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# Comparison of Pregnancy Rates in Fresh Embryo Transfer vs. Frozen Embryo Transfer in Women with Polycystic Ovary Syndrome, Undergoing in Vitro Fertilization in Jalandhar, Punjab

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***Abstract*: Anovulation being the major cause of subfertility, among the Indian women, PCOS accounts for almost, 80% of them. Such women seek advice for irregular periods & almost 25% of them seek medical advice for infertility due to anovulation. Sedentary lifestyle of adolescents & adults, ingestion of food with transfat, obesity, lack of exercise etc., aggravate these problems further. Clomiphene Citrate is the first line treatment of PCOS. But in women receiving clomiphene citrate, though 60-70% show ovulation, only 25-30% of them conceive. In case of unsuccessful CC treatment, gonadotrophin treatment is started, but it comes with the high risk of hyper stimulation (10-12%). OHSS is a medical emergency with fluid shift into the third space, due to increased capillary permeability. It is one of the most common & serious complication in an IVF clinic, ranging from 1-8%, with hospitalization rate of 0.7% usually, it is expected to occur in a fresh cycle. There is some data to suggest that Embryo Freezing (Cryopreservation) in a fresh cycle, followed by Frozen-Thawed-Embryo Transfer in a natural cycle in which the endometrium is primed, could result in a higher clinical pregnancy rate. Also the problems of superovulation leading to poor implantation could be avoided.**

**Methodology: This study was conducted at Babies World IVF centre, Chhabra hospital & Maternity Home, Jalandhar, Punjab. This study was conducted from 1st January 2017 to 31st January 2020. Study Design: It was a longitudinal observational study. Purposive sampling was adopted.**

**Sample Size: 100 cases.**

**Main Outcome Measures: Clinical pregnancy rate was the primary outcome. Secondary outcome was any adverse event like miscarriage & ectopic pregnancy.**

**Results: The occurrence of pregnancy either biochemical or clinical 46% v/s 42% & adverse event/miscarriage (39.1% v/s 14.3%) in fresh v/s frozen was not significantly associated with type of Embryo Transfer (P>0.05). However, higher proportions i.e. 30.4% had more than one foetuses among fresh embryo group compared to frozen embryo group (4.8%) (P<0.05); implicating lesser ovarian hyperstimulation among the frozen group.**

***Keywords*: Clinical pregnancy rates, Frozen-thawed embryo transfer, In vitro fertilization, Polycystic ovarian syndrome.**

## 1. Introduction

PCOS is the most common endocrinopathy among women; & about 80% of the infertile women are suffering from it. We used the criteria accepted by Eshre & ASRM in the diagnosis of PCOS- i.e., Ovarian hyper androgenism, anovulation and ultrasound image of ovaries showing typical features of dense cortex, hypervoluminous & peripherally arranged small follicles. Anovulation in the PCOS women is associated with oligomenorrhoea, hirsuitism & obesity.

The ultrasound features of PCOS are atleast 10 follicles, 2-10 mm in diameter in the cortical part of ovary & increased stromal volume. Elevated LH level & LH/FSH ratio is detected in most patients. Also Insulin resistance with hyperinsulinemia are detected in almost 70-80% of such patients. Due to insulin resistance, many of the PCOS patients develop diabetes mellitus (15% - 20%) & hypertension with cardiac risks (40%) later on in their lives.

## 2. Pathophysiology

There is an increase in the average daily production of both Androgens & Oestrogens in women with PCOS. This can be observed in the elevated serum concentrations of DHEAS, 17-OHP, testosterone, Androstenedione etc. But in contrast, the serum Eestradiol Levels in women with PCOS generally remain within the range seen in the early follicular phase; due to low level productions from limited follicular development.

## 3. Reproductive Health in PCOS

Along with anovulation, other factors such as obesity, metabolic and endocrinological abnormalities are known to affect the oocytes & also fetal development. The oocytes from PCOS women, may show reduced developmental competence, which can affect meiosis & affect fertilization potential of the

oocytes, which may lead to development of abnormal embryo leading to early miscarriages. [2] During early pregnancy, the embryo may be exposed to increased androgenization, especially on female offspring. This may, infact, disturb the epigenetic programming. Also, transgenerational effect may be related to the influence of hyperinsulinemia & also effect the intrauterine environment.

