

Reproductive Outcome Following Thawed Embryo Transfer in Management of Ovarian Hyperstimulation Syndrome

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Abstract

Background: The purpose of the study was to compare clinical pregnancy and delivery rates with fresh and frozen embryo transfer in patients admitted to Shiraz-Human Assisted Reproductive Center with ovarian hyperstimulation syndrome (OHSS).

Methods: OHSS patients randomly divided in two groups, group A (n=50) with fresh embryo transfer and group B (n=50) with frozen embryo transfer. We used vitrification method for freezing the embryos. Patient age, combination of female and male factors, total number of retrieved oocytes, number of cryopreserved embryo, number of transferred embryos, clinical pregnancy and delivery rates were recorded for all patients. All statistical calculations were done using SPSS software. Generalized linear model was used to adjust the confounding factors to compare the clinical pregnancy and delivery rates between two groups. The $p < 0.05$ was considered statistically significant.

Results: Mean (\pm SD) ages of these patients were 26.78 ± 3.5 and 28.42 ± 4.2 yrs in fresh (A) and frozen (B) embryo transfer groups respectively. Combinations of male and female factors were 28.3% and 32.1% respectively. Average numbers of oocytes retrieved in two groups were 22.14 ± 4.3 and 21.02 ± 4.9 , and after fertilization, embryos cryopreserved per patient yielded averages of 13.82 ± 3.5 and 12.5 ± 4.3 . Thaw and ET were performed and the means for transferred embryos were 3.22 ± 0.6 and 4.1 ± 0.7 . We didn't find any significant differences in implicit parameters between the two groups. The pregnancy and delivery rates in OHSS patients were significantly higher in frozen embryo transfer, 63.1% and 45.6%, compared with fresh embryo transfer, 55.1% and 35.4%, respectively.

Conclusion: The pregnancy and delivery rates in OHSS cases, both fresh and subsequently with frozen embryo transfer, were exceptionally high. There was statistically significant difference of pregnancy and delivery rates between fresh and frozen embryo transfer. As a result, an elective embryo freezing policy to moderate the severity and duration of OHSS has compromising outcomes for women at risk of OHSS.

Keywords: Cryopreservation, Embryo transfer, Ovarian hyperstimulation syndrome.

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Introduction

Ovarian hyperstimulation syndrome is an iatrogenic condition resulting from an excessive ovarian response to superovulation medi-

cation (1), potentially resulting in death in its extreme manifestation (2).

It was shown that administration of human cho

gonadotrophin (hCG) either exogenously, to induce the final maturation of the follicle prior to oocyte retrieval, or endogenously, from an establishing pregnancy can lead to this situation (3). In order to manage this mortality condition in ART, many preventive strategies have been evaluated but none of the described methods has consistently demonstrated efficacy in preventing the syndrome except for the cancellation of the treatment cycle before hCG administration (4). Since the duration of severe OHSS is greatest in those patients who conceive, elective cryopreservation of all embryos has been proposed (5).

The use of different cryopreservation techniques and protocols revealed that cryopreservation of all produced embryos in OHSS cases has been adopted with variable and contradictory results (6, 7). Therefore, more research is needed to determine whether using elective cryopreservation of embryos can reduce the rate of severe OHSS in *in vitro* insemination (IVF)/intracytoplasmic sperm injection (ICSI) cycles. In this study, we presented the efficacy of elective cryopreservation of all embryos in patients at risk of OHSS in comparison to the fresh embryo transfer cycles in patients with similar potential risk of developing the syndrome.

Methods

All couples entering our center were thoroughly checked during the stimulation induction period in order to select those who were at high risk for developing OHSS.

Each patient was identified as being at high risk of OHSS based on serum oestradiol ≥ 3000 pg/ml on the day of hCG administration and ≥ 15 follicles of intermediate (12–15 mm) and large (≥ 16 mm) size.

