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Original Research Article

Factors influencing the pregnancy outcome in normotensive women undergoing frozen embryo transfer cycles

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ABSTRACT

Background: Infertility is defined as a failure to conceive within one or more year of regular unprotected intercourse. Assisted Reproductive techniques have been developed rapidly over the past few decades helping couple who are unable to conceive naturally. The overall clinical pregnancy rate of ART is over 50%. Many factors can influence the success of a clinical pregnancy such as maternal age, ovarian reserve, duration of infertility, type of infertility, hormonal levels and endometrial receptivity. Maintaining a normal blood pressure level is generally considered positive for fertility outcomes, as elevated blood pressure can negatively impact a woman's ability to conceive, potentially leading to issues with ovulation, implantation and increased risk of miscarriage. However, there are still some unknown risk factors that could affect the pregnancy outcome of ART.

Methods: This study included 80 normotensive women who underwent frozen embryo transfer cycles. Blood pressure was measured on all the women undergoing frozen embryo transfer. Female patient's age, unique health identification, body mass index, type of infertility, blood pressure, endometrium thickness, number of embryos transferred, embryo grade and beta human chorionic gonadotropin were recorded.

Results: Among 80 normotensive women who underwent frozen embryo transfer, all factors were non-significantly associated with the clinical pregnancy rate. Factors that resulted in higher clinical pregnancy rate includes higher blood pressure, primary infertility, PCOS, overweight, day 4 embryo transfer, endometrium thickness >9 mm and morula grade embryo transfer. This could be because of limited sample size and time restrictions.

Conclusions: There is no significant association between blood pressure, type of infertility, polycystic ovarian syndrome, day of embryo transfer, embryo grade, body mass index and endometrium thickness with pregnancy rate in normotensive women undergoing frozen embryo transfer cycles. Further investigations with randomized trials are required to confirm the confounding nature of the factors analysed and their influence on pregnancy outcome in normotensive women undergoing frozen embryo transfer.

Keywords: Frozen embryo transfer, Normotensive women, Pregnancy

INTRODUCTION

Infertility treatments have increased steadily in the past 20 years and have proven effective in achieving considerable rates of successful conception and live birth rates even among women younger than 35 years. Many factors can influence the success of a clinical pregnancy. However, specific cause or any indication of infertility is still under

scrutiny, hence there are still some unknown risk factors that could affect the pregnancy outcome of Assisted Reproductive Technology.¹ In the recent times, it has been noted that blood pressure plays a crucial role in fertility outcomes for both men and women. Maintaining a normal blood pressure level is generally considered positive for fertility outcomes, as elevated blood pressure can negatively impact a woman's ability to conceive, potentially leading to issues with ovulation, implantation

and increased risk of miscarriage.² Although genetics play a role in interindividual variation in blood pressure, many lifestyle and environmental factors influence risk of hypertension, potentially through multiple pathways including inflammation, oxidative stress and endothelial dysfunction. These same pathways have been associated with adverse reproductive events, through effects on key reproductive processes such as ovulation, implantation and vascularization of the placenta thus leading to reduced implantation rates.³

Polycystic ovary syndrome (PCOS) is one of the most common reasons for infertility that affects 8%-19% of reproductive-aged women and causes 80% of anovulatory infertility.⁴ It has a range of cardiometabolic features including obesity, gestational diabetes mellitus, type 2 diabetes, hypertension, dyslipidemia and subclinical cardiovascular disease. While most metabolic risk factors of PCOS are relatively well recognized, the relationship between PCOS and blood pressure remains controversial.⁵ Several studies suggest an increased prevalence of high blood pressure among women with PCOS, while others report no difference in blood pressure among women with and without PCOS. Moreover, debate around the impact of PCOS on blood pressure independent of body mass index continues.⁶

Obesity is not only common in PCOS but is also a risk factor for high blood pressure. It has negative effects on reproductive health and associated with an increased rate of pregnancy complications, including gestational hypertension, preeclampsia, gestational diabetes, postpartum hemorrhage.⁷ There is some evidence showing that there is an increase in endometrial thickness throughout the menstrual cycle in infertile patients with PCOS, when compared to infertile patients without PCOS.⁸ An endometrial thickness <7 or >14 mm was associated with the lowest chance of pregnancy, while a thickness of 9–14 mm maximized the chance of live birth and was significantly better than a thickness of 7–8 mm.⁹

