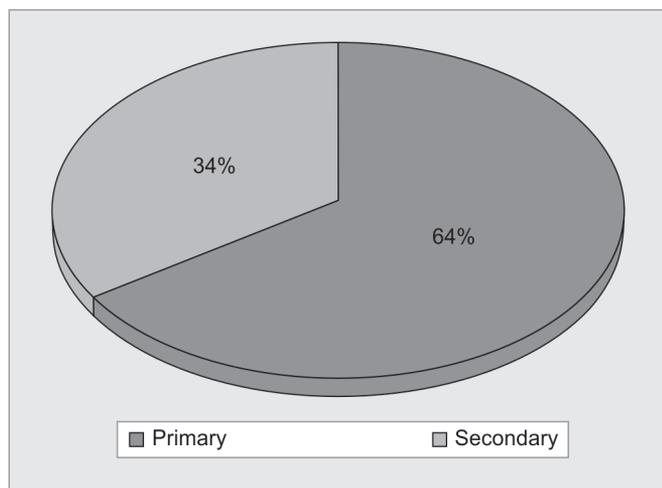
This also means that fewer blastocysts can be transferred into the uterus without compromising on the odds to achieve a pregnancy. Elective single ET has been shown to result in acceptable pregnancy rates, while simultaneously reducing the occurrence of multiple births, which are more common with the standard transfer of 2 to 3 or more day 3 embryos.<sup>4,5</sup> Newer technology, such as preimplantation genetic screening also provides a potent tool to further assess these blastocysts to choose the best quality embryos for transfer. Thus, there has been recent opinion to shift to blastocyst transfers in IVF. However, blastocyst culture is technologically more challenging. Only a subset of day 3 embryos will go on to grow to blastocysts and in certain cycles, none will.<sup>6</sup> This is also dependent on the technical expertise and quality control in individual laboratories. Thus, before switching from cleavage stage ETs to blastocyst transfers, an individual IVF laboratory needs to assess its level of technical skill, expertise, and know-how for the greater challenges involved in culturing an embryo up to blastocyst stage, and assess the risk of adversely affecting pregnancy rates in trying to switch to blastocyst transfers.

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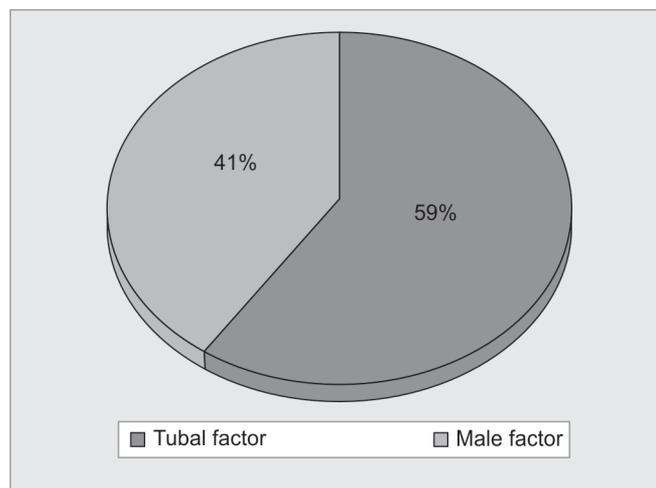
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**Graph 1:** Incidence of primary and secondary infertility within the patient group



**Graph 2:** Cause of infertility within the patient group

We retrospectively analyzed our results over 2 years of fresh blastocyst transfers in normo-responder, good prognosis patients below 37 years to assess whether it is a viable intervention strategy in terms of results.

