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Original Article

Strategies for the management of OHSS: Results from freezing-all cycles

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ABSTRACT

Objective: To compare the use of GnRH agonist (GnRHa) or hCG trigger in potential OHSS patients undergoing freeze-all programs. We also compared the clinical outcomes when fresh versus freeze-thawed embryo transfers were performed in cycles with a high number of retrieved oocytes.

Methods: The study included potential OHSS patients who received GnRHa (n=74) or hCG (n=49) trigger. The protocols were compared with respect to the clinical outcomes. We also compared the clinical outcomes of cycles in which hCG trigger was used and more than 20 MII oocytes were retrieved when: fresh embryo transfer protocol (n=153) or freeze-all protocol (n=123) were performed.

Results: A decreased serum estradiol level, a decreased number of retrieved oocytes, an increased MII retrieved rate, and decreased fertilization rate was observed in the hCG when compared with the GnRHa group. No significant differences were noted concerning clinical outcomes. When fresh cycles were compared with frozen-thawed cycles, the estradiol serum level and the number of cryopreserved embryos were higher in the frozen-thawed cycles. The clinical pregnancy rate was higher among freeze-all cycles, as well as the implantation and cumulative pregnancy rates, when compared with fresh embryo transfer cycles.

Conclusion: The use of GnRHa trigger may be a good alternative to prevent the OHSS in patients presenting an extreme ovarian response to COS, leading to similar clinical outcomes, when compared with the traditional hCG trigger. Moreover, our findings demonstrated that the strategy of freezing-all embryos not only decreases the risk of OHSS but also leads to a better pregnancy rate.

Keywords: OHSS, Freezing, Thawing, hCG, GnRHa, Trigger

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) is the dreadful complication of the controlled ovarian stimulation (COS), in which pharmacological doses of gonadotropins, create a supra-physiological hormonal environment, and promotes the growth of follicles, that under natural conditions would become atretic and regress (Setti *et al*., 2011).

The syndrome is characterized by cystic enlargement of the ovaries and a fluid shift from the intravascular to the third space due to increased capillary permeability and ovarian neoangiogenesis (Kumar *et al*., 2011). The incidence of moderate OHSS is estimated to be between 3% and 6%, and it has been recognized in two forms: the early form of OHSS, (within days after the ovulation triggering injection of hCG) although caused by hCG, it is related to an exaggerated ovarian response to COS, whereas the late form (10 days after hCG) (Abramov *et al*., 1999), is mainly related to the secretion of placental hCG. Those cases which constitute an early form followed by pregnancy are serious and long lasting (Golan *et al*., 1989).

Ovarian hyperstimulation syndrome results from an increase in vascular permeability. The hCG used to trigger oocyte maturation appears to play an integral part in the etiology of the condition and indeed, subsequent trophoblast-derived hCG dramatically worsens and prolongs the symptoms of severe OHSS (Whelan and Vlahos 2000; Aboulghar, 2009). Therefore, an important OHSS risk-reducing strategy has been to cancel the embryo transfer and freeze-all the embryos (Evans *et al*., 2014).

The cryopreservation of all embryos can prevent pregnancy-induced late OHSS; however, it cannot prevent early OHSS if hCG is used to trigger oocyte maturation (Endo *et al.*, 2002)

The use of gonadotropin-releasing hormone agonist (GnRHa) as a trigger for final oocyte maturation in antagonist in vitro fertilization (IVF) cycles has been proposed as a method for preventing ovarian OHSS (Cerrillo *et al.,* 2011; Humaidan *et al*., 2011). From a clinical point of view, the most significant benefit of GnRHa trigger is its ability to induce a quick and reversible luteolysis and thus reduce the risk of OHSS development. Recently, it was demonstrated that gonadotropin and steroid levels differ significantly during the luteal phase between patients triggered for final oocyte maturation with a GnRHa or with hCG (Fatemi *et al*., 2013).

To date, there is no consensus in the literature on the use of GnRH to prevent the development of OHSS. Moreover, not much is known about clinical outcomes when GnRH agonist or hCG are used to trigger ovulation in freeze-all IVF cycles. Therefore, the goal for the present study was to compare the use of either GnRHa or hCG to trigger the final follicular maturation in freeze-all programs. We also aimed to compare the clinical outcomes when fresh versus freeze-thawed embryo transfers were performed in cycles with a high number of retrieved oocytes.

