ORIGINAL RESEARCH

A Prospective Study of Factors Affecting Pregnancy Rate Following Fresh Embryo Transfer

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Abstract

Background: Couples often want to know their chance of having a positive outcome if they opt for assisted reproductive techniques (ARTs) and hence this prospective study was aimed to examine variable predictor parameters for its success.

Aim: The aim of the study was to assess factors favoring positive outcome following fresh embryo transfer (FrET).

Materials and methods: In this study, 200 couples—both with primary and secondary infertility—undergoing embryo transfer (ET) following controlled ovarian hyperstimulation and those obtaining at least one transferable embryo were included.

In addition to the routine infertility data, the couples underwent long protocol which started on day 21 of menstrual cycle and continued till oocyte retrieval in next cycle. After that either FrET or were planned for "freeze all" embryos followed by frozen embryo transfer (FET). The success was measured in terms of serum beta human chorionic gonadotropin (β-HCG) on day 15 and by transvaginal ultrasonography (TVS) at 6 weeks. The data was analyzed using descriptive and inferential statistical analysis.

Results: Primary infertility was the most common type of infertility (84.5%). Although 42% patients became HCG positive but clinical pregnancy was achieved only in 39.5%. Clinical pregnancy was achieved in 84.8% of cases of primary infertility and 15.20% of cases of secondary infertility. Among the causes of infertility, combined factor infertility (41.5%) was the most common cause. The pregnancy rate was less when very high doses (5.1%) and longer duration (39.2%) of gonadotropins were used. There was a significant association between clinical pregnancy and the total number of oocytes ($p = 0.008$), the total number of metaphase II (MII) oocytes ($p = 0.003$), and the total number of embryos (0.002) but not with endometrial thickness (ET) in the range of 8– 14mm ($p = 0.702$).

Conclusion: Familiarity with the predicting factors can help to prevent overtreatment and balance the decision to achieve pregnancy either through ART or by natural conception.

Keywords: Assisted reproductive techniques, Infertility, Fresh embryo transfer.

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Introduction

Infertility is defined by the World Health Organization as failure to become pregnant after one year of unprotected intercourse.[1] The major causes of infertility include ovulatory dysfunction (20–40%), tubal and peritoneal pathology (30–40%), and male factors (30–40%); uterine pathology is relatively uncommon and the remainder is largely unexplained.[2]

In vitro fertilization (IVF) involves a sequence of events that begin with different stimulation protocols with controlled ovarian hyperstimulation with exogenous gonadotropin. Oocyte retrieval is done from the ovaries under the guidance of ultrasonography, fertilization in the laboratory, and transcervical transfer of embryos into the uterus. The goal of IVF is to maximize pregnancy rates while, at the same time, minimizing multiple gestations, high-order multiple gestations in particular.[3]

Various factors that have been suggested for prediction of success with assisted reproductive technique (ART) include female age, body mass index (BMI), duration and cause of infertility, antral follicle count (AFC), progesterone and estradiol level on the day of human chorionic gonadotropin (HCG) administration, number of oocytes retrieved and injected oocytes, embryo quality, endometrial receptivity, and sperm characteristics.

Although many studies have used multivariable models to identify predictors of IVF outcome, there is still no real consensus.[4–6] Couples often want to know their chance of having a positive outcome if they opt for ART, and hence, from the patient's perspective, the probability of live birth is more important. Therefore, this prospective study aimed to examine variable parameters that might be predictors of success following ART so that more data can be obtained for patient counseling.

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Materials and Methods

This was a prospective observational study over a period of 2 years involving couples, both with primary and secondary infertility; registering at Edappal Hospitals Pvt Ltd, Edappal, Kerala, India, and undergoing IVF treatment at Centre for Infertility Management and Assisted Reproduction (CIMAR).

After obtaining approval from the scientific committee and institutional ethics committee, a total of 300 couples were enrolled with their written informed consent. All cases of embryo transfer (ET) following controlled ovarian hyperstimulation and those who were obtaining at least one transferable embryo were included. Cases having donor egg or donor ETs, canceled cycles, male partner with severe oligoasthenoteratospermia (OAT), frozen embryo transfer (FET), and with documented karyotype abnormalities such as translocations were excluded.

