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Pregnancy Rate in Frozen Cycles Transfer After Freeze-All Policy to Prevent Ovarian Hyper Stimulation Syndrome

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ABSTRACT

Background: Ovarian hyperstimulation syndrome **(**OHSS) is an iatrogenic state, potentially lethal, and still one of the major complications encountered during controlled ovarian stimulation (COS) in IVF. It occurs in approximately 1–14 % of assisted reproductive technique (ART) cycles and is related to increased vascular permeability. There are two forms of (OHSS) early and late form.

Objectives: The aim of the study was to evaluate the pregnancy rate from frozen thawed embryo transfer after freeze-all policy for patient with high risk for OHSS.

Patients and methods: This is cohort study conducted at Genetics and Infertility Unit, Department of Obstetrics and Gynecology, Zagazig University and a private center (retro- prospective and record-based data). The study was carried out on 150 infertile cases. They were divided into three groups. Group A: Frozen thawed embryo transfer after freeze-all policy for cases of high risk to OHSS, group B: frozen thawed embryo transfer for cases with normal ovarian response and group C: Fresh embryo transfer in cycles with 6 or more oocytes with antagonist protocol.

Results: The main results of the study revealed that there was high statistically significant difference between groups regarding pregnancy outcomes. Pregnancy rate in frozen cycles is better than fresh cycles in normal responders with no late OHSS and less severe early OHSS. **Conclusion:** Freeze-all policy improves pregnancy rate in high and normal responders. Also, it prevents late OHSS.

Keywords: Frozen Embryo Transfer, Cryopreservation, OHSS, ART.

INTRODUCTION

In vitro fertilization (IVF) has been performed for more than 40 years, and it is estimated that more than 1 % of all births are generated from assisted reproductive therapies [(1)].

Pharmacological doses of gonadotropins create a supra-physiological hormonal environment and promote the growth of follicles, which may lead to ovarian hyperstimulation syndrome (OHSS) [(2)]**.** OHSS is an iatrogenic state, potentially lethal, and still one of the major complications encountered during controlled ovarian stimulation (COS) in IVF. It occurs in approximately 1–14 % of assisted reproductive technique (ART) cycles and is related to increased vascular permeability. There are two forms of (OHSS), early and late form [(1)]**.** Early onset OHSS, which is caused by administration of human chorionic gonadotropin (HCG) for oocyte maturation. The occurrence of late onset OHSS depends on the rise in human chorionic gonadotropin (HCG) levels following implantation of the transferred embryos [(3)]**.**

Cryopreservation has become an alternative and good option for patients performing an IVF cycle, mainly in those with an increased risk for OHSS. Considering the possible side effects of COS, recent studies have shown better IVF outcomes when performing elective frozen embryo transfer (FET), using the freeze-all strategy to prevent OHSS and even in normal responders [(1)].

In the past decade, the number of frozen-thawed embryo transfer cycles per started in vitro fertilization (IVF) cycle increased steadily, and at the same time, the percentage of frozen-thawed embryo transfers that resulted in live births increased. Currently, cryopreservation of human embryos is more important than ever for the cumulative pregnancy rate after IVF [(4)]**.**Quality of the frozen embryos and their potential for implantation are similar to those observed with fresh embryos [(1)]. Thus, it would be plausible to discuss the freeze-all policy, which is performed with the elective cryopreservation of all viable embryos in a fresh IVF or IVF/ICSI cycle and the future transfer of frozen–thawed embryos**.** In this study, we compared clinical pregnancy rate (CPR) in FET cycles after freeze-all policy to prevent OHSS and in FET cycles utilizing surplus embryos.

The aim of the study was to evaluate the pregnancy rate from frozen thawed embryo transfer after freeze-all policy for patient with high risk for OHSS.

PATIENTS AND METHODS

This was a prospective study to compare the clinical pregnancy rate and implantation rate between 3 groups.

Group A: Frozen thawed embryo transfer after freeze-all policy for cases of high risk to OHSS with antagonist protocol, group B: Frozen thawed embryo transfer for cases with normal ovarian response and

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group C: Fresh embryo transfer in cycles with 6 or more oocytes with antagonist protocol.

Sample size has been calculated by open Epi assuming that pregnancy rate in frozen thawed embryo transfer cycles' group is 57% and in control group is 34%, confidence level 95% and power 80%. Therefore, the total sample size was 150 cases and was divided into three groups (50 cases in each group).

Patient selection:

Inclusion criteria: (1) women aged 20-40 years. (2) BMI between 18 and 30 Kg/m². (3) Normal endometrial cavity confirmed by hysteroscopy done within 6 months before the trial. (4) The criteria of being at high risk for OHSS include patients with polycystic ovaries, history of OHSS in previous ART cycle, more than 25 oocyte retrieved and cases with 15-25 oocyte retrieved if they developed ultrasound-detected ascites on day 3.