MATERIALS AND METHODS

Experimental Design

This is case-control study analyzing potential OHSS patients (>20 retrieved MII) undergoing freeze-all cycles. The cycles were split into those receiving the GnRHa (n=74) or the hCG (n=49) trigger. The protocols were compared with respect to the clinical outcomes. We also compared the clinical outcomes of cycles in which hCG trigger was performed when the fresh embryo transfer protocol (n=153) or (ii) the freeze-all protocol (n=123) was performed.

The clinical parameters evaluated were: (i) pregnancy rate, (ii) single pregnancy rate, (iii) multiple pregnancy rate, (iv) implantation rate, (v) miscarriage rate and (vi) cumulative pregnancy rate.

The pregnancy test was performed 12 days after embryo transfer. All women with a positive test were submitted to a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed when the

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fetal heartbeat was detected. Pregnancy rates were calculated per transfer. Miscarriage was defined as pregnancy loss before 20 weeks.

A written informed consent, in which patients agreed to share the outcomes of their cycles for research purposes was obtained, and the study was approved by the local institutional review board.

Controlled Ovarian Stimulation and Oocyte Retrieval

Controlled ovarian stimulation was achieved by using recombinant FSH (Gonal-F; Serono, Geneva, Switzerland), at a daily dose, starting on day three of the cycle. Pituitary blockage was performed using a GnRH antagonist (Cetrotide, Serono, Geneva, Switzerland), starting when at least one follicle ≥14 mm was visualized.

Follicular growth was monitored using transvaginal ultrasound examination starting on day four of gonadotropin administration. Recombinant hCG (Ovidrel, Serono, Geneva, Switzerland) or GnRHa (Leuprolide acetate, Lupron; TAP Pharmaceuticals, Lake Forest, USA) was administered to trigger the final follicular maturation. The oocytes were collected 35 hours after follicular maturation trigger through transvaginal ultrasound ovum pick-up.

Preparation of oocytes and Morphology assessment

Retrieved oocytes were maintained in culture medium (Global® for Fertilization, LifeGlobal, Connecticut, USA) supplemented with 10% Human Synthetic Albumin (HSA, Irvine Scientific, Santa Ana, USA), covered with mineral oil (Ovoil™ - Vitrolife, Kungsbacka, Sweden) at 37°C and 6% CO2 for 5 hours. Surrounding cumulus cells were removed with exposure to a HEPES buffered-medium containing hyaluronidase (80 IU/mL, Irvine Scientific, Santa Ana, USA). The remaining cumulus cells were then mechanically removed by gentle pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, Virginia, USA). The oocytes were checked for oocyte maturation and those which have released the first polar body (metaphase II oocytes – MII) were considered mature and used for ICSI.

Intracytoplasmic Sperm Injection

Intracytoplasmic Sperm Injection was performed on all MII oocytes using the technique described by Palermo et al. (1992). The oocytes were individually placed in 4-µL droplets of buffered medium (Global® w/HEPES, LifeGlobal, Connecticut, USA), and sperm was placed in a central 4-µL droplet of polyvinylpyrrolidone solution (PVP, Irvine Scientific, Santa Ana, USA) in a 50 X 40-mm glass culture dish (WillCo-dish®, New Jersey, USA) covered with warm mineral oil (Ovoil™, Vitrolife, Kungsbacka, Sweden), on a heated stage (37.0 ± 0.5°C) of an inverted microscope.

Assessment of Fertilization and Embryo Quality

After the ICSI procedure, the presumptive embryos were individually maintained in a 50-µL drop of culture medium (Global®, LifeGlobal, Connecticut, USA) supplemented with 10% human serum albumin (HAS) and covered with mineral oil in a humidified atmosphere with 6% CO_2 at 37°C until transfer, which occurred on the fifty day of development.

Approximately 18h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Subsequently, embryos were transferred to new drops of culture medium to be individually cultured for 48 hours. The quality of the embryos was evaluated under an inverted microscope.

Embryo freezing or embryo transfer

For freeze-all cycles, on day three, embryos were vitrified and cryo-stored. Both vitrification and the warming procedure were performed using the Cryotop method, described elsewhere (Quaas *et al.*, 2013). On day five, for both fresh and freeze-all protocols, one or two embryos were transferred.