Since factors affecting the outcome of fresh and FET may vary, hence we studied 200 couples of fresh embryo transfer (FrET).

The demographic details, nature, duration, and etiology of infertility were obtained. Records of basal necessary investigations such as basal transvaginal ultrasound (TVS) and basal hormonal assays, glycosylated hemoglobin done on day 2 or day 3 of the menstrual cycle were obtained as part of the institutional protocol. The couples underwent a long protocol and were called in the third week, around day 21 of the menstrual cycle (spontaneous or progesterone withdrawal cycle). On that day, TVS was done. Also, cervical culture and mock ET using an ET catheter were done.

The female partner was put on long luteal phase hypothalamo–pituitary ovarian axis down regulation with gonadotropin-releasing hormone agonist leuprolide till the day of HCG injection. The couples were asked to report on day 2/day 3 of the next menstrual cycle for TVS with the assessment of AFC and ET.

After confirmation of good suppression, controlled ovarian stimulation with daily intramuscular injections of exogenous gonadotropins recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin was done. Couples were then advised to report after 7 or 8 days for evaluation of response to gonadotropin therapy. Exogenous gonadotropins, along with leuprolide acetate were continued till at least 2 or 3 follicles of size 17–18 mm or more along with 4–5 follicles of size around 14–15 mm and ET of 7–14 mm, was obtained. Then for final follicular maturation, an intramuscular injection of HCG 10,000 IU IM was given. Serum estradiol, progesterone, and luteinizing hormone (LH) levels were obtained on the day of HCG administration early in the morning.

Transvaginal ultrasound-guided oocyte retrieval was done under intravenous (IV) sedation (using propofol or ketamine) after 36 hours of HCG injection. For fresh cycles, micronized progesterone injection 100 mg IM (injection Hald 100 mg, Intas Pharmaceuticals Ltd, India) or micronized progesterone vaginal gel 8% (Crinone gel 8%, Merck Serono Ltd, United Kingdom) was given at the time of ovum pick up and ET was performed on day 2/day 3 after the oocyte retrieval. Only good-quality embryos (grade I) were transferred and similar grade I embryos that were in excess or surplus were cryopreserved with vitrification protocols.

Clinical Pregnancy was confirmed by visualization of gestational sac using TVS at 6 weeks of ET. It included intrauterine pregnancy as well as ectopic pregnancy.

The main aim of the study was to determine factors affecting pregnancy rates following ART cycles. The primary objective of the study was to assess the relative contribution of different factors favoring positive outcome following fresh embryo transfer. The secondary objective was to determine its success rate in our institution. Data on various parameters recorded were subjected to descriptive and inferential statistical analysis performed by using statistical package for social sciences (SPSS) software, version 20.0.

Table 1: Basic parameters, clinical pregnancy in relation to age, BMI, duration of infertility, AFC, day-2 FSH, day-2 E2, and P4 in fresh embryo transfer group of subjects of fresh embryo transfer group (FrET)

Parameter	*Group*	*Number (%)*	*Clinical pregnancy (%)*	*p-value*
Age (years)	≤20	3 (1.5)	2 (2.4)	0.018
	21–25	22 (11)	11 (13.9)	
	26–30	73 (36.5)	33 (41.8)	
	31–35	75 (37.5)	26 (32.9)	
	36–40	27 (13.5)	7 (8.9)	
BMI (kg/m^2)	<30	174 (87)	69 (87.3)	0.908
	≥30	26 (13)	10 (12.7)	
Duration of infertility (years)	<5	90 (45)	40 (50.6)	0.161
	5–10	84 (42)	31 (39.2)	
	>10	26 (13)	8 (10.1)	
AFC	<5	11 (5.5)	1 (1.3)	0.04
	5–10	40 (20)	14 (17.7)	
	11–15	83 (41.5)	34 (43)	
	>16	66 (33)	30 (38)	
Day-2 FSH (mIU/mL)	<7	170 (85)	71 (89.8)	0.12
	≥7	30 (15)	8 (10.2)	
Day-2 E2 (pg/mL)	<20	77 (38.5)	38 (48.1)	0.001
	20–40	86 (43)	34 (43)	
	41–60	25 (12.5)	6 (7.6)	
	>60	12 (6)	1 (1.3)	
Day-2 P4 (ng/mL)	≤1	158 (79)	66 (83.5)	0.203
	>1	42 (21)	13 (16.5)	