### 4. Ovulation Stimulation in PCOS

The polycystic ovaries respond variedly to ovulation induction by gonadotrophins. The initial response is slow, but with increasing dosage, may suddenly lead to an overresponse, culminating with OHSS. Usually lower doses of gonadotrophin are required, preferably follicle stimulating hormone, due to an initial high levels of Leutinizing hormone.

Due to exaggerated response, more oocytes are retrieved. But the fertilization rates may be reduced. OHSS & failed fertilization may lead to cycle cancellations. Hence the efficacy of Frozen Embryo Transfer. Also, the miscarriage rates in women with PCOS following IVF may be high, as compared to women with normal ovaries. This may be attributed to the hyperandrogenic environment, metabolic disorders, poor implantation & poor placentation. Excessive response in PCOS women may be due to an increased number of antral follicles. These follicles are not atretic, but they get stimulated in an exaggerated manner to the exogenous gonadotrophins. Serum Anti Mullerian Hormone (AMH) is a glycoprotein produced from granulose cells, which is elevated in PCOS. There is now evidence correlating AMH, oligo-anovulation and hyperandrogenemia. Elevated AMH can predict the occurrence of OHSS & can be resorted to guide the stimulation protocols, so as to avoid over-response or OHSS.

### 5. Clinical Outcomes Among PCOS Patients with or without IVF Study Setting

Jalandhar is a district consisting of five tehsils/subdivisions viz. Jalandhar I, Jalandhar II, Nakodar, Phillaur and Shahkot and five sub-tehsils, viz. Adampur, Bhogpur, Kartarpur, Goryan and Nurmahal. The district is divided into 11 development blocks, viz., Jalandhar East, Jalandhar West, Bhogpur, Adampur, Nakodar, Shahkot, Phillaur, Nurmahal, Lohian, Rurka Kalan and Mehatpur. [21]

It totally consists of 21.9 lakh population with 10.5 lakhs being females. The district has 21 hospitals, 28 primary health centres, 124 dispensaries, 6 CHCs/hospitals, 4 Unani institutions and 8 homeopathic. There are 2792 doctors and 14 family planning centres. (“Demography.” Demography, Jalandhar Web Portal, India, jalandhar.nic.in/demography)

There are nearly fifteen centres enrolled as Assisted Reproductive Technology (ART) Clinicsunder ‘National Registry of ART Clinics and Banks’ under ICMR in Jalandhar and our study setting is one of the NABH accredited advanced fertility centre with state of art equipment. [40] (ICMR. List of Enrolled Assisted Reproductive Technology (ART) Clinics Under National Registry of ART Clinics and Banks in India. [14] ICMR, New Delhi, 2018, pp. 1-51,

https://www.icmr.nic.in/sites/default/files/whats_new/New-list-of-approved-ART-Clinics-14-09-2018.pdf. Accessed 16 Mar. 2020.) [32]


Fig. 1. Map of Jalandhar district depicting study setting with marked study area
Source: (“Demography.” Demography, Jalandhar Web Portal | India, jalandhar.nic.in/demography

### 6. Study Place

The study was conducted at Babies World IVF centre, Chhabra Hospital and Maternity Home, Jalandhar, Punjab. It is an advanced fertility centre with state of art equipment with NABH Accredited IVF lab, bearing number ISO 9001:2015. [33]. It is run by a team of experts & dedicated professionals, namely Infertility Consultants, Andrologist, Microbiologist, Counsellors, Programme Coordinator, ART Specialist, Gynaecologist & technical staff.

It is a sterile well equipped lab with hepafilter room, Co2, incubator, Olympus Microscope with Narshige Micromanipulator, CASA, Laser hatching system and has an IVF theatre, which deals only with ovum pick-up and embryo transfer which reduces the risk of the embryos being exposed to the detrimental fluctuations in environment. It is backed by ultrasound, hormonal assays and endoscopic facilities. Endoscopic Procedures are done in a separate general operation theatre. The andrology lab is located within the IVF unit and can deal with all types of male infertility. Sperm banking facilities are also available thus offering all aspects of infertility treatment under one roof. A viewing gallery is provided to enable patients and doctors to observe the actual IVF/ICSI/IUI procedures. It also has semen collection room equipped with audio-visual facilities provided for the male partner for semen collection. On an average more than 100 cycles of IVFs are conducted in a year. [4]

(“Infrastructure.” Babiesworldivfcentre.com, www.babiesworldivf.com/infrastructure.)

*Study Period:* This study was conducted for a period of three years from February 1st 2017 to January 31st 2020).

