



## Embryo transfer duration and IVF success

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**OBJECTIVE(S):** To study the influence of time taken for embryo transfer on the success rates of IVF.

**METHOD(S):** A prospective study was done on 103 women recruited IVF at our unit in whom fresh embryo transfer was done. These cases were thoroughly studied as regards their age, duration of infertility, ease of transfer, and duration between loading and discharge of embryo and its statistical relationship with pregnancy and implantation rates.

**RESULTS:** Pregnancy rate and implantation rate were found to be more among the women having less than 60 seconds duration between loading and discharge of embryo than in women where this duration was more. This difference was found to be statistically significant ( $p < 0.05$ ). Furthermore, a significantly higher pregnancy and implantation rates were seen with easy embryo transfers.

**CONCLUSION :** The time taken for loading and discharge of embryo could be an important prognostic factor for implantation and pregnancy rates in invitro fertilization programmes as the shorter the time higher the pregnancy rate.

**Key words :** duration of embryo transfer, pregnancy rates, implantation rates, invitro fertilization, easy embryo transfer

### Introduction

In the last decade major advances have been made in the field of ovarian stimulation and embryo culture but the technique of embryo transfer (ET) has remained substantially the same. Over the recent years there has been an increasing interest in the methodology of embryo transfer and its influence on success rates of IVF cycles.

A lot of research is going on regarding the prognostic factors for success rates of ET like atraumatic transfers, use of soft catheters, influence of blood, mucus or bacteria in catheters, use of no touch techniques, performing mock transfer and transfers under ultrasound guidance <sup>1,2</sup>.

Difficult ET is associated with impaired implantation <sup>3</sup> probably due to endometrial damage <sup>4</sup>, presence of blood in the catheter <sup>5</sup>, and triggering of uterine contractions due to cervical manipulation.

There is hardly any literature on the effect of time employed for performing ET on the success rates of IVF cycles. Logically, the time employed for performing ET may be an important prognostic factor for implantation and pregnancy rates since external factors like changes in environment, temperature, exposure to light, composition of catheter and embryo handling may have detrimental effects on the survival and growth of the embryo.

### Methods

A prospective study was done on all women recruited for IVF at our unit between January and March 2005. A total of 103 cases were studied as regards their age, duration of infertility, ease of ET, number of retrieved, inseminated, fertilized and transferred oocytes, and interval between loading and discharge of embryo and its relationship with pregnancy and implantation rates.

All the women recruited for the program were down regulated with GnRH analogue triptoreline acetate (Decapeptyl, Ferring laboratories) as per the schedule of the long protocol. Controlled ovarian stimulation was done with recombinant FSH (Gonal-F, Serum Institute of India) and highly purified urinary menopausal gonadotrophin (Menotrophin, Serono Laboratories, Switzerland) as per the protocol. Ovulation

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was initiated with hCG (Inj. Profasi 5,000/10,000 units as required) and transvaginal ultrasound guided oocyte retrieval was scheduled 36 hours after the above injection. The luteal phase was supported with micronised progesterone (Uterogestan, Solvay, India) 200 mg in the morning and 400 mg at night inserted vaginally.

Embryos were cultured upto day 2, 4-8 cell stage in tri gas incubators (Galaxy UK) in culture media (Scandinavian).

A mock ransfer was performed 3-4 weeks before oocyte pick up. The number of embryos transferred depended on the prognosis of the patient and the quality of embryos according to the scoring system and usually ranged from three to four.

Embryo transfer was carried out 48 hours after oocyte retrieval. The patient was placed in dorsal position. Speculum was introduced to expose the cervix and the anterior lip of cervix was held with an Allis forceps. Vagina and cervix were cleaned with cotton.

The embryo transfer catheter (Cook, Australia Jansen Anderson Bulb tip) was loaded with the best embryos. Drawing up of the embryos was done with the help of a disposable tuberculin syringe using the “three drop procedure” (embryos are separated by a bubble of air from

**Tablem1. Differences in conceptional and nonconceptional cycles.**

Parameter	Conceptional cycles (n=34) (mean ± SD)	Nonconceptional cycles (n=69) (mean ±SD)	t	P
Mean age (years)	32.88 ± 3.63	34.00 ± 5.04	1.156	>0.05 NS
Mean duration of infertility (years)	9.68 ± 3.64	19,51 ± 6.92	0.656	>0.05 NS
Mean time interval between loading and discharging of embryo (seconds)	49.41 ± 12.35	57.19 8.±10	3.824	<0.05 S
ILDE (Excluding noneasy transfer) (seconds)	49.41 ± 12.35	57.52 ± 8.79	4.002	<0.05 S

NS - Not Significant, S - Significant, ILDE - Interval between loading and discharging of embryo

It reveals that the women who conceived (33.09%) and those who did not were comparable as regards their mean age and mean duration of infertility. It was observed that shorter mean interval between loading and discharging of embryos (ILDE) (49.41 ± 12.35 seconds) in conceptual cycles was statistically significant (P<0.05) when compared with longer mean ILDE (57.19 ± 8.10 seconds) in nonconceptual cycles.

As noneasy transfer takes longer time for ET the ILDE was calculated excluding the noneasy transfer for both

the preceding and following drop of medium with embryo).

The catheter was introduced into the uterine cavity with a no touch technique and the embryo transferred. Ultrasound guidance for ET was not employed except in two cases. The catheter was carefully removed after 10 seconds, flushed and checked under the microscope for any retained embryos.

