Comparison of Intravenous Calcium Infusion with Coasting for Prevention of the Early Onset Ovarian Hyperstimulation Syndrome

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Abstract: *Objectives:* To compare the effects of intravenous calcium infusion and coasting in the prevention of the early onset ovarian hyperstimulation syndrome (OHSS) in high-risk women underwent ICSI. *Study design:* Prospective randomized trial. *Patients and Methods:* Sixty women at risk of ovarian hyperstimulation syndrome during ICSI cycles were randomly scheduled into two equal groups. In group I, (Intravenous calcium group), intravenous 10% calcium gluconate, 10 mL in 200 mL of normal saline (0.9%) was administered on the day of ovum pickup, days 1, 2 and 3 after ovum pickup; while in group II (Coasting group) gonadotrophins administration was ceased until serum estradiol levels checked every 24 hours, reached below 3000 pg/ mL before HCG administration. The main outcome measures were rates and grades of OHSS, and the pregnancy rate in both groups. *Results:* Ten patients (33.33%) in calcium group and 15 patients (50%) in coasting group developed OHSS. The incidence of mild {8 (26.67%) vs 10 (33.33%); p= 0.573}, moderate {2 (6.67%) vs 4 (13.33%); p= 0.389} and severe {Zero vs 1 (3.33%); p= 0.313} OHSS were nonsignificantly lower in calcium group compared to coasting group. The clinical pregnancy rate was nonsignificantly higher in calcium group compared to coasting group (40% vs 23.33%; p= 0.165). The mean number of retrieved (p =0.0002), metaphase II (p = 0.007) oocytes and the mean number of good-quality embryos (p = 0.0002) were significantly higher in calcium group than coasting group. *Conclusions:* Intravenous calcium infusion was as effective as coasting in the prevention of early severe OHSS in high-risk patients, but yielded more retrieved oocytes and higher pregnancy rates.
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1. Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic life-threatening complication of controlled ovarian hyperstimulation (COH). The OHSS is typically associated with gonadotropine stimulation; however, it may rarely occur during ovarian stimulation with clomiphene citrate or even in spontaneous pregnancy [(1)]. The overall incidence of OHSS among high-risk women is estimated to be in the range of 1-10% [(2)], while the severe form may occur in 1-3% of all cycles [(3)].

Ovarian hyperstimulation syndrome is characterized by cystic enlargement of the ovaries and a shift of protein-rich fluid from the intravascular space to the third space compartments. Loss of fluid into the third space causes a profound fall in intravascular volume, haemoconcentration, thrombosis, suppression of urine formation and a fall in plasma oncotic pressure which results in further loss of intravascular fluid. In addition secondary hyperaldosteronism occurs and causes salt retention [(4)]. The pathophysiological hallmark of the OHSS is a sudden increase of vascular permeability secondary to the elaboration of exactly unknown hyperpermeability factors in response to ovulation triggering with human chorionic gonadotropin (HCG) [(5)]. Prostaglandins, the renin-angiotensin-aldosterone system, histamine, prolactin, inhibin and inflammatory mediators have been involved in the pathogenesis of OHSS [(6)]; however, vascular endothelial growth factor (VEGF) has been identified as the major permeability mediator [(5)].

Once OHSS occur, only conservative management is available, with potentially life-threatening complications, necessitating costly long-term hospitalizations, therefore, preventive measures are the must. The key to the primary prevention of OHSS during COH is the identifications of the risk factors, individualized the ovarian stimulation protocol, judicious administration of gonadotropins, and careful monitoring of follicular development and serum E2 level [(7)]. Preventive strategies of OHSS includes: cycle cancellation with holding of HCG trigger, coasting, decreasing the dose of HCG trigger, GnRH agonist ovulatory trigger, avoiding embryo transfer and cryopreservation of all embryos , *in vitro* maturation, albumin infusion at the time of egg retrieval, metformin, dopamine agonists and GnRH antagonist [(8, 9, 10)]. Unfortunately, apart from cycle cancellation, none of the currently used strategies completely prevents OHSS. Therefore, there are constant efforts for development of the best effective

strategy. Limited publications reported that intravenous calcium infusion effectively prevents the development of moderate or severe OHSS in high-risk women with no detrimental effect on the pregnancy rate [11, 12, 13].