Exclusion criteria: those with any of the following criteria: (1) patients with a history of recurrent pregnancy loss. (2) Implantation failure (3 previous ETs without pregnancy). (3) Severe male factor infertility (oligospermia < 0.5 million/mL, azoospermia and necrospermia. (4) Patients with less than 6 oocytes retrieved.

Basic patient characteristics: women's age at the time of embryo freezing and FET, indications for IVF, methods of endometrial preparation, endometrial thickness, number and quality of embryos transferred, duration of cryopreservation, and pregnancy outcomes were recorded.

Study protocol:

For fresh cycles, patients underwent COS with daily HMG (Fostimon® 75 IU; IBSA) starting on day 2 or 3 of menses, with doses ranging from 150 to 450 international units per day, according to the patients' stage. A GnRH antagonist (Cetrotide, Merck Serono) was used for pituitary suppression when a leading follicle achieved 14 mm. Final oocyte maturation was induced with a human chorionic gonadotropin (hCG) (Choriomon 10000 iu , IBSA) when two or more follicles reached a diameter of 18 mm.

The patients underwent transvaginal ultrasound-guided oocyte retrieval 36 hours after the trigger, followed by intra cytoplasmic sperm injection.

On the fifth day after oocyte retrieval, embryo quality was evaluated, and 1– 2 good quality blastocysts, were transferred. Luteal phase support started on the day of oocyte retrieval. All patients receive progesterone injections (100 mg prontogest MARCYRL), intravaginal progesterone 400 – 800 mg a day for 14 days. When a pregnancy test was performed, progesterone was continued until 12 weeks.

For frozen-thawed embryo transfer cycles, all embryos were cryopreserved on day 5, by vitrification using cryotop and sage vitrification media. The endometrial preparation protocol started on the 2nd day of each patient's menstrual cycle. E2 valerate was orally administered at a dose of 6 mg/day. After at least 12 days of E2 replacement, an ultrasound scan and hormone level measurements were performed. If the endometrium was > 7 mm, the frozen-thawed ET was scheduled. The progesterone replacement with intramuscular injection started 5 days before ET. Estradiol valerate and progesterone were continued until the 12th week of pregnancy. If the endometrium was < 7 mm after endometrial priming the ET was canceled.

Hormonal analysis: Blood samples were collected on the day of the ovulation trigger, for E2 and progesterone. Pregnancy was determined by hCG levels measured 11 days after ET.

Pregnancy outcome: Clinical pregnancy was defined by the observation of intrauterine embryo heart motion by 7 weeks of gestation. The implantation rate was calculated as the ratio of the number of observed embryo heart beats to the number of transferred embryos. The main outcome measure was clinical pregnancy rate. Implantation rates were the secondary outcome measures.

Ethical considerations:

An approval of the study was obtained from Zagazig University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical analysis

Data entry, processing and statistical analysis were carried out using MedCalc version, 18.2.1 (MedCalc, Ostend, Belgium) (Statistical Package for the Social Sciences (SPSS version 20.0). Tests of significance, Kruskal-Wallis, Wilcoxon's and Chi square, were used. Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. P-values ≤ 0.05 (5%) was considered to be statistically significant, while P-value < 0.01 was considered highly significant.

RESULTS

There was no significant difference between groups regarding age. The mean ages of groups A, B and C were 27.6 ± 8.9, 25.9 ± 7.3 and 27.1 ± 8.1 years, respectively. There was no significant difference between groups regarding BMI and groups parity (Table 1).

Table (1): Demographic and baseline clinical characteristics of the study groups

	Demographic data	Group A (n=50)	Group B (n=50)	Group c (n=50)	P value
Age (years)	**Mean ± SD**	27.6 ± 8.9	25.9 ± 7.3	27.1 ± 8.1	**0.636**
	Range	25 – 30	23 – 28	26 – 30	
BMI (kg/m^2)	**Mean ± SD**	22.04 ± 2.4	22.1 ± 2.1	21.4 ± 2.1	**0.186**
	Range	19.9-30	20-28	19.8-29	
Parity	**Mean ± SD**	**0.8 ± .3**	**1.3 ± .53**	**0.7 ± .2**	**0.152**
	Range	**0-1**	**0-2**	**0-1**	

F- Tests were used.

There was no significant difference between groups regarding Baseline FSH and LH (Table 2).

Table (2): Baseline FSH and LH of the study groups

	Demographic data	Group A (n=50)	Group B (n=50)	Group c (n=50)	P value
Baseline FSH (mIU/ml)	**Mean ± SD**	6.8 ± 2.3	6.5 ± 2.7	6.7 ± 1.06	**0.235**
Baseline LH (mIU/ml)	**Mean ± SD**	8.01 ± 3.2	5.05 ± 2.1	5.08 ± 2.01	**0.741**

F- tests were used.

