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RESEARCH ARTICLE

Analysis of *In Vitro* Fertilization—Intracytoplasmic Sperm Injection Results from Fresh Day 2 and Day 3 Embryo Transfers at a Tertiary *In Vitro* Fertilization Center

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ABSTRACT

Embryo transfers (ET) on day 2 or day 3 following fertilization have been the standard of practice since the initial days of human *in vitro* fertilization (IVF). Recent advances in culture media, as well as embryo culture techniques, have prompted in a shift in strategy to day 5 blastocyst transfers following IVF. However blastocyst transfers, although resulting in slightly better pregnancy rate, are known to be associated with certain disadvantages, such as higher costs, higher cycle cancellation rates, and in vitro damage to embryos. Thus we reviewed our results with day 2 and day 3 ETs to see whether outcomes were adequate to justify a return to day 3 embryo transfer policy. Our data shows a 46% clinical pregnancy rate and 1.9% incidence of multiple pregnancy rate with cleavage transfers. Thus in our setting with a lot of poor resource patients, we feel day 2 or 3 transfer provides a good strategy for IVF cycles.

Keywords: Clinical pregnancy rate, Day 3, Embryo transfer, *In vitro* fertilization.

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INTRODUCTION

Approximately 8–12% of couples in the reproductive age group are infertile.[1] Fertility research began with the pioneering work of Pincus, Austin, and Chang. The first IVF baby was born in 1978.[2] IVF is now a routine clinical procedure, with over 5 million babies born worldwide. Recent decades have witnessed extensive research in the field, including refinement of ovarian stimulation protocols, sperm evaluation, and embryo culture and assessment. However, pregnancy rates in IVF cycles remain poor globally.[3] Thus, continued research is underway to optimize results for infertile couples.

Embryo transfer (ET) has been traditionally done at the cleavage stage, on day 2 or 3 following fertilization. With the development of blastocyst culture media, it has become feasible to culture embryos up to blastocyst stage.[4] Blastocyst growth in itself indicates embryo quality and competence.[5] Thus, there has been recent advocacy for blastocyst culture and transfer following IVF.

However, blastocyst culture is more expensive and technically challenging. Fewer day 3 embryos will grow to blastocysts, increasing the risk of no embryos being available for transfer at ET. Thus, day 5 ET is associated with higher cycle cancellation rates.[6] Variable technical expertise and quality control in individual laboratories is also a variable. Another school of thought is that even with continued improvements in culture media, the natural environment of the endometrial cavity likely provides the best possible culture conditions for embryos.

Extended *in vitro* culture comes with its own disadvantages, the most important being epigenetic modifications to the embryos.[7] Moreover, the decision to culture to blastocyst is also dependent on the availability of a large number of embryos, usually associated with strong ovarian stimulation and related adverse effects of hormonal hyperstimulation.[8]

Hence, there has been recent advocacy to transfer embryos after 2–3 days of in vitro culture, representing a return to earlier ET strategy. This would, of course, be contingent upon achieving acceptable pregnancy rates. To further explore this question, we retrospectively analyzed our day 3 fresh ET results over the last 2 years to review our standard of practice to re-assess whether a day 3 embryo transfer strategy was still a viable option in the era of blastocyst transfer.

MATERIALS AND METHODS

The study was carried out at a tertiary fertility center in Navi Mumbai between August 2015 and September

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2017. Most of our IVF cycles are frozen embryo transfer cycles, but for this study, we decided to analyze fresh ET results. Institutional ethics committee clearance was obtained. Informed consent was taken from all patients. Only patients below 37 years being treated for tubal, male factor or unexplained infertility were included in the study. Patients with polycystic ovarian disease (PCOD) were excluded. All patients had earlier undergone ovarian stimulation with highly purified human menotropin (HP-hMG) (Menogon, Ferring Pharma, Switzerland) or recombinant FSH (Gonal F, EMD Serono, USA), and monitored by serial transvaginal ultrasound (TV-USG) folliculometry and serum estradiol estimation, and daily GnRH antagonist injections (Cetrotide 0.25 mg, EMD Serono, USA) started once lead follicle measured 14 mm. Ovulation induction is done with r-hCG (Ovitrelle 250 mg, Merck Serono, UK) when at least 2–3 follicles measured 17 mm or more. Ovum pick-up (OPU) performed under USG guidance 34–38 hours after trigger. IVF or ICSI had been done in all patients as per standard protocol. ET done under USG guidance on day 2 or day 3 following fertilization as per standard protocol, where 2–3 embryos were transferred per patient. 108 patients were included in the final data analysis. Luteal support given with intramuscular progesterone injections on every 4th day (Gestone 100 mg, Nordic Pharma, UK) and vaginal micronized progesterone in the intervening days (Crinone Gel, 8%, Merck Serono, UK). Pregnancy was documented by the demonstration of fetal cardiac activity at 5–7 weeks gestation. Data were analyzed as mean and standard deviation or percentages, as appropriate.