Statistical analyses

The cycle characteristics, clinical outcomes and laboratory outcomes were compared between the groups using Chi squared and student t-test for categorical and continuous variables, respectively. Continuous variables are expressed as the mean ± the standard deviation, and percentages were used for categorical variables

All results considered 5% to be significant at the critical level ($P < 0.05$). Data analysis was carried out using the Minitab (version 14) Statistical Program.

RESULTS

When demographic variables, stimulation characteristics and response to COS were compared between cycles in which the trigger was performed by hCG or GnRHa, a decreased serum estradiol level, a decreased number of retrieved oocytes, an increased MII retrieved rate, and decreased fertilization rate were observed in the hCG group. No significant difference was noted for the other evaluated parameters (Table 1).

No significant difference was observed in the clinical outcomes when the trigger was per-

Table 1: Characteristics from freeze-all cycles when the trigger was performed by using hCG or GnRH agonist

Cycles' Characteristics	hCG	GnRH agonist	*P*
Number of cycles	49	74	
Number of patients	45	69	
Patient's age	31.8 ± 3.8	31.9 ± 3.6	0.887
Estradiol level (E2) on trigger day	4039 ± 2112	5328 ± 3036	0.006
Total dose of FSH for COS	2259 ± 714	2067 ± 481	0.101
Aspirated follicles	34.5 ± 11,7	41.3 ± 17.9	0.015
Retrieved oocytes	25.3 ± 9,6	30.8 ± 11.3	<0.001
Retrieved oocytes rate	73.4%	74.5%	0.59
MII number	19.6 ± 7.8	22.0 ± 8.1	0.123
MII rate	77.2%	71.3%	<0.001
Normal fertilization rate	79.3%	84.0%	0.011
Number of cryopreserved embryos	9.2 ± 4.5	9.9 ± 4.9	0.422

formed by either hCG or GnRHa (Table 2).

When fresh cycles were compared with frozen-thawed cycles, the estradiol serum level on the trigger day and the number of cryopreserved embryos were higher in the frozen-thawed cycles. The other demographic variables, stimulation characteristics and response to COS variables did not differ between the groups (Table 3).

The clinical pregnancy rate was higher among freeze-all cycles, as well as the implantation and cumulative pregnancy rate, when compared with fresh embryo transfer cycles (Table 4).

Table 2: Clinical outcomes from freeze-all cycles when the trigger was performed by using hCG or GnRH agonist

Cycle outcomes	hCG	GnRH agonist	*P*
Number of cycles	49	74	
Clinical pregnancy rate	44.8%	50.0%	0.483
Single pregnancy rate	72.7%	75.6%	0.856
Twin pregnancy rate	22.7%	24.3%	0.585
Triplet pregnancy rate	4.5%	0	0.935
Miscarriage rate	29.7%	14.6%	0.164
Implantation rate	39.0%	37.1%	0.885
Cumulative pregnancy rate	53.0%	59.5%	0.483

DISCUSSION

GnRHa can be used as an alternative trigger to hCG in cycles that have been suppressed with a GnRH antagonist. Prior studies of GnRHa triggering have reported complete prevention of severe early OHSS and good pregnancy rates following fresh embryo transfer even in high-risk patients (Humaidan 2009; Humaidan *et al*., 2009; Radesic & Tremellen, 2011). Nevertheless, in a very elegant previous study (Iliodromiti *et al*., 2013) it was reported that in women undergoing ovarian stimulation and who develop an excessive ovarian response, the use of a GnRHa trigger combined can provide the opportunity to proceed to fresh embryo transfer with adequate clinical pregnancy rates. However, this procedure will not completely eliminate the risk of OHSS; therefore, in cases of extreme ovarian response, the GnRHa trigger followed by a freeze-all policy to completely avoid OHSS is recommended.

For the present study, the used of GnRHa trigger was compared with the hCG trigger in freeze-all protocols, and no differences were found for any evaluated parameter, with exception of fertilization and number of MII - which was increased and the MII retrieved rate, which was decreased in the GnRHa group.