To our knowledge, this is the first study conducted to compare coasting with the pathophysiological approach of intravenous calcium infusion for the prevention of OHSS in high-risk women underwent ICSI.

2. Patients and methods

This prospective randomized study was performed in the Assisted Reproductive Technologies Unit, Benha University Hospital and in private IVF centers from June 2011 till October 2012. The study protocol was approved by the Local Ethics Committee and written informed consents were obtained before the study started. The study included 60 women among those, who had undergone IVF/ICSI procedure for different indications and were at risk of OHSS. The inclusion criteria were; age 18-35 years, serum FSH level within normal limits (≤ 12 mIU/ml) with development of at least 20 follicles (12-14 mm in the mean diameter) in both ovaries and serum estradiol (E2) level ≥ 3000 pg/ml, on the day of HCG administration. Exclusion criteria were: endocrinopathies; systemic diseases e.g., bronchial asthma, severe renal, hepatic or cardiac diseases; Hypercalcemia and hypercalciuria (e.g., in hyperparathyroidism, vitamin D over dosage, decalcifying tumors such as plasmacytoma, bone metastases); using medication (e.g., GnRH antagonists, insulin-sensitizing drugs and digitalis glycosides); and patients need cycle cancellation.

Stimulation protocol

All women underwent controlled ovarian hyperstimulation (COH) with luteal phase GnRH agonist long down regulation protocol. Long-term desensitization protocol was done using subcutaneous Triptorelin acetate 0.1 daily (Decapeptyl GynTM; Ferring, Kiel, Germany) starting from day 21 of cycle preceding stimulating one. Adequate pituitary desensitization was established on 2nd or 3rd day of the menstrual cycle with the following criteria: thin endometrium, no follicular activity or cysts >1 cm and serum estradiol (E2) level <50 pg/ml. Ovarian stimulation with gonadotropins was started on day 3 of the next cycle using highly purified urinary follicular stimulating hormone HP-uFSH (Fostimon®, IBSA Switzerland) at a daily dose 75-150 IU. The starting dose was individualized according to patient age, ovarian reserve tests and modified according to her response after the first 6 days of stimulation. Follicular development was monitored by serial Transvaginal ultrasound scans and serum E2 level. Serum E2 concentrations were measured with Enzyme-Linked Immunosorbent Assay (ELISA, DRG Instruments GmbH, Germany)

When the inclusion criteria were acheived, patients were randomly scheduled into two equale groups. Randomization was done according to a computer generated random numerical table. Treatment allocation was concealed by using sequentially numbered opaque sealed envelopes, opened sequentially by a third person (study nurse). In group I (Coasting group), when the leading three follicles were ≥ 15mm in diameter, gonadotropins administration was ceased until serum estradiol levels checked daily, reached below 3000 pg/ mL before HCG administration; while in group II (Intravenous calcium group), intravenous 10% calcium gluconate, 10 mL in 200 mL of normal saline (0.9%) was administered on the day of ovum pickup, days 1, 2 and 3 after ovum pickup. Intravenous infusion was performed over 30 minutes. When at least two follicles ≥ 18 mm in mean diameter were observed, final oocyte maturation was triggered with 10,000 IU human chorionic gonadotropin (HCG) (Choriomon®_, IBSA, Switzerland) was administered as a single intramuscular injection. Oocyte pickup was performed 36-38 hours after HCG administration using transvaginal guided follicle aspiration under general anesthesia. After fertilization through intracytoplasmic sperm injection (ICSI), 2-3 good-quality embryos were transferred transcervically 48-72 hours after egg retrieval for both groups. A good-quality embryo was defined as embryo in G1 and G2 grade, having four blastomeres on day 2 or ≥8 blastomeres on day 3, less than 20% fragmentation, and no multinuclear blastomeres [14]. Luteal phase support (LPS) was started at the day of ovum pick up by the vaginal administration of progesterone (Prontogest® 200mg suppositories, Nile Company, Pharmaceuticals, Egypt) thrice daily for 16 days and was continued for up to 12 weeks if pregnancy occurred. Serum β-hCG was checked 2 weeks after embryo transfer for diagnosis of biochemical pregnancy, confirmed clinically two weeks later by sonographic detection of the gestational sac.