Factors such as maternal age, ovarian reserve, duration of infertility, type of infertility, hormonal levels and endometrial receptivity can influence the success of a clinical pregnancy. However, there are still some unknown risk factors that could affect the pregnancy outcome of ART. Therefore, this retrospective study was conducted to determine some of the clinical and embryological factors that may influence pregnancy outcome in normotensive women undergoing frozen embryo transfer cycles.

METHODS

This retrospective cohort study included normotensive women who underwent frozen embryo transfer cycles at Institute of Reproductive Medicine and Women's Health, The Madras Medical Mission from May 2023 to January 2025. A total number of 80 patients were included in the study. The patient's data including age, unique health identification, body mass index, type of infertility,

endometrium thickness, blood pressure, number of embryos transferred, embryo grade, day of embryo transfer and beta human chorionic gonadotropin (β -hCG) result were collected. Normotensive women aged 20-45 years with autologous oocytes undergoing intracytoplasmic sperm injection (ICSI) and frozen embryo transfer (FET) cycles, men who underwent surgical sperm retrieval were included. Women aged > 45 years, with missing blood pressure, who underwent fresh embryo transfer, oocyte recipients and hypertensive women were excluded.

Measurement of blood pressure

Blood pressure was measured by staff nurses to all the patients on their first visit to the hospital. The patient was asked to rest for 10 minutes before measuring the blood pressure. Blood pressure was measured using CIRCA Digital Blood Pressure Monitor. Normal blood pressure was defined as a systolic blood pressure of 90-129 mmHg and diastolic blood pressure of 60-84 mmHg and high normal blood pressure was defined as a systolic blood pressure of 130-139 mmHg and diastolic blood pressure of 85-89 mmHg according to 2020 ISH global hypertension practice guidelines.

Ovulation induction

All patients included in the study underwent controlled ovarian hyperstimulation using GnRH antagonist protocol. Dosage of gonadotrophins used for stimulation was decided based on women's age, body mass index, anti mullerian hormone (AMH) level and antral follicle count. Ovulation induction was started on day 2/3 of menstrual cycle, followed by transvaginal ultrasonography and serum estradiol monitoring to track the ovarian response. Once the presence of follicles reaches the size of 18-22 mm in diameter was confirmed, hCG trigger was administered.

Oocyte retrieval

In our centre, oocyte retrieval under transvaginal ultrasonography was performed 35 hours post hCG trigger. The follicular fluid is collected in a 14 mL preincubated round bottom tube and poured into the preincubated screening dish. The screening dish is screened under stereo zoom microscope for oocyte cumulus complexes. Oocyte cumulus complexes (OCCs) were rinsed in a pre-equilibrated (37°C) G-MOPS media, followed by transferring them to a centre-well dish containing pre-equilibrated G-IVF media overlaid with OV-oil (Incubation dish) and cultured for 2-3 hours in a CO₂ incubator to achieve synchronization between cytoplasmic and nuclear maturation of the oocytes.

Sperm preparation

Fresh or frozen semen samples were used for ICSI. Semen samples were prepared using discontinuous density

gradient method. Pre-equilibrated 80% and 40% density gradient media and G-MOPS were used to separate highly motile sperms. Semen sample was overlaid on top of 40% and 80% density gradient media and centrifuged at 1500-2000 rpm for 10 minutes, followed by discarding the supernatant and the pellet was thoroughly mixed with approximately 1 ml of G-MOPS media and centrifuged at 1200-1500 rpm for 5 minutes, the supernatant was discarded and the neat pellet was overlaid with 0.3 ml of G-MOPS media and swim up was performed.