RESULTS

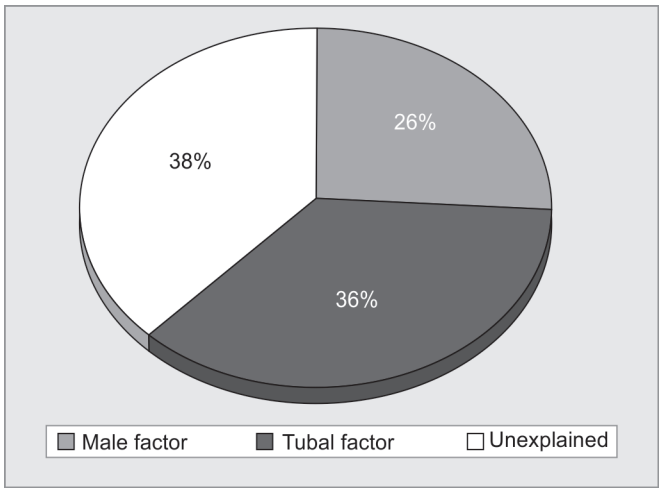
Seventy-one percent of patients (n = 77) had presented with primary infertility and 29% with secondary infertility (n = 31) as shown in Graph 1. Thirty-six percent of patients had presented with tubal factor infertility, 26% with male factor infertility, and 38% with unexplained infertility as shown in Graph 2.

Other results have been summarized in Table 1.

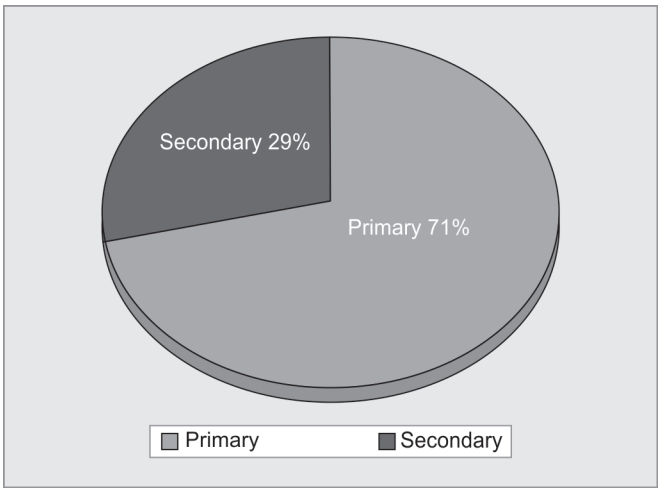
Data analysis revealed that the mean age of patients was 31.9 years and mean AMH level was 2.45 ng/mL. A mean of 5.3 oocytes had been retrieved per patient out of which mean 2.4 were transferred per patient. Mean endometrial thickness on the day of ET was 9.8 mm. We documented a mean fertilization rate of 63.9%, implantation rate of 21.1% and the pregnancy rate of 46.3% in the cohort. There were only 2 multiple pregnancies in the group (both twins), to result in a multiple birth rate of 1.9%. An average of 1.1 top quality embryos was available for freezing at the end of embryo transfer.

DISCUSSION

Our study included patients who presented with infertility due to tubal factor, male factor, or unexplained infertility. In our study, we achieved a clinical pregnancy rate of 46.3%, which is quite acceptable in terms of standard results, internationally, where pregnancy rates at the best centers are



Graph 2: Cause of infertility



Graph 1: Incidence of primary and secondary infertility

Table 1: Summary of patient parameters and cycle results

Parameter	*Result*
Age (years)	31.9 ± 4.51
AMH (ng/Ml)	2.45 ± 2.35
Follicles aspirated	6 ± 3.71
Oocytes retrieved	5.3 ± 3.4
Embryos generated	3.9 ± 2.3
Fertilization rate	80.5%
Endometrial thickness (mm)	9.8 ± 1.2
No. of embryos transferred	2.4 ± 0.6
Implantation rate	21.1%
Pregnancy rate	46.3%
Multiple pregnancy rate	1.9 %
No. of embryos stored by vitrification	1.1 ± 1.7

around 50–60%.[9] Our data indicate that in good prognosis patients below the age of 37 years with acceptable ovarian reserve and normo-ovarian function, day 3 cleavage stage ETs can achieve reasonably good pregnancy outcome. This is true even when only a moderate number of embryos were transferred per patient (2.4 ± 0.6).

It was also interesting to note that the rate of multiple pregnancies was quite low (1.9%), with there being no case of higher order multiple pregnancies (triplets or more). We did not transfer more than 3 embryos in any cycle. Instead, nearly half of our ETs consisted of one or two cleavage stage embryos. We believe these strategies were instrumental in restricting the number of multiple pregnancies.

Although it could be argued that blastocyst transfers might have resulted in even higher pregnancy rates,[10] studies have shown that ETs are equally effective at day 3 or day 5.[11]

Blastocyst transfers are associated with risk of epigenetic modifications to the embryos, higher costs, more technical expertise, fewer embryos available for transfer, and greater risk of cycle cancellation due to no embryos being available for transfer at ET. Thus, given the acceptable pregnancy rate we achieved with day 3 transfers, we feel it offers an effective and viable alternative to blastocyst transfers, especially in a poor resource nation such as ours, where the cost of IVF treatment represents a substantial burden to many patients.

Well-designed randomized controlled trials between day 3 cleavage stage ETs and blastocyst transfers are required to provide more conclusive data on this issue.

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