*Study Design:* It was a longitudinal observational study

*Sampling:* Purposive sampling was adopted

*Sample size:* 100 cases.

*Sample size calculation:*

Based on a study conducted by Bharathi RV et al., considering polycystic ovarian syndrome (PCOS) prevalence of 6% of the cases (p), q=100-p i.e., 94%, at 95% confidence interval and permissible error (L) as 5%, total sample size of ≈ 87 was calculated using the formula n=z2(pq/L2). The total sample size of 87 ≈ 100 was considered for the study after making an allowance of 15% of the required sample size lost to follow up. (Bharathi RV, Swetha S, Neerajaa J, Madhavica JV, Janani DM, Rekha SN, Ramya S, Usha B. An epidemiological survey: Effect of predisposing factors for PCOS in Indian urban and rural population. Middle East Fertility Society Journal. 2017 Dec 1;22(4):313-6.) [24]

*Inclusion Criteria:*

All the study subjects who were diagnosed with polycystic ovarian syndrome (PCOS) as a cause of infertility at Babies World IVF centre, Chhabra Hospital & Maternity Home, Jalandhar, Punjab between the study period.

*Exclusion Criteria:*

Patients with noted history of major medical illnesses, uterine anomalies or adnexal pathology were excluded. Those with history of cycles cancelled prior to oocyte retrieval were also excluded.

*Study subjects:*

After considering inclusion and exclusion criteria,50 patients with PCOS who underwent fresh embryo transfer and 50 patients who underwent frozen embryo transfer for infertility treatment at Babies World IVF centre, Chhabra Hospital & Maternity Home, Jalandhar, Punjab between the study periods were considered for the study.

*Ethical issues and ethical committee clearance [Annexure-I]*

The ethical approval was taken by the Institutional Ethics Committee of Babies World IVF centre, Chhabra Hospital & Maternity Home, Jalandhar, Punjab. [16]

*Informed Consent [Annexure-2]:*

Patients were explained about the study procedure and importance of the study in their own language of understanding and written informed consent was taken from them.

Procedure of data collection

It was a longitudinal observational study conducted among 100 PCOS patients who underwent infertility treatment at Babies World IVF centre, Chhabra Hospital & Maternity Home, Jalandhar, Punjab and full-filled the inclusion and exclusion criteria during the study period from February 1st 2017 to January 31st 2020 were included. Based on the inclusion and exclusion criteria, a total of 100 patients (50 with fresh embryo transfer and another 50 with frozen embryo transfer) were enrolled after obtaining written informed consent from patients and ethical committee clearance from the institutional ethical committee board. [27]

*Data collection:*

All information pertaining to the patients viz., name, age, gender, address, relevant past and present medical history, height, weight, other examination findings were obtained under three headings:

1. Socio-demographic data: Name, age, gender, address
2. History: Details on presenting complaints, history of previous treatment received, family history, marital history, menstrual history, previous conception and also data related to risk factors for poor ovarian response (POR) viz., history of chronic smoking, drinking, previous ovarian surgery, previous chemotherapy [6].
3. General Physical Examination (GPE) and Systemic Examinations: Routine physical examination and anthropometry measurements, along with specific gynecological examinations were conducted. BMI in $kg/m^2$ was calculated and categorized according to WHO Asia-Pacific guidelines.
4. Investigations: Results of transvaginal scans, Laparoscopic findings, tubal evaluation, along with baseline hormone profile: serum FSH (follicle stimulating hormone), LH (luteinizing hormone) and AMH levels along with the parameters of semen analysis of the male partner were obtained.
5. Method of estimation of AMH levels: Generation 2 ELISA kit with sensitivity being 0.025 ng/ml, and intra- and inter-assay variation of the assay being 7% AMH levels were measured.