Denudation

Oocyte denudation is a prerequisite for ICSI and oocyte cryopreservation where cumulus cells were removed through both enzymatic and mechanical method to assess the nuclear maturity of oocyte before ICSI. After 2-3 hours of incubation, OCCs were denuded before ICSI by briefly exposing them to 80 IU/ml HYASE for not more than 30 seconds and gentle pipetting is done using glass Pasteur pipette, followed by gentle pipetting with 170 µm and 140 µm flexipipette in the pre-equilibrated G-MOPS media to remove the cumulus cells. The oocytes were transferred to pre-equilibrated G-IVF media overlaid with OV-Oil (Pre-ICSI dish) post denudation.

Intracytoplasmic sperm injection

RI micromanipulator with inverted microscope was used to perform ICSI. An ICSI dish with microdroplets of G-MOPS and a streak of PVP was prepared, overlaid with OV-Oil and equilibrated at 37°C for 30 minutes. Prepared semen sample was loaded into PVP streak and oocytes were transferred from pre-ICSI dish to G-MOPS droplet. Both the injection and holding needle were aligned at 30-35 degree angle and made sure that they are free of air bubbles.

Once the required morphologically normal spermatozoa were immobilized and aspirated into the injection needle and the oocytes were held with the holding needle, ICSI was performed on all mature oocytes (metaphase 2) according to standard protocol. Once the procedure of ICSI was completed, the injected oocytes were cultured in a G-TL culture media overlaid with OV-Oil in a tri-gas bench top incubator at 37°C.

Embryo assessment

Embryo assessment was done according to ESHRE/ALPHA consensus. Fertilization was assessed 17±1 hours post ICSI, where fertilization was confirmed with the presence of two polar bodies and two pronuclei with precursor bodies. On day 2 (4-cells stage) and day 3 (8-cells stage), the embryos were assessed and graded based on number of blastomeres, blastomere size, fragmentation and multinucleation. On day 4, the embryos were assessed and graded based on compaction of blastomeres. On day 5, the embryos were assessed and graded based on the grade of expansion, grade of inner cell

mass and grade of trophectoderm cells according to Gardner's blastocyst grading system.

Vitrification and thawing

Vitrification of embryos on Day 2/3/5 was carried out for all patients undergoing frozen embryo transfer, followed by thawing of vitrified embryos before or on the day of embryo transfer using kitazato vitrification and thawing protocol.

Protocol for vitrification

In a sterile prelabelled 60 mm petri dish, 300 microliters of equilibration solution and 300 microliters of vitrification solution were transferred and equilibrated at room temperature for 30 minutes. The embryos were exposed in equilibration solution for 10-15 minutes (12 mins for cleavage stage embryos and 15 minutes for blastocyst), followed by exposing the embryos in vitrification solution for 1 min and the embryos were aspirated from vitrification solution and placed onto the cryolock with vitrification solution as minimal as possible. The cryolock was plunged, sealed, placed in a goblet and stored in a storage dewar at -196°C.

Protocol for thawing

0.5 ml of thawing solution was equilibrated at 37°C for 30 mins, 300 microliters of diluent solution and 300 microliters of washing solution were transferred into a sterile prelabelled 60 mm petri dish and equilibrated at room temperature for 30 minutes. The sealed cap of the cryolock was carefully removed and immediately plunged into thawing solution for 1 minute, followed by exposure of embryos in diluent solution for 3 minutes and washing solution for 6 minutes. The embryos were transferred to pre-equilibrated culture media (G-TL media) and cultured till the procedure of embryo transfer.

Embryo transfer

Embryo transfer will be done under transabdominal ultrasonography. When the patient's bladder is full, the patient will be put in a lithotomy position and draped with sterile towels. Cusco's speculum will be gently inserted into the vagina and cleaned with sterile gauze and normal saline. Under ultrasound guidance, outer catheter will be inserted into the cervical canal just above the internal OS. The embryos will be loaded from transfer dish (containing pre-equilibrated G2 media) into the inner catheter (with volume <15 µl) by indirect method. The inner catheter will be gently inserted through the outer catheter and the embryos are transferred into the uterus.

Pregnancy

On 16th day after embryo transfer, the β-human chorionic gonadotropin test was performed for all patients to confirm the clinical pregnancy.