The lower MII retrieved rate observed when GnRHa was used may be explained by the fact that differences exist between the GnRHa-induced surge and that of the natural cycle or the traditional hCG trigger. The LH surge of the natural cycle is characterized by three phases, with a total duration of 48 h (Hoff *et al.*, 1983), whereas the GnRHa-induced surge of gonadotropins consists of two

Table 3: Characteristics from OHSS cycles when fresh embryos were transferred or all of the embryos were cryopreserved

Cycles' Characteristics	Freeze-all	Fresh embryo transfer	*P*
Number of cycles	123	153	
Number of patients	114	141	
Patient´s age	32.6 ± 2,8	33.4 ± 2.9	0.431
Estradiol level (E2) on trigger day	4543± 2232	3326 ± 1657	0.003
Total dose of FSH for COS	2147 ± 606	2298 ± 756	0.456
Aspirated follicles	38.3 ± 14,7	37.9 ± 6.9	0.645
Retrieved oocytes	28.2 ± 10,9	27.4 ± 11.2	0.352
Retrieved oocytes rate	73.5%	73.1%	0.652
MII number	20.8 ± 8,0	20.4 ± 6.7	0.546
MII rate	73.8%	74.5%	0.336
Normal fertilization rate	80.7%	77.7%	0.451
Number of cryopreserved embryos	9.3 ± 4.6	5.2 ± 3.1	<0.0001

Table 4: Clinical outcomes from OHSS when fresh embryos were transferred or all of the embryos were cryopreserved

Cycles' outcomes	Freeze-all	Fresh embryo transfer	*P*
Number of cycles	123	153	
Clinical pregnancy rate	47.9%	41.1%	0.004
Single pregnancy rate	74.5%	68.2%	0.589
Twin pregnancy rate	23.7%	31.7%	0.384
Triplet pregnancy rate	1.7%	0	0.754
Miscarriage rate	15.8%	15.6%	0.789
Implantation rate	38.6%	24.8%	0.028
Cumulative pregnancy rate	62.3%	-	
Estimated cumulative pregnancy rate	68.4%	55.7%	0.049

phases only, with a duration of 24–36 h (Itskovitz *et al*., 1991). This leads to a significantly reduced total amount of gonadotropins being released from the pituitary when GnRHa is used. Conversely, the traditional hCG trigger continues to stimulate ovarian steroid hormone production for up to 5 days. For the GnRHa trigger, these differences may have an effect on oocyte maturation. The higher fertilization rate observed in this group may also be due to the "more physiological" environment for oocyte development, that is created when the GnRHa triggers follicular maturation.

When fresh and freeze-all protocols, following hCG trigger were compared, a significantly increased pregnancy rate was seen. In agreement with these findings, there is growing evidence in the literature suggesting that the supraphysiologic hormonal environment created by COS may decrease endometrial receptivity and embryo implantation (Devroey *et al.*, 2004; Shapiro *et al.*, 2014). In fact, it has been suggested that the freeze-all policy has emerged as an alternative to fresh embryo transfer to improve cycle outcomes (Roque *et al*., 2013; Roque *et al*., 2015).

In our study, the whole cohort of embryos were cryopreserved, and the embryo transfer was performed later in a most receptive and well-prepared endometria. The potential advantage of this method is that it provides a more physiologic hormonal milieu in which embryo transfers take place (Barnhart, 2014).

Barnhart *et al*. (2014) reported that ovarian stimulation may have unintended consequences. As many aspects of IVF have become optimized, other aspects, such as possible alterations in endometrial development, early embryo development, implantation, early placentation, and OHSS risk have become the focus of modifiable factors that may further enhance safety and success.

When comparing the cycles' characteristics of freeze-all and fresh embryo transfer cycles, as expected, the estradiol serum level on the trigger day and the number of cryopreserved embryos were higher in the frozen-thawed cycles. Indeed the estradiol level is one of the main parameters in the decision to freeze the whole cohort of embryos in women undergoing ovarian stimulation and who develop an excessive ovarian response.

In conclusion, our findings suggest that the GnRHa trigger may be a good alternative to prevent OHSS in patients presenting an extreme ovarian response to COS, leading to similar clinical outcomes when compared with the traditional hCG trigger. Moreover, our findings demonstrated that the strategy of freezing all embryos not only decreases the risk of OHSS but it also leads to a better pregnancy rate.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

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