The ovarian stimulation protocol was similar for all patients. We used a down-regulation protocol with GnRH analogue and urinary gonadotrophin. All patients were given 10,000 IU of hCG (Gonasi, IBSA, Switzerland) 34–36 hr before oocyte retrieval. The oocytes were inseminated with the husband's spermatozoa and then incubated in the standard condition. After 14–18 hr from insemination, the fertilization was assessed by the presence of two pronuclei. In group A, patients' zygotes were immediately cryopreserved, while the zygotes of group B couples were kept in culture medium for a subsequent 72 hr. In group A, according to vitrification method, embryos were equilibrated in solution composed of 750 μ l

DMSO (Sigma, Germany), 750 μ l Ethylene Glycol (Sigma, Germany), Hams F10 (Gibco, Paisely, UK) or HTF+10% serum albumin (Gibco, Paisely, UK) up to 10 ml for 8 or 10 min at room temperature. Embryos were then exposed to the vitrification solution composed of 1.5 ml DMSO, 1.5 ml Ethylene Glycol, 0.5 M sucrose (Sigma, Germany), Hams F10 or HTF +10% serum albumin up to 10 ml for 1 min at room temperature, and then immediately loaded into cryotop and plunged in liquid nitrogen. Warming was done in two steps. First, the embryos were released from the straw into solution 1:1 M sucrose, Hams F10 or HTF+10% serum albumin up to 10 ml for 1 min, and then the embryos were transferred into a solution 2: 0.5 M sucrose, Hams F10 or HTF+10% serum albumin up to 10 ml for 3 min at room temperature. In the next step, embryos were washed into Hams F10 or HTF+10% serum albumin 5–10 times. Finally, embryos were deposited in a culture dish medium (Quinn's Advantage Cleavage Medium; Sage IVF) and observed under the inverted microscope to confirm survival and to assess their morphology. Embryos that presented one or more viable cells were incubated at 37°C, 5% O₂ and 7.3% CO₂. After 24 hr in culture and previous to vaginal embryo transfer (VET), embryos were observed and classified in three developmental stages: embryos with no cell division (the number of cells was the same as 24 hr before), cleavage cell stage embryos (embryos with more cells after 24 hr in culture) and embryos that had developed into morula or early blastocyst stage. Embryo Glue media (Vitrolife, Sweden) was used for embryo transfer. Transfer was guided with an abdominal scan using a Wallace catheter (Smith Medical International Ltd.UK).

Endometrial Preparation: Endometrium was prepared by hormone replacement treatment. Ovarian down regulation was achieved using a GnRH agonist (Ginecrin depot, Abbott Laboratories, Spain) in the luteal phase. With menstruation, patients' treatment began with 2 oestradiol patches/2 days (Estradot® 75 mg; Novartis, Spain). After 8 to 10 days, a transvaginal scan and a serum oestradiol measurement were performed. Endometrium was assumed to be prepared when its thickness was over 7 mm and serum oestradiol was above 100 pg/ml. From that moment, 200 mg/8 hr of progesterone could be used (Utrogestan®, Seid, or Progeffik®, Effik, Spain) and embryo transfer program was initiated. Both oestradiol patches and progesterone were used until the pregnancy

test was carried out 15 days after commencement of progesterone use. If the pregnancy test was positive, hormonal treatment could be continued until 12th week of gestation.

Clinical pregnancy was defined by the ultrasound observation of an intrauterine sac four weeks after embryo transfer.

Statistical Analysis: All statistical analysis were done using SPSS software. Generalized linear model was used to adjust the confounding factors to compare the clinical pregnancy rates between the two groups. The $p < 0.05$ was considered statistically significant.

Results

There was no significant difference in demographic data between the two groups (Table 1). Mean (\pm SD) ages of these patients were 26.78 ± 3.5 and 28.42 ± 4.2 yrs and combinations of male and female factors were 28.3% and 32.1% in fresh (A) and frozen (B) embryo transfer groups respectively.

Average numbers of oocytes retrieved in these groups were 22.14 ± 4.3 and 21.02 ± 4.9 , and after fertilization, embryos cryopreserved per patient yielded averages of 13.82 ± 3.5 and 12.5 ± 4.3 in fresh (A) and frozen (B) embryo transfer groups respectively. Thaw and ET were performed and the means for transferred embryos were 3.22 ± 0.6 and 4.1 ± 0 . We didn't find any statistical differences in implicit parameters between the two groups.

Generalized linear model confirmed that clinical pregnancy and delivery rates in OHSS patients were significantly higher in frozen embryo transfer, 63.1% and 45.6%, compared with fresh embryo transfer, 55.1% and 35.4%, respectively (Table 1).