Primary outcome measures were rates and grades of OHSS, while the secondary outcomes were the stimulation cycle characteristics and the pregnancy rate in both groups. Monitoring was started from day of HCG administration (day 0) till day 10 after HCG for clinical, laboratory and sonographic signs of the early onset OHSS. The criteria set by Humaidan *et al.* [15] were used to categorize the patients into three groups of mild, moderate and severe forms of OHSS. The mild form was characterized by pelvic discomfort, abdominal

distension, and ultrasonic evidence of ascites in pouch of Douglas, and enlarged ovaries; moderate by ultrasonic evidence of ascites in pouch of Douglas and pelvis, hematocrit > 45% besides the same manifestations of mild OHSS; and severe OHSS by the following objective criteria (severe ascitis, hematocrit >45%, white blood cells >15,000/mm3, and low urine output [<600 mL/24 h]) and subjective criteria (abdominal distension, pelvic discomfort, breathing difficulty, ovarian enlargement, and pregnancy occurrence).

Statistical analysis

Data obtained were statistically analyzed using The Statistical Package for Social Sciences (SPSS, Chicago, USA) software version 15.0 for Windows. Results were expressed as mean± SD, numbers, ranges and percentages. Means were compared using the unpaired student's t test while proportions were compared using the chi-square test. A p value of less than 0.05 was considered statistically significant.

3. Results

Table 1 shows that there were no statistically significant differences between both groups as regards to the mean age, body mass index, the mean period and cause of infertility, past history of OHSS, and baseline serum ionized calcium and hormonal profile.

Table 2 shows that there were no statistically significant differences between both groups regards, the dose and duration of gonadotropin stimulation, serum E2 and endometrial thickness on the day of HCG administration and the number of transferred embryos. The mean number of retrieved oocytes (17.3 ± 4.1 vs 12± 5.6; p=0.0002), metaphase II oocytes (12 ± 3.8 vs 9 ± 4.4; *p*=0.007), fertilization rate (78.33 ± 19.2 vs 61.3 ± 16; *p* =0.0004) and the mean number of good-quality embryos (5.20 ± 2.60 vs 3.07 ±1.44; *p* =0.0002) were significantly higher in Calcium group compared to coasting group.

Table 3 shows that, the clinical pregnancy rate was higher in Calcium than coasting group, but the difference was not statistically significant (40% vs 23.33%; *p* =0.165, respectively). Twelve pregnancies occurred in Calcium group, 2 of these were twin pregnancies. Seven pregnancies occurred in coasting group, 2 of these were twin pregnancies. Implantation rate was higher in Calcium than coasting group, but the difference was not statistically significant (17.14% vs 9.86%; *p* =0.205, respectively). Ten patients (33.33%) in calcium group and 15 patients (50%) in coasting developed OHSS. No significant differences were observed between both groups as regards to the incidence of mild {8 (26.67%) vs 10 (33.33%); *p* = 0.573}, moderate {2 (6.67%) vs 4 (13.33%); *p* = 0.389} and severe {0 vs 1 (3.33%); *p* = 0.313} OHSS. The mean duration of coasting in our study was 2.1±0.8 (1-4) days. Only two patients required coasting for more than three days. Total number of retrieved oocytes was 3 and 4 in these patients and neither became pregnant.