*Controlled ovarian stimulation, ovarian response & Trigger:*

Among the patients with PCOS and infertility controlled ovarian hyperstimulation (COH) and embryo transfer implemented by the gonadotrophin-releasing hormone antagonist - GnRH-antagonist protocol required less time and has been considered to be more cost-effective than the standard GnRH agonist long protocol, and is known to be also associated with a significantly lower incidence of ovarian hyperstimulation syndrome. [13] Thus, the GnRH-antagonist protocol was recommended and used in our study. (Chen R, Chen S, Liu M, He H, Xu H, Liu H, Du H, Wang W, Xia X, Liu J. Pregnancy outcomes of PCOS overweight/obese patients after controlled ovarian stimulation with the GnRH antagonist protocol and frozen embryo transfer. Reproductive Biology and Endocrinology. 2018 Dec 1;16(1):36). [18]

*Standard Antagonist (flexible) protocol:*

- After the basal Day 2 Transvaginal scan (TVS) for Antral follicle count, the patient was administered an initial dose of 225-375 IU human menopausal gonadotropin (HMG) or Follicle Stimulating Hormone (FSH purified or recombinant). The doses were adjusted based on the response to gonadotropin as assessed by serum E2 levels and sonographic monitoring of follicular growth till it reached 18-20 mm in size starting from the Day 2

of the cycle.

- Once the follicles reached 13-14mm, Injection Cetorelix 250 µg subcutaneously was added in the morning time.
- The ovarian response was monitored by serial trans-vaginal scans for increasing size of the follicles and serum E2 levels on days 7, 9 and 11.

*Trigger:*

- When three or more follicles of size 18 mm or more were seen, final oocyte maturation trigger was given with inj. hCG Injection Ovitrelle 250 µg*2 subcutaneously, or with inj. Leupride acetate 0.5 mg (Sun Pharmaceutical Ind Ltd, Mumbai). [20]

*Oocyte Retrieval:*

- Under sedation, oocyte retrieval was performed trans-vaginally using a 35 cm 17G oocyte aspiration needle 34–36 hr after the trigger. For most of them it was done at 36 hrs but for those with poor reserve it was scheduled at 34 hrs.

*Fertilization:*

- The retrieved Eggs were incubated at 37◦C in HEPES, later denuded and their quality was assessed by embryologist.
- The oocytes were classified based on nuclear maturation grading into metaphase II (mature) or non-metaphase II categories. The latter category included oocytes at the metaphase I and prophase I stage. The oocytes that did not develop to metaphase II after 8 hours of incubation were discarded.
- ICSI dishes were prepared and performed by a senior embryologist.
- Fertilization was defined as the formation of zygotes with two pronucleiafter 16–18 hours(normal fertilization) and later the zygotes were evaluated using the Z-score system.[6]
- The embryos were cultured using Fert culture media at 37°C, 5% CO2 in the Benchtop incubator.
- On day 2 (44–46 h after insemination or sperm injection) and on day 3 (66–72 h insemination or sperm injection), cleavage embryos were assessed by embryologist and were graded according to the criteria described by Veeck.[14]

*Embryo transfer:*

*Fresh embryo transfer:*

- Following oocyte retrieval, Embryo Transfer was done on Day 3/5 i.e., 3 days after fertilization or with blastocyst transplantation 5 days after fertilization. [20]

*Frozen embryo transfer:*

- The frozen embryos, frozen at 6 cell stage by Rapid freeze protocol such as vitrification were transferred for frozen embryo transfer group following the preparation of the endometrium and/or down regulation.

All patients were prescribed 600 mg of micronized progesterone for 2 weeks as luteal phase support. On the day of hCG administration, serum estradiol, LH and progesterone levels were measured [20]

Furthermore, the 50 patients who underwent frozen embryo transfer and another 50 who underwent fresh embryo transfer as a part of in-vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI), were followed up and compared for the clinical outcomes in terms of pregnancies and also for the adverse events like miscarriage and ectopic pregnancy.

*Positive Pregnancy:*

- Serum β-hCG> 50 mIU/l was considered as positive. Clinical pregnancy was defined as a viable intrauterine pregnancy (positive cardiac activity) on transvaginal scan performed at 6 weeks.

*Study outcomes:*

- The primary outcome of the study was clinical pregnancy rate.
- The secondary outcome was any adverse event like miscarriage and ectopic pregnancy.