Statistical analysis

To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean and standard deviation were used for continuous variables. To find the significant in the multivariate analysis, the Chi square test was used. In all the above statistical tools, the probability value of 0.05 is considered as significant level. The collected data were entered in Microsoft excel 2021 and analyzed with JMP Statistical software.

RESULTS

The demographic data and factors influencing the pregnancy outcome in normotensive patients undergoing Frozen Embryo Transfer were shown in Table 1. The mean age of the patient was 31.8±4.5 years. The mean body mass index of the patient was 27.79±5.3. The mean endometrial thickness of the patient was 8.89±1.1. The mean systolic blood pressure of the patient was 114.56±12.4. The mean diastolic blood pressure of the patient was 73.83±8.5 (Table 1).

Blood pressure

Table 2 summarize the comparison between blood pressure and clinical pregnancy rate. Among 80 patients, 77.5% had normal blood pressure and 22.5% had high normal blood pressure. In patients with normal blood pressure, 41.9% were resulted in clinical pregnancy and in patients with high blood pressure, 44.4% were resulted in clinical pregnancy. Clinical pregnancy rate was higher in patients with high blood pressure when compared to patients with normal blood pressure (p=0.8497) (Table 2).

Type of infertility

Table 3 summarize the comparison between type of infertility and clinical pregnancy rate. Among 80 patients, 66.25% had primary infertility and 33.75% had secondary infertility. In patients with primary infertility, 41.16% were resulted in clinical pregnancy and in patients with secondary infertility, 33.33% were resulted in clinical pregnancy. The clinical pregnancy rate was higher in patients with primary infertility when compared to patients with secondary infertility (p=0.2365) (Table 3).

Polycystic ovarian syndrome

Table 4 summarize the comparison between PCOS and clinical pregnancy rate. Among 80 patients, 25% had polycystic ovarian syndrome and 75% doesn't have polycystic ovarian syndrome. In patients with polycystic ovarian syndrome, 55% were resulted in clinical pregnancy and in patients without polycystic ovarian syndrome, 38.33% resulted in clinical pregnancy. The clinical pregnancy rate was higher in PCOS patients when compared with non-PCOS patients (p=0.1916) (Table 4).

Body mass index

Table 5 summarize the comparison between body mass index and clinical pregnancy rate. Among 80 patients, 3.75% comes under slim category, 27.5% comes under normal category, 38.75% comes under overweight category and 30% comes under obese category. In patients under slim category, 0% were resulted in clinical pregnancy, under normal category, 36.36% were resulted in clinical pregnancy, under overweight category, 51.61% were resulted in clinical pregnancy and under obese category, 41.66% were resulted in clinical pregnancy. The clinical pregnancy was higher in overweight patients when compared to other groups (p=0.3059) (Table 5).

Day of embryo transfer

Table 6 summarize the comparison between day of embryo transfer and clinical pregnancy rate. Among 80 patients, 35% patients underwent day 3 embryo transfer, 50% patients underwent day 4 embryo transfer and 15% patients underwent day 5 embryo transfer. In patients with day 3 embryo transfer, 42.85% were resulted in clinical pregnancy, with day 4 embryo transfer, 47.5% were resulted in clinical pregnancy and with day 5 embryo transfer, 25% were resulted in clinical pregnancy. The clinical pregnancy rate was higher in patients who underwent day 4 embryo transfer when compared to other groups (p=0.3839) (Table 6).

Endometrium thickness

Table 7 and Figure 6 summarize the comparison between endometrium thickness and pregnancy rate. Among 80 patients, 56.25% patients had endometrium thickness less than 9 mm, 7.5% patients had endometrium thickness equal to 9 mm and 36.25% had endometrium thickness more than 9 mm. In patients with ET < 9 mm, 42.2% were resulted in positive clinical pregnancy, with ET=9 mm, 50% were resulted in positive clinical pregnancy and with ET > 9mm, 58.6% resulted in positive clinical pregnancy. The clinical pregnancy rate was higher in patients with endometrium thickness more than 9 mm when compared to other groups (p=0.3863) (Table 7).