Table 1: Baseline clinical and hormonal characteristics of patients in the study groups

Variables	IV calcium (n=30)	Coasting (n=30)	*p*-value
Mean age (years)	28.35 ±3.65	28.5 ±3.26	0.866
BMI (kg/m2)	25.8 ± 3.4	26.4 ± 3.5	0.503
Period of infertility (year)	7.49 ± 4.1	6.07 ± 3.47	0.155
Cause of infertility [n (%)]			
Male factor	10 (33.33%)	11 (36.67%)	
PCOS	8 (26.67%)	9 (30%)	
Tubal disease	3 (10%)	4 (13.33%)	0.968
Endometriosis	3 (10%)	2 (6.67%)	
Male and female factors	4 (13.33%)	3 (10%)	
Unexplained infertility	2 (6.67%)	1 (3.33%)	
History of OHSS [n (%)]	1 (3.33%)	2 (6.67%)	0.554
Baseline hormonal profile			
FSH(mIU/ml)	6.17 ±2.74	6.60 ± 2.68	0.541
LH (mIU/ml)	7.99 ±2.46	7.03 ± 2.70	0.154
PRL (ng/ml)	11.66 ± 3.90	12.75 ±3.56	0.261
E2(pg/ml)	34.81 ± 14.70	36.75 ± 13.69	0.598
Baseline serum ionized calcium (mg/dl)	5.0 ±0.4	5.1 ±0.3	0.277

Values are expressed as mean ±SD and [n (%)]; [number (percentage)]; BMI: Body mass index; kg/m2: kilogram per square meter; PCOS: polycystic ovarian syndrome; OHSS: ovarian hyperstimulation syndrome; FSH: follicle-stimulating hormone, LH: Luteinizing hormone, E2: estradiol, PRL: Prolactin.

Table 2: Stimulation cycle characteristics in the study groups

Variables	IV calcium (n=30)	Coasting (n=30)	*p* value
Basal antral follicles count (n)	15.5 ±2.2	15.9 ±3.1	0.568
Number of gonadotropin ampoules (75 IU/amp)	22.5 ±4.2	21.9 ±5.2	0.623
Duration of gonadotropin stimulation (days)	9.7 ± 1.1	9 ± 1.8	0.075
On the day of HCG			

injection			
Serum E2 (pg/ml)	3177 ± 402	3115 ± 732	0.686
Endometrial thickness (mm)	10.17 ± 2.05	9.44 ± 1.73	0.141
Duration of coasting (days)	-	2.1 ± 0.8 (1-4)	-
Number of retrieved oocytes	17.3 ± 4.1	12± 5.6	0.0002*
Number of metaphase II oocytes	12 ± 3.8	9 ± 4.4	0.007*
Number of fertilized oocytes	9.4 ± 4.4	6.6 ± 2.1	0.003*
Fertilization rate (%)	78.33 ± 19.2	61.3 ± 16	0.0004*
Number of good-quality embryos	5.20 ± 2.60	3.07 ±1.44	0.0002*
Number of transferred embryos	2.33 ±0.83	2.37 ±0.67	0.837

Values are expressed as mean ±SD and [n (%)]; number (percentage); mm: millimeter; HCG: Human chorionic gonadotropin; E2: Estradiol; pg/mL: picogram per milliliter; OHSS: ovarian hyperstimulation syndrome; * statistically significant.

Table 3: Clinical outcomes of patients in the study groups

Variables	IV calcium (n=30)	Coasting (n=30)	*p* value
Implantation rate	12/70 (17.14%)	7/71 (9.86%)	0.205
Clinical pregnancy rate	12/30 (40%)	7/30 (23.33%)	0.165
Multiple pregnancies	2/12 (16.67%)	2/7 (28.57%)	0.539
OHSS			
Mild	8/30 (26.67%)	10/30 (33.33%)	0.573
Moderate	2/30 (6.67%)	4/30 (13.33%)	0.389
Severe	-	1/30 (3.33%)	0.313
Hospital admission	-	1/30 (3.33%)	0.313

Values are expressed as ratio and percentage; OHSS: ovarian hyperstimulation syndrome.

4. Discussion

The current study showed that, the incidence of clinically significant moderate to severe OHSS was 6.67% versus 16.66% in calcium and coasting groups respectively. However, the published rates of clinically significant OHSS ranges from 3.1- 8% reported by **Delvigne and Rozenberg** [16], to 31% reported by **Engmann *et al.*** [17]. This wide rang of variation may be attributed to differences in the clinical criteria used to classify OHSS, differences in the strategies used for OHSS prevention, small number of patients involved in each of these studies and the occurrence of OHSS in two distinct forms (early and late) [18].