There was no significant difference between groups regarding indications of ART (Table 3).

Table (3): Indications among studied patients

Indications	Group A (n=50)	Group B (n=50)	Group C (n=50)	P value
Male	**25(50%)**	**28(56%)**	**25(50%)**	
Ovarian	**8(1 6%)**	**4(8%)**	**2(4%)**	**0.752**
Tubal	**3(6%)**	**3(6%)**	**4(8%)**	
Unexplained	**10(20%)**	**10(20%)**	**13(26%)**	
Combined factors	**4(8%)**	**5(10%)**	**6(12%)**	

F tests were used.

There were no differences between the groups with respect to duration of COS (days). However, the study group had significantly higher serum E2 and total dose of rFSH. Metaphase 2 oocyte and P levels on the day of oocyte triggering suggests a higher probability of pregnancy table (4).

Table (4): Characteristics of treatment protocols among studied patients in fresh cycle.

Stimulation protocol	Group A (n=50)	Group B (n=50)	Group C (n=50)	P value
Total dose of FSH (IU) Mean ± SD	1590± 71	3120± 180	2900± 100	0. 02
Duration of COS (days) Mean ± SD	10.1± 1.8	10.4± 1.9	11.2± 1.3	0.931
E2 on trigger day (pg/ml) Mean ± SD	5302.5± 412	2326.1± 20	1900.5 ± 73	0.001
P on trigger day (ng/ml) Mean ± SD	1.6± 0.31	1.51± 0.24	1.3± .09	0.04
• **Mean No of retrieved oocytes Mean ± SD**	50.3± 21.3	25.1± 13.4	24.7± 11.2	0.001
Mean No. of fertilized embry Mean ± SD	18.2± 6.3	16.1± 7.2	15.1± 4.1	0.001
Metaphase2 oocyte Mean ± SD	35.4± 17.2	19.7± 9.26.3	18.5± 9.1	0.001

Student-t tests were used.Bold are statistically significant at $p < 0.05$.

There were no differences between the groups with respect to mean no. of transferred embryos and mean no. of good embryos to be transferred more in freezing embryo transfer group (Table 5).

Table (5): Antagonist protocol among studied patients

Stimulation protocol	Group A (n=50)	Group B (n=50)	Group C (n=50)	P value
Mean no. of transferred embryo Mean ± SD	1.95 ± .64	1.8 ± .75	1.9 ± .15	**0.08**
Mean no of good embros to be transferred Mean ± SD	**1.5 ± 1.41**	**1.77 ± .96**	**1.27 ± .68**	**0.417**

Student-t tests were used.Bold are statistically significant at $p < 0.05$.

There was highly significant difference between groups regarding pregnancy outcomes. Clinical pregnancy rate and implantation rate are more in freezing embryo transfer group (Table 6).

Table (6): Pregnancy outcomes among studied patients

Stimulation protocol	Group A (n=50)	Group B (n=50)	Group C (n=50)	P value
• **Positive pregnancy test**	**31 (62%)**	**27 (54%)**	**21 (42%)**	**0.001**
• **Biochemical pregnancy (n%)**	**2 (4%)**	**2 (4%)**	**3 (6%)**	**0.02**
• **Clinical pregnancy (n%)**	**29 (58%)**	**25 (50%)**	**18 (36%)**	**0.03**
• **Number of sacs(mean ± SD)**	**1 .2 ± .53**	**.41 ± 1.3**	**1 .1 ± .57**	**0.536**
• **Implantation rate (n%)**	**43 (43%)**	**37 (37%)**	**34 (34%)**	**0.001**

Student-t tests were used.Bold values are statistically significant at $p < 0.05$.

There was significant difference between fresh and frozen emberyo transfer in group B (Table 7)

Table (7): Comparison between pregnancy rate in fresh and frozen embryo transfer in group B

	FRESH ET (n=50)	FER (n=50)	P value
Positive	17 (34%)	27(54%)	**0.001**
Negative	**33 (66%)**	**23 (46%)**	

Bold values are statistically significant at $p < 0.05$.

As regarding to OHSS in high responders group in our study, there was 20 cases with ascites detected by ultrasound only, 1 case with moderate OHSS, one case with severe OHSS and there was no critical cases. There was no detected late OHSS cases in our study.

DISCUSSION

Ovarian hyperstimulation syndrome (OHSS) is a serious and potentially fatal complication affecting women during ovulation induction [(5)].