Results

The annual average subject inflow opting for ARTs is around 2500 in our setup. A total of 200 subjects for FrET were included in the study. There were no dropouts from the study during this time period. The mean age of subjects was 29.97 ± 4.64 years and the mean BMI was 24.91 ± 4.25. Most of the subjects had a duration of infertility of 5 years and an AFC of 11–15 days. Day-2 FSH was below 7 mIU/mL, day-2 LH was below or equal to 5 mIU/mL, and day 2 P4 was ≤1 ng/mL in most of the subjects. The mean day-2 E2 of the subjects was 28.75 ± 23.3. The baseline parameters and their relation with clinical pregnancy are mentioned in Table 1.

Primary infertility was the most common type of infertility (84.5%). Although 42% of patients became HCG positive (biochemical pregnancy), clinical pregnancy was achieved only in 39.5%. Clinical pregnancy was achieved in 84.8% of cases of primary infertility and 15.20% of cases of secondary infertility. Among the causes of infertility, combined factor infertility (41.5%) was the most common cause followed by male factor (38.5%), female factor (15.5%), and unexplained (4.5%). Decreased ovarian reserve was the most common cause of female factor infertility (26.3%) followed by polycystic ovaries (24.6%). Other causes were endometriosis (19.3%), tubal factors (17.5%), pelvic factors (8.8%), and others (3.5%). With respect to male factors OAT, asthenoteratospermia, azoospermia, and oligospermia were seen in 59.4, 16.3, 13.8%, and 10.6%, respectively. Clinical pregnancy rates were highest when male factor-only infertility was the cause of infertility (46.8%). Polycystic ovaries (31.6%) and OAT (66.7%) had shown maximum pregnancy rates among female and male factors respectively as depicted in Table 2.

Table 2: Clinical pregnancy in relation to cause of infertility, male, and female factors in fresh embryo transfer group

Parameter	*Group*	*Clinical pregnancy (%)*	*p-value*
Cause of infertility	Male factor	37 (46.8)	0.09
	Female factor	11 (13.9)	
	Combined	27 (34.2)	
	Unexplained	4 (5.1)	
Female factor	Polycystic ovaries	12 (31.6)	0.74
	Decreased ovarian reserve	6 (15.8)	
	Endometriosis	7 (18.4)	
	Tubal factor	6 (15.8)	
	Pelvic factor	6 (15.8)	
	Others	1 (2.6)	
Male factor	Oligospermia	7 (11.1)	0.84
	Asthenoteratospermia	7 (11.1)	
	OAT	42 (66.7)	
	Azoospermia	7 (11.1)	

Table 3: Stimulation details and clinical pregnancy in relation to dose and days of gonadotropin administered

Parameter	*Group*	*Number (%)*	*Clinical pregnancy (%)*	*p-value*
Total dose of gonadotropin (IU)	<2500	52 (26)	30 (37.97)	0.002
	2501–3500	65 (32.5)	23 (29.1)	
	3501–4500	65 (32.5)	22 (27.8)	
	>4500	18 (9)	4 (5.1)	
Number of days of gonadotropin	<10	63 (31.5)	31 (39.2)	0.069
	11–12	81 (40.5)	30 (38)	
	>12	56 (28)	18 (22.8)	

The pregnancy rate was less (5.1%) when very high doses of gonadotropins were used (p = 0.002) and most of the pregnant subjects received gonadotropin for above 10 days (39.2%) (p = 0.069) as shown in Table 3.

As shown in Table 4, although the pregnancy rate was highest in subjects with ET in the range of 8–14 mm, it was not statistically significant (p = 0.702). There was a significant association between clinical pregnancy and the total number of oocytes (p = 0.008), the total number of metaphase II (MII) oocytes (p = 0.003), and a total number of embryos (0.002). The pregnancy rate was high in subjects who had more than 2 embryos transferred (51.9%).