The current study showed that intravenous calcium infusion and coasting were comparably effective in the prevention of early onset severe OHSS in high-risk patients. The preventive role of IV calcium infusion on OHSS was first described by **Yakovenko *et al.,*** in their randomized study comparing this pathophysiological approach with IV infusion with 6% hydroxystarch solution [11]. These observations were confirmed by another study [12]. **Gurgan *et al.,*** in a retrospective comparative study, reported that IV infusion of calcium gluconate compared to no treatment in controls (matched by age and BMI) resulted in a significantly lower rate of development of OHSS for patients with polycystic ovary syndrome [13]. Various studies reported that coasting (i.e., withholding gonadotropin administration and postponing the hCG injection, while continuing GnRH agonists) considerably reduced the incidence of OHSS in high-risk patients, but unlike cancellation of cycle, it dose not completely prevent its occurrence [19]. The majority of cases of OHSS in both groups was mild and does not require special treatment, suggesting that the used strategies may reduce the severity as well as the incidence of OHSS.

The number of retrieved oocytes, good-quality and metaphase II oocytes were significantly lower in coasting group. This agreed with those of **Egbase *et al.,*** [20] and **Aflatoonian *et al.,*** [21] reported that the total number of retrieved oocytes was lower in the coasting group compared to early unilateral follicular aspiration (EUFA) and Cabergoline therapy, respectively. These conclusions may be attributed to atresia of smaller follicles. This disagreed with **Abdellh *et al.,*** [22] reported that the late-onset coasting like that used in this study avoided an abrupt arrest in follicular development and a rapid decline in plasma E2. Consequently, oocyte quality is not markedly compromised.

Highly significant fertilization rate and good-quality embryos, with nonsignificant higher implantation and clinical pregnancy rates were observed in calcium group compared to coasting group. This agreed with various studies that confirm the preventive role of calcium infusion on OHSS without detrimental effects on implantation and clinical pregnancy rates [11, 12, 13]. These positive effects of calcium infusion might be due to enhanced endometrial receptivity [13]. On the other side, data from nonrandomized trials showed that coasting reduces the incidence of OHSS in high-risk patients without affecting cycle outcome [16, 23]. However , **Tortoriello *et al.,*** [24] and **Ulug *et al.,*** [25] suggest that coasting for > 3 days may reduces the implantation and pregnancy rates and may be a cause of cycle cancellation. These conclusions agree with our study as, only two patients required more than three days of coasting with no pregnancy.

Intravenous calcium infusion reduces the incidence and severity of OHSS through alteration of renin–angiotensin system [13] that was among the first systems investigated as a potential contributor to the findings in OHSS [26]. Calcium infusion control renin secretion indirectly, as calcium inhibits and decreases calcium amplifies cyclic adenosine monophosphate (cAMP) stimulated renin secretion from the juxtaglomerular cells [27]. Decreased rennin secretion resulted in decreased angiotensin II synthesis. Consequently, the stimulatory effect of angiotensin II on VEGF production and aldosterone secretion would be attenuated [28]. However, ***Yakovenko et al.,*** suggested that membrane depolarization resulted in development of OHSS clinical manifestations and calcium induced membrane stabilization is expected to alleviate OHSS manifestations [12]. **Velasco *et al.,*** showed that coasting decreases the incidence of OHSS by decreasing VEGF protein secretion and gene expression in granulosa cells, through inducing apoptosis in moderate or small sized follicles [29]. However, ***Ajonuma et al***. suggested that the reduction in VEGF is secondary to the fall in serum estradiol levels [30].

The strategy of using IV calcium infusion for OHSS prevention is easy to use, cheap; relatively safe in well-selected patients, has high success rates, no adverse effects on cycle outcome and has no teratogenic effect.

The main limitations of this study were the small sample size, the lack of serum calcium, renin, prorenin, and VEGF measurement, and the patients were not evaluated for late onset OHSS.

In conclusion, IV calcium infusion was as effective as coasting for prevention of early severe OHSS. This method is a pathophysiological approach, time-saving and lead to more retrieved oocytes, with higher implantation and pregnancy rates than coasting. However, further studies with more cases, are needed to justify these results.

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