Controlled ovarian stimulation with its supraphysiologic hormonal levels, may decrease ER and implantation rate. Embryo cryopreservation is a routine procedure in most IVF centers. Growing evidence in the literature shows that frozen-thawed ET is associated with good outcomes because endometrial development can be controlled more precisely during its priming for frozen-thawed embryo transfer [(6)]. Since the pathophysiological changes in the development of OHSS is mediated by hCG, the condition can, to a high extent, be prevented by using a short GnRH antagonist protocol for stimulation and GnRH agonist to trigger final oocyte maturation with freeze-all policy [(7)].

Therefore, the freeze-all policy can be implemented as an alternative, to avoid the deleterious effects of COS on embryo endometrium synchrony and decrease risk for OHSS [(6)].

This study was conducted to evaluate the pregnancy rate in frozen cycles transfer for cases with high risk for OHSS after freeze-all policy and to evaluate pregnancy rate in frozen cycles in normal responders. The study was a cohort study that included 150 cases selected according to the inclusion criteria. Patients had been divided into 3 groups. Group A: Frozen- thawed embryo transfer after freeze-all policy for cases with high risk of OHSS. Group B: Frozen-thawed embryo transfer for cases with normal ovarian response. Group C: Fresh embryo transfer in cycles with 6 or more oocytes retrieved.

In our study, there was no significant difference between groups regarding age. The mean age of groups A, B and C were 34.8 ± 5.7, 34.4 ± 5.3 and 34.2 ± 5.1 years respectively. There was no significant difference between groups regarding BMI, parity, baseline FSH level, LH level or the duration of COS. However, group (A), which was selected according to inclusion criteria and had a high risk for OHSS, had statistically significant lower total dose of FSH, higher serum E2 on the day of oocyte triggering and higher oocytes retrieved and fertilized embryos. Regarding the higher progesterone level in group A, it was found that the level of progesterone is correlated to the number of oocytes retrieved. **Venetis *et al.*** [(8)] study

also showed that the serum estradiol level on triggering day and the number of oocytes were related to the level of serum progesterone elevation.

In the current study, there was a significant difference between groups regarding pregnancy outcomes. Best outcome was observed in group A, while outcome in frozen embryo cycles (group A and B) was better than outcome in fresh embryo cycles (group C). This is in agreement with **Roque *et al.*** [(1)] study, which included 530 normal responder cases. They were categorized into 351 in the fresh ET group and 179 in the freeze all group. The implantation rate was 19.9% and 26.5% while the clinical pregnancy rate was 35.9% and 46.4% in the fresh ET and the freeze-all group respectively. The outcomes were significantly improved in the freeze-all policy group. The authors speculated that endometrial receptivity might have been impaired by controlled ovarian stimulation and that the freeze-all method could improve the outcomes. However, in our study embryo transfer was done in day 5. In **Roque *et al.*** [(1)] study, embryo transfer was done in day 3. In addition, approximate results were found by **Ming *et al.*** [(9)] in their retrospective study, which compared fresh embryo transfer cycles with frozen –thawed transfer cycles. A significantly higher clinical pregnancy (29.69% and 19/64 versus 10.81% and 8/74) and implantation (13.33% and 22/165 versus 5.13% and 8/156) rates can be achieved using the freeze-all strategy with frozen thawed embryo transfer. Also **Shapiro *et al.*** [(10)] designed a randomized controlled clinical trial on 53 patients with fresh embryo transfer (fresh group) and 50 patients with frozen embryo transfer. They found that the clinical pregnancy rate per transfer was 84.0% and 54.7% and the implantation rates were 70.8% and 38.9% in the frozen embryo transfer group and the fresh embryo transfer group respectively. In contrast with our study, **Kalem *et al.*** [(11)] study aimed to investigate and compare the pregnancy rate in IVF cycles of frozen thawed embryo transfers and fresh embryo transfers in a group of 254 women with a high risk of ovarian hyperstimulation syndrome. Results suggested that fresh and frozen-thawed embryo transfers have similar IVF results in patients with a high risk of OHSS. In their study, they considered those patients with a total follicle count of 15 or more and/or an estradiol (E2) value of over 3000 pg/mL on the day of ovulation induction as high responders and with a high risk of OHSS. In our study, we considered that women with more than 20 oocyte as high responders.

In the Middle East, we have higher percent of PCOS, which yields higher number of oocytes [(12)]. Regarding OHSS in high responders group in our study, there was 20 cases with ascites detected by ultrasound only, 3 cases with moderate OHSS (6%), one case with severe OHSS (2%) and there was no critical cases. This in agreement with the incidence of moderate OHSS that was reported to range between 3.1% - 8% in IVF cycles with probalbility to rise to 20% in high risk population [(13, 14)]. There was no detected late OHSS cases in our study. Freeze all policy, with withholding pregnancy, makes management of early OHSS more flexible and effective.

CONCLUSION

Freeze-all policy improves clinical pregnancy rate in high responders and normal responders and prevent late OHSS.

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