Most of the subjects on the day of HCG administration had serum E2 levels in the range 1500–2500 pg/mL and a serum progesterone level of 1 ng/mL as shown in Table 5.

Table 4: Cycle parameters and clinical pregnancy in relation to it

Parameter	*Value*	*Number (%)*	*Clinical pregnancy (%)*	*p-value*
Endometrial thickness (mm)	<8	7 (3.5)	3 (3.8)	0.702
	8–14	190 (95)	74 (93.7)	
	>14	3 (1.5)	2 (2.5)	
Total number of oocytes	<5	15 (7.5)	3 (3.8)	0.008
	5–10	52 (26)	16 (20.3)	
	11–15	38 (19)	14 (17.7)	
	16–20	28 (14)	13 (16.5)	
	>20	67 (33.5)	33 (41.8)	
Total number of MII oocytes	<5	33 (16.5)	6 (7.6)	0.003
	5–10	75 (37.5)	28 (35.4)	
	11–15	52 (26)	23 (29.1)	
	16–20	24 (12)	14 (17.7)	
	>20	16 (8)	8 (10.1)	
Total number of embryos	<5	54 (27)	11 (13.9)	0.002
	5–10	82 (41)	35 (44.3)	
	11–15	41 (20.5)	22 (27.8)	
	16–20	18 (9)	7 (8.9)	
	>20	5 (2.5)	4 (5.1)	
Number of embryos transferred	≤2	98 (49)	38 (48.1)	0.83
	>2	102 (51)	41 (51.9)	
Luteal support	E + P	121 (60.5)	53 (67.1)	0.204
	E + P + HCG	76 (38)	26 (32.9)	
	P	3 (1.5)	0 (0)	

E, estrogen; P, progestrone

Table 5: Hormone assay details on HCG day of subjects and clinical pregnancy in relation to it

Hormone	*Serum level*	*Number (%)*	*Clinical pregnancy achieved in %*
Estradiol (pg/mL)	<1500	47 (23.5)	20.30
	1500–2500	68 (34)	35.40
	2501–3500	36 (18)	20.30
	>3500	49 (24.5)	24.10
Progesterone (ng/mL)	<1	127 (63.5)	81
	1–1.5	40 (20)	12.70
	1.6–2	21 (10.5)	3.8
	2.1–2.5	5 (2.5)	1.3
	>2.5	7 (3.5)	1.3

Discussion

Although many studies reported on potential predictors of pregnancy chances after ART, there is no consensus to pinpoint which predictors are clinically most relevant and on what factors one should base the decision to start treatment or not.

It is recognized by many that with the increase of maternal age, the ART outcome becomes increasingly worse. We noticed a drastic fall in the pregnancy rate after 35 years. Our study showed a significant relation between age and clinical pregnancy rate in fresh cycles (Table 1, p = 0.01). These results confirm a negative association between maternal age and pregnancy rate which is comparable to the studies done by Hao et al. and Wang et al.[7,8] Although 87% of the pregnant women had a BMI below 30 and 12.7% had a BMI above or equal to 30, it was not statistically significant (p = 0.9) which may be due to large number of cases with BMI below 30.The decrease in pregnancy rate with increasing BMI was also shown in studies done by Sarais et al. and Ferlitsch et al.[9,10] Our results showed a decrease in pregnancy rate as the duration of infertility increased especially when the duration of infertility was above 10 years, possibly associated with increasing in age and decreasing gamete quality.

Antral follicle count and clinical pregnancy are positively associated, and the association was significant (p = 0.04). The majority of pregnant cases had an AFC of 11–15. Our data agree with studies conducted by Kannamannadiar et al. and Holte et al.[11,12] Pregnancy rates were found to be more (89.8%) in subjects with FSH of below 7 mIU/mL when compared to subjects with a value above or equal to 7 (10.2%) but there was no statistical significance (p = 0.12). This trend of decrease in pregnancy rate with an increase in FSH which was shown in our study was also seen in studies done by Sabatini et al. and Abdalla and Thum, but contrary to our study, they got a statistically significant association. Our study is not in agreement with the study done by Jellad et al. in which they found no relation between FSH and fertilization or pregnancy rate.[13–15] There was a decrease in pregnancy rate with an increase in day-2 E2 levels and the association was of significance (p = 0.001). Coming to day-2 P4 it was observed that pregnancy rates were high (83.5%) when day-2 P4 was less than or equal to 1 ng/mL when compared to subjects with P4 above 1 ng/mL but showed no significant association (p = 0.20).