Grade of embryo

Table 8 summarize the comparison between grade of embryo transferred and pregnancy rate. Among 80 patients, 78 patients were transferred with good quality embryos, where 26.9% patients had cleavage stage grade A embryo, 57.7% patients had morula and 15.4% had expanded blastocyst. In patients with cleavage stage grade A embryos, 28.5% resulted in clinical pregnancy, with morula, 51.1% resulted in clinical pregnancy and with expanded blastocyst, 33.3% resulted in clinical pregnancy. The clinical pregnancy rate was higher in patients who underwent morula transfer when compared to other groups (p=0.1783) (Table 8).

Table 1: Demographic data and factors influencing pregnancy outcome.

Variables	Number of patients	Mean±SD
Patient's age	80	31.8±4.5
Body mass index	80	27.79±5.3
Endometrial thickness	80	8.89±1.1
Systolic blood pressure	80	114.56±12.4
Diastolic blood pressure	80	73.83±8.5

Table 2: Blood pressure and pregnancy rate.

Factor		Total number of patients	Positive (β-hCG)	Negative (β-hCG)	P value
Normal blood pressure	Count	62	26	36	0.8497
	%	77.5	41.9	58.1	
High blood pressure	Count	18	8	10	
	%	22.5	44.4	55.6	

Table 3: Type of infertility and pregnancy rate.

Factor		Total number of patients	Positive (β-hCG)	Negative (β-hCG)	P value
Primary infertility	Count	53	25	28	0.2365
	%	66.25	41.16	52.84	
Secondary infertility	Count	27	9	18	
	%	33.75	33.33	66.67	

Table 4: Polycystic ovarian syndrome and Pregnancy Rate.

Factor		Total number of patients	Positive (β-hCG)	Negative (β-hCG)	P value
PCOS	Count	20	11	9	0.1916
	%	25	55	45	
Non-PCOS	Count	60	23	37	
	%	75	38.33	61.67	

Table 5: Body mass index and pregnancy rate.

Factor		Total number of patients	Positive (β-hCG)	Negative (β-hCG)	P value
Slim	Count	3	0	3	0.3059
	%	3.75	0	100	
Normal	Count	22	8	14	
	%	27.5	36.36	63.64	
Overweight	Count	31	16	15	
	%	38.75	51.61	48.39	
Obese	Count	24	10	14	
	%	30	41.66	58.34	

Table 6: Day of transfer and pregnancy rate.

Factor		Total number of patients	Positive (β-hCG)	Negative (β-hCG)	P value
Day 3	Count	28	12	16	0.3839
	%	35	42.85	57.15	
Day 4	Count	40	19	21	
	%	50	47.5	52.5	
Day 5	Count	12	3	9	
	%	15	25	75	

Table 7: Endometrium thickness and pregnancy rate.

Factor		Total number of patients	Positive (β -hCG)	Negative (β -hCG)	P value
Endometrium thickness <9 mm	Count	45	19	26	0.3863
	%	56.25	42.2	57.7	
Endometrium thickness =9 mm	Count	6	3	3	
	%	7.5	50	50	
Endometrium thickness >9 mm	Count	29	17	12	
		36.25	58.6	41.4	

DISCUSSION

The goal of the study is to evaluate the factors influencing the pregnancy outcome in normotensive women undergoing frozen embryo transfer. In this study we evaluated factors including blood pressure, type of infertility, polycystic ovarian syndrome, day of embryo transfer, embryo grade, body mass index, endometrium thickness. The results obtained from this study shows that there is no significant association between blood pressure, type of infertility, polycystic ovarian syndrome, day of embryo transfer, embryo grade, body mass index and endometrium thickness with pregnancy rate.

However, the study by Chen et al reported that in women with a successful pregnancy outcome, systolic blood pressure and diastolic blood pressure were lower than in those who did not achieve pregnancy because the study showed that even rather small differences in SBP and DBP in women without hypertension undergoing IVF/ICSI treatment are associated with the livebirth rate.¹² But the study shows that the clinical pregnancy rate is higher in patients with high normal BP when compared to patients with normal blood pressure, with no statistical significance (p value=0.8497, where p<0.05 is statistically significant). It could be because of the sample size in our study.