Discussion

In spite of the above-mentioned facts, the inci-

dence of severe forms of ovarian hyper-stimulation syndrome is only 1% to 2% (8, 9); OHSS remains the major significant source of morbidity and mortality in patients undergoing assisted reproduction technologies. The incidence and the duration of the syndrome are strictly related to the surge of pregnancy hormones and the number of implanted embryos (10, 11). Because no method of treatment has been able to eliminate severe OHSS from the practice of assisted reproduction technologies, prevention of such risk or cycle cancellation remain the best strategies. Cryopreservation of all derived embryos has been successfully adopted to reduce the onset of the severe form of OHSS (8). Avoiding fresh embryo transfer in patients at high risk of developing OHSS prevents the late onset of the syndrome by simply eliminating the rise of hCG associated with successful embryo implantation. In this manner, some papers highlighted the reduction in the pregnancy chances because frozen-thawed embryo replacement may be associated with a lower pregnancy rate (12). Skaiker confirmed the results of intravenous albumin and transfer of fresh embryos with cryopreservation of all embryos for subsequent transfer in prevention of ovarian hyperstimulation syndrome (13). They did not observe significant differences between examined groups. Queen et al. showed that transfer of cryopreserved-thawed zygotes in 15 patients yields excellent pregnancy rates with reduction of OHSS symptoms (7). For comparison between transfer of cryopreserved and fresh embryo, a prospective randomized study was designed by Ferraretti and et al. (14). Their results suggest that elective cryopreservation of zygotes prevents the risk of OHSS and does not affect live birth and pregnancy rates. The last study in which elective cryopreservation of all embryos was compared with fresh ET was done by Fitzmaurice et al. (15). Although several studies have indicated that cryopreservation of all em-

Table 1. Demographic data of group A and group B patients

	Group A	Group B	P-value
Patient number	50	50	--
Patient age	26.78 ± 3.5	28.42 ± 4.2	0.3
Combination of female & male factors	28.6%	32.1%	0.5
Number of oocytes retrieved	22.14 ± 4.3	21.02 ± 4.9	0.4
Number of embryos cryopreserved	13.82 ± 3.5	12.5 ± 4.3	0.31
Number of embryo transferred	3.22 ± 0.6	4.1 ± 0.7	0.07
Clinical pregnancy rates	45.6%	63.1% *	0.02
Delivery rates	35.4%	55.1% *	0.03

* Significant difference with group A

bryos reduced the risk of OHSS while achieving acceptable pregnancy rates (9, 16, 17), Cochrane review concluded that there was insufficient prospective evidence to conclude that cryopreservation prevented OHSS, compared with albumin administration or no cryopreservation (18). One reason why the paper published on this topic reached different conclusions could be the fact that embryo cryopreservation practice varies widely among assisted conception units. The variation includes policies regarding identification of the specific stage and the protocol used to freeze embryos, identification of the stage for thawing and replacing them, and consequently significant differences in success rates. Our results showed that cryopreservation of embryos reduces the patient's risk of developing the severe form of OHSS, and conserves the pregnancy potential in the form of stored embryos. To explain the different outcomes between the fresh and thawed embryo transfers, multiple factors can be considered. First, the improved techniques of vitrification result in better survival and developmental potential after thawing (19). Also, as we know, natural endometrial preparation is manipulated hormonally; several studies have revealed that ovarian stimulation severely decreased endometrial receptivity for embryo (20, 21). In Addition, embryo transfer during implantation window is a critical factor in the success of pregnancy but it is usually missed in most fresh cycles, although it is achievable through post-thaw embryo cycles (22). Checa also explained that the multiple eggs generated by ovarian stimulation would increase the release of estradiol from the ovary, which affect the receptivity of endometrial tissue. Some recent studies have shown that ovarian stimulation causes changes to the endometrial DNA pattern, which are not evident in the normal receptive endometrium (23). Therefore, the developmental potential of cryo-thawed embryos is a concern due to synchronization of endometrial receptivity. It was shown that better synchronization was achieved between the embryo and endometrium in frozen-thawed natural cycles than in stimulated cycles (20, 22).

Conclusion

In conclusion, for the patients who are at risk of OHSS, the embryos would be vitrified for frozen embryo transfer in order to reduce complication of fresh embryo transfer in OHSS cycle and improve the pregnancy rate.

Conflict of interest

Authors declare no conflict of interest

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