*Operational Definitions:*

- Fertilization rate was calculated based on the number of mature oocytes fertilized into zygote with two pro-nuclei.
- Top quality embryos grading was based on Cleavage Stage Embryo scoring described by Veeck as mentioned below:

*Grades Description:*

- Grade 1: Embryo with blastomeres of equal size, no cytoplasmic fragments
- Grade 2: Embryo with blastomeres of equal size, minor cytoplasmic fragments or blebs.
- Grade 3: Embryo with blastomeres of distinctly equal size, none or few cytoplasmic fragments.
- Grade 4: Embryo with blastomeres of equal or unequal size, significant cytoplasmic fragmentation.
- Grade 5: Embryo with blastomeres of any size, severe or complete fragmentation. [11]

*Anthropometric Measurements:*

*Measurement of Height:* The height was measured with stadiometer. Subjects were made to stand without footwear on the flat surface, with evenly distributed weight on both feet and heels together, and the head positioned so that the line of vision is perpendicular to the body (Frankfurt line). The arms were hung freely by the sides, the head, back, buttocks, and heels in contact with the vertical board. The individual was asked to inhale deeply and maintain a fully erect position. The movable headboard was brought onto the topmost point on the head with sufficient pressure to compress the hair. The height was recorded to the nearest 0.5 cm. [8]

*Measurement of weight:* The weight was measured in kilograms using standardized bathroom weighing machine with person standing erect on centre of the platform, with the body

weight evenly distributed between both the feet with light clothing and looking straight. The weight was recorded to the nearest 0.5 kg. [15]

*Body Mass Index (BMI):* It is also called as Quetelet's index used to assess obesity and computed by weight (kg) divided by height in meters square.

$$BMI = \frac{\text{Weight in Kilograms}}{\text{Height in meter}^2}$$

Table 1
Classification of BMI -WHO Asia-Pacific guidelines [37]

| MI | Category |
|---|---|
| 18.5 | Underweight |
| 8.5 - 22.9 | Normal range |
| 3 - 24.9 | Overweight |
| 5 - 29.9 | Obese – I |
| ≥ 30 | Obese – II |

## 7. Statistical Analysis

All the data were entered into Microsoft excel sheet. Data was analyzed with the Statistical Package for the Social Sciences version 20.0 software. [36] The frequencies of the variables were computed. The continuous data viz., age, estrogen levels, AMH levels, FSH and LH levels were expressed in means and standard deviations for normal distribution or medians and range for skewed distributions. The continuous data were compared between the different comparison groups viz., fresh v/s frozen embryo transfer groups, laser assisted hatching versus no laser assisted hatching groups and the groups with and without PCOS using independent t – test. The ordinal data viz., number of follicles, total numbers of oocytes available, transferred, fertilized and fertilization rate were expressed in medians and were compared between the different comparison groups using Mann Whitney U test. [68] The discrete data viz., occurrence of outcomes like pregnancies, adverse events during pregnancies and number of foetuses, etc., were expressed in proportions and were compared using chi-square test or Fisher's exact test. A P value <0.05 was considered significant for all statistical tests.

*A. Comparison of clinical outcomes among frozen v/s fresh embryo transfer patients with PCOS*

Table 2
Age-group distribution among fresh and frozen embryo transfer group

| **Age group in years** | **Fresh Embryo n (%)** | **Frozen Embryo n (%)** | **$\chi^2$ – value (*P*-value)** |
|---|---|---|---|
| ≤30 | 18 (36.0) | 15 (30.0) | 1.09 (0.58) |
| 31-40 | 24 (48.0) | 23 (46.0) | |
| 41-50 | 08 (16.0) | 12 (24.0) | |
| Total | 50 (50.0) | 50 (50.0) | |

In both fresh and frozen embryo groups, most of them i.e., 48.0% and 46.0% belonged to 31 – 40 years' age group followed by 36.0% and 30.0% of them were younger than 30 years and 16.0% and 24.0% belonged to 41-50 years' age group respectively. There was no significant difference among the groups (P>0.05). The mean age of the study participants among the fresh and frozen embryo groups were 33.98±5.80 years and 34.94±7.00 years respectively and were comparable (P>0.05, t=-0.75). The participants ranged from a minimum of 25 years to a maximum of 49 years in the fresh embryo group and in the other group it ranged from 23 years to 50 years.

Table 3
Distribution of study subjects based on BMI classification among both fresh and frozen embryo transfer groups with PCOS patients

| **BMI (kg/m$^2$)** | **Fresh Embryo n (%)** | **Frozen Embryo n (%)** | **$\chi^2$ – value (*P*-value)** |
|---|---|---|---|
| Underweight & Normal | 08 (16.0) | 04 (8.0) | 8.71(0.03)* |
| Overweight | 11 (22.0) | 03 (6.0) | |
| Obese class I | 24 (48.0) | 29 (58.0) | |
| Obese class II | 07 (14.0) | 14 (28.0) | |
| Total | 50 (50.0) | 50.0 (50.0) | |