The patient was advised to rest for 30 minutes and then discharged with luteal support and asked to report on the 15<sup>th</sup> day of ET for estimation of serum βhCG.

The time taken from loading of the catheter and actual transfer of embryo was noted in seconds in each case using a stop watch.

The cases were thoroughly followed and studied regarding the pregnancy and implantation rates. Statistical analysis was performed by means of χ<sup>2</sup> test and students t-test following the standard criteria of applicability. Statistical significance limit was defined as p<0.05.

**Results**

Table 1 gives the correlation of conceptional and non-conceptional cycles with women’s mean age, mean duration of infertility, and interval between loading and discharging of embryo in seconds.

the conceptional and nonconceptional cycles and here too a statistically significant difference (P<0.05) was observed; shorter ILDE was seen in conceptional cycles.

Table 2 shows the correlation of ILDE of less than 60 seconds and more than 60 seconds with mean age, mean duration of infertility, oocyte and embryo characteristics, and percentage of noneasy transfers. It was observed that the two ILDEs were well matched as regards the mean age, mean duration of infertility, and the transferred oocyte and embryo characteristics.

**Table 2. Results in conceptional and nonconceptional cycles.**

Parameter	ILDE <60 seconds (n=64)	ILDE >60 seconds (n=39) (mean ± SD)	t (mean ± SD)	P
Mean age (years)	33.98 ± 4.95	33.05 ± 4.04	0.990	>0.05 NS
Mean duration of infertility (years)	9.86 ± 6.55	10.85 ± 5.08	0.807	>0.05 NS
Oocyte and embryo characteristics				
(a) Mean aspirated	8.55 ± 5.19	8.72 ± 6.61	0.145	>0.05 NS
(b) Mean inseminated	7.23 ± 2.88	7.69 ± 2.89	0.785	>0.05 NS
(c) Mean fertilized	5.72 ± 2.45	6.03 ± 2.26	0.641	>0.05 NS
(d) Mean transferred	3.75 ± 0.83	3.87 ± 0.56	0.799	>0.05 NS
(e) Mean class I embryos	3.16 ± 1.25	3.33 ± 0.86	0.744	>0.05 NS
Percentage of noneasy transfers	3.13%	1.94%		<0.01

ILDE - Interval between loading and discharging of embryo NS-Not Significant S-Significant

**Table 3. Correlation of time interval between loading and discharge of embryos with pregnancy and implantation rates taking into account only easy transfers.**

Parameter Studied	ILDE<60 sec.	ILDE>60 sec.	$\chi^2$	P
Pregnancy rate	43.75%	15.38%	8.808	<0.05
Implantation rate	29.68%	6.979%	6.976	<0.05

ILDE - Interval between loading and discharge of embryos

The percentage of noneasy transfers was higher in the less than 60seconds ILDE cases as compared to that in more than 60 seconds and this was statistically highly significant (P<0.01)

Table 3 depicts the pregnancy and implantation rates in relation to the ILDE taking into account only easy transfers. Pregnancy and implantation rates were found to be higher among women having ILDE<60 seconds as compared to those with ILDE>60 seconds and the difference was statistically significant (P<0.05).

## Discussion

Assisted reproduction is one of the fastest growing areas of medicine having expanded far beyond the imagination of those who pioneered the techniques that led to the birth of Louisa Brown.

ET is the final and most important step in ART and there have not been any major changes in its technique since its inception. But over the last few years lot of studies have been done on the influence of methodology of ET on the pregnancy and implantation rates.

A number of studies <sup>4-8</sup> have demonstrated that factors like embryo misplacement, uterine contractions, endometrial damage and presence of blood or bacteria have a detrimental effect on embryo implantation. Further it is shown by workers that there are some environmental factors which may prove to have some adverse effects on the oocytes and embryos such as exposure to light <sup>9,10</sup>, temperatures other than 37°C <sup>11</sup>, polyvinyl chloride <sup>12</sup>, polychlorinated biphenils <sup>13</sup> and others <sup>14</sup>.

The importance of keeping the embryo temperature close to 37°C and oxygen and carbon dioxide concentrations under specific ranges cannot be overlooked <sup>15</sup> and a number of technological advances have been made in this direction. However, it may be difficult to control the above mentioned environmental factors from moment of embryo loading and upto their discharge into the uterus. Therefore it has been speculated that the time taken from loading to discharge of the embryo may have an effect on the implantation rates of embryos.

In our study an inverse association between the duration of ILDE and the pregnancy and implantation rates was observed. High pregnancy and implantation rates were seen with ILDE less than 60 seconds than with a longer duration ILDE (P<0.05). Since difficult transfers are associated with longer

ILDEs, we have compared the data only for easy transfers.

It can furthermore be stated that lower pregnancy and implantation rates seen with increased duration of ILDE could be related to a greater exposure of the embryos to detrimental environmental factors like light, temperature and composition of transfer catheter. However, it cannot be ruled out that a relatively prolonged endocervical manipulation leading to uterine contractions, contamination of catheter and endometrial damage may also play a role.

The limitation of our study was a small study cohort and also that it was not a controlled study. A controlled ultrasound guided study with larger cohort should be planned to find out the exact prognostic value of the duration of ILDE on implantation and pregnancy rates. The duration of ILDE may act as an important prognostic factor (independent of transfer difficulty) for implantation and pregnancy rates.

### **Conclusion**

The longer the ILDE duration (more than 60 seconds) the lower the implantation and pregnancy rates. Wherever possible ILDE should be shortened to under 60 seconds.

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