**MATERIALS AND METHODS**

The study was done at an IVF center in Navi Mumbai between August 2015 and September 2017. Fresh day 5 blastocyst transfer data were analyzed. Institutional Ethics Committee approval was obtained. Informed consent was obtained from the patients. Patients below 37 years of age were included in the analysis. All patients were suffering from tubal or male factor infertility. Ovarian stimulation had been done with highly purified human menopausal gonadotropin (Menogon, Ferring Pharma, Switzerland) or recombinant follicle stimulating hormone (Gonal F, EMD Serono, USA). Stimulation monitoring was done by serial transvaginal ultrasound and serum E<sub>2</sub> estimation. Daily gonadotropin-releasing hormone antagonist (Cetrotide 0.25 mg, EMD Serono, USA) started when lead follicle was 14 mm on ultrasonography (USG). Ovulation trigger given with recombinant human chorionic gonadotropin (hCG) (Ovitrelle 250 µg, Merck Serono, UK) once at least 2 to 3 follicles were 17 mm or more. Ovum pickup was done under USG guidance 36 hours after hCG. As per routine protocol, IVF or intracytoplasmic sperm injection had been done in all patients. As per standard protocol, USG-guided ET done on day 5 following fertilization, with 1 to 2 blastocysts were transferred per patient; 32 patients were part of the final data analysis. Luteal support was given with progesterone in oil injections on every 4th day (Gestone 100 mg, Nordic Pharma, UK), as well as vaginal micronized progesterone in the interim days (Crinone Gel, 8%, Merck Serono, UK). Pregnancy was established by the documentation of fetal cardiac activity at 5 to 7 weeks of gestation.

**Table 1:** Summary of patient parameters and cycle results

Parameter	Result
Age (years)	30.7 ± 3.47
AMH (ng/mL)	2.61 ± 0.96
Follicles aspirated	8.91 ± 3.51
Oocytes retrieved	7.28 ± 3.17
Embryos generated	4.0 ± 2.31
Fertilization rate	54.3%
Endometrial thickness (mm)	9.97 ± 1.81
No. of embryos transferred	1.38 ± 0.49
Implantation rate	32.8%
Pregnancy rate	37.5%
Multiple pregnancy rate	None
No. of embryos stored by vitrification	1.25 ± 1.7

**RESULTS**

Data analysis revealed that 66% of patients (n = 21) had presented with primary infertility and 34% with secondary infertility (n = 11) as seen in Graph 1; 59% patients had presented with tubal factor infertility, whereas 41% presented with male factor infertility, as seen in Graph 2. Other main results are summarized in Table 1.

Data analysis showed that mean age of patients was 30.7 years and mean anti-Mullerian hormone (AMH) level was 2.61 ng/mL. A mean of 7.28 oocytes had been retrieved per patient. An average of 1.3 embryos had been transferred per patient. Mean endometrial thickness on day of ET was 9.97 mm. We recorded a mean fertilization rate of 54.3%, implantation rate of 32.8%, and pregnancy rate of 37.5% in the cohort. There were no multiple pregnancies in the group. An average of 1.25 top quality embryos were available for freezing at the end of ET.

**DISCUSSION**

Our study had only included patients who had presented with infertility due to tubal or male factor. We had

also designed the study to only include patients below the age of 37 years. Our data show that an acceptable pregnancy rate of 37.5% was achieved with blastocyst transfers on day 5 following fertilization. While it is true that pregnancy rates with blastocyst transfers can be even higher, these results demonstrate that for centers practicing day 3 ETs, it is possibly a viable alternative to switch to blastocyst transfers and achieve acceptable pregnancy rates.

The technical expertise required for blastocyst culture translates to a slightly steeper learning curve for the IVF embryologist. However, once the related skills are acquired, blastocyst transfers do appear to be a viable strategy for acceptable results in an IVF program. Moreover, none of the ETs resulted in a multiple pregnancy. We believe that it was possible to achieve this because of our conservative approach to ETs, where an average of only 1.3 blastocysts were transferred per patient.

Thus, in view of our acceptable pregnancy outcome and complete absence of multiple pregnancies, we feel that with adequate training in embryo culture techniques, blastocyst culture and ET on day 5 following IVF is a viable strategy to ensure good results and, at the same time, mitigate the risk of multiple births. However, our

sample size was small. Thus, larger, well-designed trials are required to provide more evidence in support of our findings and conclusions.

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