* indicates statistically significant association at P<0.05 [16]

In the fresh embryo transfer group, most of them belonged to obese class I (48.0%) followed by overweight (22.0%), underweight and normal (16.0%) and obese class II (14.0%). In the frozen embryo transfer group, most of them belonged to obese class I (58.0%), followed by obese class II (28.0%), underweight and normal (8.0%) and overweight (6.0%). The distribution proportions of BMI categories varied significantly among the groups (P<0.05). The mean height in fresh embryo transfer group was 2.00±0.0 m and 1.98±0.14 m in the frozen embryo transfer group and it did not vary significantly among the groups (t = -1.00, P= 0.32, 95% CI [-0.06 to 0.02]). The mean weight among fresh and frozen embryo transfer groups were 67.97±11.34kg and 72.60±7.96kg respectively and it was significantly higher in the frozen embryo group (t = -2.37P= <0.02, 95% CI [0.75 to 8.52]). The mean BMI among both fresh and frozen embryo transfer groups were 26.28±4.07kg/m$^2$ and 28.16±3.15kg/m$^2$ and it was significantly higher among frozen embryo group compared to fresh embryo group (t= 2.59P=0.01, 95% CI [0.44 to -3.33]).

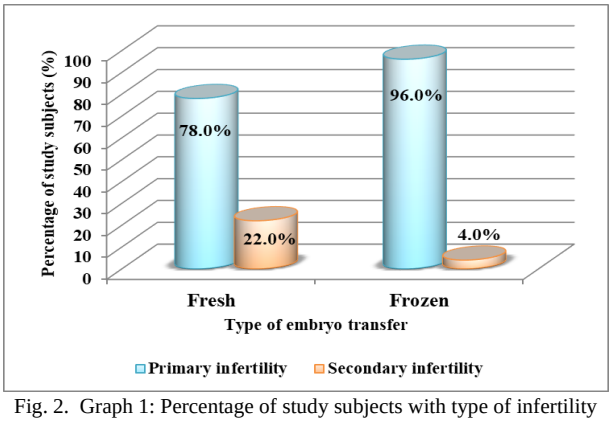


Fig. 2. Graph 1: Percentage of study subjects with type of infertility

Among fresh embryo transfer group, 78.0% of them had primary infertility and22.0% had secondary infertility. In case of frozen embryo transfer, 96.0% of them had primary

infertility and 4.0% of them had secondary infertility. The proportions of those with primary infertility (96.0%) were significantly higher in frozen embryo transfer group compared to fresh group (78.0%) (P<0.05).

The mean duration of infertility was 7.56±2.74 years and it ranged from a minimum of 3 years to a maximum of 18 years. It was 7.96±3.32 years in fresh embryo group and 7.16±1.95 years in frozen embryo group and there was no significant difference among the duration of infertility among both groups (t – value [95% C.I]: 0.15[-1.88 to 0.28]; *P*=0.15).

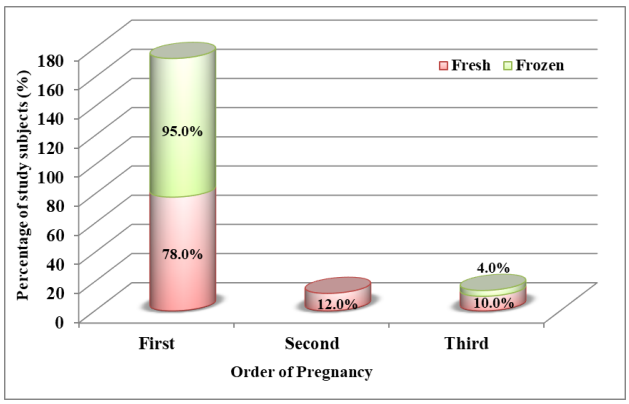


Fig. 3. Graph 2: Percentage of study subjects with different order of planned pregnancy among both fresh and frozen embryo transfer groups with PCOS patients

Majority of the study subjects among both fresh and frozen embryo transfer group had first order of pregnancy (95.0% v/s 78.0%) followed by second (12.0% v/s 0.0%) and third (10.0% v/s 4.0%).