Subjects who received lower doses of gonadotropins showed a higher pregnancy rate (37.97%) in comparison with those who received higher doses (5.1%) and the relation was found to be significant (Table 3). Our findings were consistent with studies done by Baker et al. and Martin et al. who also demonstrated an inverse relationship between gonadotropin requirements and pregnancy rates.[16,17] With respect to the number of days for which gonadotropins were administered, pregnancy rates were highest (39.2%) in the subjects who received gonadotropins for below 10 days when compared to subjects who received them for above 12 days (22.8%) but still statistical significance could be not seen (p = 0.06). The decrease in pregnancy rate with increasing days of stimulation was also demonstrated in a study done by Chuang et al. but unlike our study they got a significant p value.[18] This is contrary to a study done by Martin et al who found no difference in pregnancy rates when women were stimulated for less than 9, 10–11, and more than 12 days.[17]

Table 4 gives data regarding clinical pregnancy in relation to various cycle parameters. The pregnancy rate was maximum (93%) in the group with ET in the range 8–14 mm but at the same time, the percentage of subjects who were non-pregnant in this group was also high (95.9%). There was no significant association demonstrable between ET and clinical pregnancy in fresh cycles (p = 0.7) which was in concordance with a study done by Rashidi et al. and not in concordance with studies done by Ma et al., Richter et al., Zhao et al. where they showed higher pregnancy rate when the thickness was above14 mm.[19–22] The disparity may be due to the presence of a large number of subjects within the group having endometrium (95%). The finding between clinical pregnancy and a total number of oocytes showed a significant association with a p = 0.008 similar to study done by Spitzer et al.[23] In a study done by Cai et al., they concluded that the quality of embryos rather than number of oocytes played a role in predicting treatment outcome which is contrary to our study as we found a positive association though all subjects included in our study had grade 1 ET.[24] Maximum pregnancy rate (35.4%) was seen in the group with 5–10 MII oocytes and the relation between both was found to be statistically significant in our study (p = 0.003). Our finding was in agreement with a study done by Rana et al who also showed an increase in live birth rates with an increasing number of mature eggs.[25] Data relating pregnancy rate and number of number of embryos obtained also showed a significant relation (p = 0.002). The pregnancy rate was slightly higher 51.9% when the number of embryos transferred was more than two when compared to 48% when less than two embryos were transferred.

Furthermore, E2 and progesterone together were given for luteal support in the majority of pregnant subjects (67.1%). No significance was noted between E2 levels on HCG day of the study subjects and clinical pregnancy (Table 5). This observation is comparable to a study done by Ng et al. who concluded that high serum estradiol concentration may adversely affect pregnancy rates but contrary to studies done by Prasad et al, Gahlot, and Siddhartha et al. which may be due to the effect of confounding factors such as age and ET.[26–29] Our study showed the highest pregnancy rates (81%) in subjects who had P4 level below 1 ng/mL showing a statistically significant association between P4 and clinical pregnancy. These findings were consistent with studies done by Huang et al. and Kyrou et al. who also demonstrated a decrease in live birth rate and quality of embryos as the progesterone levels increased.[30,31]

Conclusion

In our study, positive predictors for FrET cycles were decreased age of the female partner, the lower total dose of gonadotropins administered, lower serum progesterone level on the day of HCG administration, AFC, the total number of oocytes, total number of MII oocytes, and the total number of the embryo. Whereas factors like BMI, day-2 FSH below 7 IU, estradiol level on the day of HCG, and ET were not significantly associated with higher clinical pregnancy rates. The two main limitations of the study were that parameters such as age and ovarian reserve were not matched while comparing various factors which might have led to confounding effects and also there was no comparison of factors in types of stimulation protocols used in fresh cycles.

In spite of these limitations, the familiarity with the predicting factors regarding the probability of achieving a pregnancy after a FrET can help to prevent overtreatment and balance the decision to achieve pregnancy either through ART or by natural conception.

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