The study by Bergin et al reported that patients with secondary infertility appear to have a higher likelihood of ongoing pregnancy when compared to those with primary infertility when attempting a single euploid embryo transfer.¹³ Our study shows that the clinical pregnancy rate is higher in patients with primary infertility than in patients with secondary infertility, with no statistical significance (p value=0.2365, where p<0.05 is statistically significant). The study by Liu et al reported that patients with PCOS had higher clinical pregnancy rate when compared with non-PCOS patients because the PCOS women included in the study were younger and had higher BMI compared with women without PCOS.¹⁴ The study also shows that patients with PCOS had higher clinical pregnancy rate when compared with non-PCOS patients, with no statistical significance (p value=0.1916, where p<0.05 is statistically significant). The study by Funabiki et al reported that pregnancy rate was significantly lower in patients with high BMI when compared to patients with normal BMI and low BMI group showed no significant

impact on fertility because high BMI group had a significant reduction in the number of oocytes retrieved, in the number of good quality embryos and a significant increase in the number of bad quality embryos.¹⁵ But the study reported that patients with high BMI (Overweight) resulted in higher clinical pregnancy rate when compared to low BMI, normal BMI and Obese patients, with no statistical significance (p value=0.3059, where p<0.05 is statistically significant) because the patients with high BMI had good quality embryos transferred.

The study by Sun-Hee et al reported that the pregnancy outcomes of day 4 and day 5 embryo transfers were similar and implantation rate was significantly higher in day 5 embryo transfer patients because the embryos transferred on day 5, 85.9% were of good quality and 79.9% of the embryos transferred on day 4 were good.¹⁶ Our study showed that patients who underwent day 4 embryo transfer had higher clinical pregnancy when compared to day 3 and day 5 embryo transfers, with no statistical significance (p value=0.3839, where p<0.05 is statistically significant). It could be because of the number of patients who underwent day 4 embryo transfer is higher than the number of patients who underwent day 5 embryo transfer.

The study by Zhiquin et al reported that patients with endometrial thickness 9 to 14 mm had higher clinical pregnancy rate when compared to patients with endometrium thickness<8 mm because lower endometrial thickness on the embryo transfer day significantly affects IVF outcomes in cleavage embryo transfer cycles independent of other factors.¹⁷ The study also showed similar results that patients with endometrial thickness>9 mm had higher clinical pregnancy rate when compared with endometrium thickness <9 mm and equal to 9 mm, with no statistical significance (p value=0.3863, where p<0.05 is statistically significant) following the above-mentioned reason. The study by Ryh-Sheng Li et al reported that the pregnancy rate and live birth rate was comparable between morula embryo transfer and blastocyst embryo transfer.¹⁸ However, the term birth rate was higher in patients who underwent morula embryo transfer when compared to blastocyst embryo transfer because embryos could be exposed to the natural environment, in the uterus, for the maximum time and minimally in vitro, before implantation. This reduction in the time of embryos existing ex vivo reduced the susceptibility to the interruption of epigenetic regulatory

mechanism. Our study also showed similar results where morula embryo transfer resulted in higher clinical pregnancy rate when compared to cleavage stage and blastocyst embryo transfer, with no statistical significance (p value=0.1783, where p<0.05 is statistically significant).

CONCLUSION

Considering the factors analysed, the study concludes that factors including blood pressure, type of infertility, PCOS, BMI, day of embryo transfer, endometrium thickness, grade of embryo transferred were non-significantly associated with the clinical pregnancy rate in this study. This could be because of limited sample size and time restrictions. However, factors including higher blood pressure, primary infertility, PCOS, overweight, day 4 embryo transfer, endometrium thickness >9 mm and morula grade embryo transfer resulted in higher pregnancy rate. This study doesn't predict any significant factor that influence the clinical pregnancy outcome in normotensive women undergoing frozen embryo transfer. Further investigations with randomized trials are required to confirm the confounding nature of the factors analysed and their influence on pregnancy outcome in normotensive women undergoing frozen embryo transfer.

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