The table 4, shows the average serum levels of different hormones viz., leutinizing hormone, follicle stimulating hormone, estrogen and anti-mullerian hormones among the two different groups of different type of embryo transfers. The levels of LH (Fresh v/s frozen: 10.68 v/s 10.99IU/L; t=0.63, *P*>0.05) did not vary significantly among the two different type of embryo transfer groups. However, levels of FSH (Fresh v/s frozen: 8.38 v/s 13.23IU/L; t=10.93, *P*<0.05), estrogen (Fresh v/s frozen: 12.00 v/s 51.5 ng/ml; U=83, *P*<0.05) and AMH (Fresh v/s frozen: 5.23 v/s 4.24 ng/ml; t=-3.79, *P*<0.05) indicate that, FSH and estrogen levels were significantly higher in frozen group compared to fresh embryo transfer group and it was vice versa in AMH levels (*P*<0.05).

The proportions of those study subjects who underwent IVF (78.0% v/s 74.0%) and ICSI (22.0% v/s 28.0%) as a part of treatment did not differ significantly among the groups (*P*>0.05). However, significantly higher proportions in frozen embryo group (46.0%) underwent laser hatching (*P*<0.05).

The endometrial thickness in fresh v/s frozen groups were 9.37 mm and 10.73 mm respectively and the thickness was significantly higher in frozen embryo group compared to fresh embryo transfer group (t=7.51, *P*<0.05 ).The number of follicles in fresh v/s frozen groups were 12.5 and 18, oocytes were 10and15, number of embryos transferred were 3 in each

Table 4
Average levels of different hormones at the baseline among different groups of fresh and frozen embryo transfers

| Hormonal Levels | Mean±SD | | t – value [95% C.I]/ U- value | *P*- value |
|---|---|---|---|---|
| | Fresh Embryo (n = 100) | Frozen Embryo (n = 100) | | |
| LH (IU/L) | 10.68±3.17 | 10.99±1.41 | 0.63 [-0.67 to 1.28] | 0.53 |
| FSH (IU/L) | 8.38±2.01 | 13.23±2.40 | 10.93 [3.96 to 5.72] | <0.001* |
| Estrogen¥ (ng/ml) | 12.00 (2 - 72) | 51.5 (34 - 72) | 83.00 | <0.001* |
| AMH(ng/ml) | 5.23±1.74 | 4.24±0.65 | -3.79 [-1.51 to -0.47] | <0.001* |

LH - Leutinizing Hormone; FSH - Follicle Stimulating Hormone; AMH - Anti-Mullerian Hormone
¥- Median values with range and Mann Whitney U test applied as test of significance
* indicates statistically significant difference at P<0.05

Table 5
Comparison of modes or part of treatment among different groups of fresh and frozen embryo transfers with PCOS patients

| Mode/ part of fertility treatment | Fresh Embryo n (%) | Frozen Embryo n (%) | $\chi^2$ – value/ Fisher's exact (*P*-value) |
|---|---|---|---|
| **IVF** | 39 (78.0) | 37 (74.0) | 0.22 (0.64) |
| **ICSI** | 11 (22.0) | 14 (28.0) | 0.48 (0.49) |
| **Laser Hatching** | 00 (0.0) | 23 (46.0) | (<0.001)* |

* indicates statistically significant association at *P*<0.05

Table 6
Average endometrial thickness, number of follicles, oocytes, embryos available and fertilized along with the fertilization rate among both the groups of fresh and frozen embryo transfer with PCOS patients

| Particulars | (Mean±SD/Median with range) | | t – value [95% C.I]/ U- value | *P*- value |
|---|---|---|---|---|
| | Fresh Embryo n = 100 | Frozen Embryo n = 100 | | |
| Endometrial Thickness (mm)¥ | 9.37±0.72 | 10.73±1.06 | 7.51 [1.00 – 1.72] | <0.001* |
| Number of follicles | 12.5 [9 – 20] | 18 [12 – 31] | 272.0 | <0.001* |
| Number of oocytes | 10 [7 – 15] | 15 [9 – 22] | 367.5 | <0.001* |
| Number of embryos fertilized | 9 [7 – 14] | 12 [7 – 20] | 554.5 | <0.001* |
| Number of embryos transferred | 3 [3 – 3] | 3 [3- 3] | 1250.0 | 1.00 |
| Fertilization rate (%) | 87.5 [66 – 100] | 83 [52.9 – 100] | 915.0 | 0.02* |

¥ - Mean values with standard deviation and independent t-test applied as test of significance
* indicates statistically significant difference at *P*<0.05

group, and fertilized were9and12 and fertilization rates were 87.5 % and 83 %respectively and except the embryos transferred, all were significantly higher in frozen embryo group compared to fresh embryo transfer group($P$<0.05) but for fertilization rate where it was significantly higher in fresh embryo group compared to frozen ($P$<0.05).

Table 7
Association of biochemical and clinical pregnancy with type of embryo transfer among PCOS patients

| Pregnancy | Fresh Embryo (n=50) n (%) | Frozen Embryo (n=50) n (%) | $\chi^2$ – value (*P*-value) |
|---|---|---|---|
| **Biochemical pregnancy** | | | |
| Yes | 23 (46.0) | 21 (42.0) | 0.16 (0.68) |
| No | 27 (54.0) | 29 (58.0) | |
| **Clinical Pregnancy** | | | |
| Yes | 23 (46.0) | 21 (42.0) | 0.16 (0.68) |
| No | 27 (54.0) | 29 (58.0) | |

Higher proportions i.e., 63/50, 46.0% were positive for both biochemical and clinical pregnancies among fresh embryo transfer group compared to frozen embryo transfer (21/50, 42.0%) group. However, the association between the occurrence of pregnancy either biochemical or clinical and type of embryo transfer was not statistically significant ($P$>0.05).

Table 8
Association of occurrence of any adverse event (miscarriage) with the type of embryo transfer

| Adverse events during pregnancy | Fresh Embryo (n=23) n(%) | Frozen Embryo (n=23) n(%) | *P*-value¥ |
|---|---|---|---|
| Yes | 09 (39.1) | 03 (14.3) | 0.09 |
| No | 14 (60.9) | 18 (85.7) | |

¥ - Fisher's exact test applied

The experience of adverse event (miscarriage) did not vary across the groups (fresh v/s frozen embryo group: 39.1 % v/s 14.3 %; $P$>0.05).

Table 9
Association of study subjects based on number of fetuses with the type of embryo transfer

| Number of fetuses | Fresh Embryo (n=23) n (%) | Frozen Embryo (n=21) n (%) | $\chi^2$ – value (*P*-value) |
|---|---|---|---|
| One | 16 (69.6) | 20 (95.2) | 4.86 (0.03)* |
| More than one | 07 (30.4) | 01 (4.8) | |

* indicates statistically significant association at P<0.05

Among the positive pregnancies, significantly higher proportions i.e., 30.4 % had more than one foetuses among fresh embryo group compared to frozen embryo group (4.8%)($P$<0.05).

*B. Clinical outcomes among fresh v/s frozen embryo transfer groups with PCOS*

Among fresh v/s frozen embryo transfer group with PCOS, the mean ages were 33.98 and 34.97 years, duration of infertility was 7.96 and 7.16 years and embryos transferred were 3 in each respectively and those factors were comparable in both the groups. However, the mean BMI among frozen embryo group (28.16±3.15 kg/m$^2$) was significantly higher compared to fresh embryo transfer group (26.28±4.07 kg/m$^2$) ($P$<0.05). Both the groups had male factors (fresh-30.0%, frozen-30.0%) and tubal factors (fresh-39.0%, frozen – 36.0%) as common causes of infertility.

The endometrial thickness (9.37 v/s 10.73 mm), number of follicles (12.5 v/s 18) and oocytes (10 v/s 15) were significantly higher in frozen embryo group compared to fresh embryo transfer group ($P$<0.05). But the fertilization rates were 87.5 % and 83 % respectively and were significantly higher in fresh embryo group compared to frozen embryo transfer group ($P$<0.05).

The occurrence of pregnancy either biochemical or clinical 46.0% v/s 42.0% and adverse event/ miscarriage (39.1 % v/s 14.3 %) in fresh v/s frozen was not significantly associated with type of embryo transfer ($P$>0.05). However significantly higher proportions i.e., 30.4 % had more than one foetuses among fresh embryo group compared to frozen embryo group (4.8%) ($P$<0.05) implicating lesser ovarian hyper-stimulation among the frozen group.

## 8. Conclusion and Recommendations

The fresh or frozen embryo transfers, both have similar outcomes in terms of occurrence of pregnancy either single or multiple. But adverse events like miscarriage or ectopics were greatly lesser in fresh embryo transfer group.

However, in PCOS patients, though there was no influence of frozen embryo transfer on occurrence of pregnancy, it exhibited lesser ovarian hyperstimulation by leading to less number of multiple gestations. Thus frozen embryo transfer among PCOS patients might be considered as a good option.

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