

## PROTOCOL OF A THESIS FOR PARTIAL FULFILMENT OF MASTER DEGREE IN OBSTETRICS & GYNAECOLOGY

- Title of the Protocol:

**GnRH agonist trigger VS HCG trigger for final  
oocyte maturation in GnRH antagonist  
protocol ICSI cycles.**

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**What is already known on this subject? AND  
What does this study add?**

In the last decades, GnRH antagonist has been introduced to the market to be used for pituitary desensitization in IVF/ICSI treatment cycles. GnRH antagonist protocol is shown to be an effective alternative to the standard long GnRH agonist protocol [Check et al., 1993].

The introduction of GnRH antagonist protocol has offered an alternative to HCG-induced ovulation triggering. The administration of GnRH agonist, which induces an endogenous rise in both LH and FSH concentrations, has been shown to effectively induce ovulation [Griesinger et al., 2006, 2007].

In this study, we are assessing the effectiveness of GnRH agonist trigger to reduce OHSS and its effects on oocyte maturation, embryo quality, fertilization and clinical pregnancy rate.

**1. INTRODUCTION**

In normal menstrual cycle, gonadotropin-releasing hormone (GnRH) is secreted from the mediobasal hypothalamus in the follicular phase of the cycle in a periodic pulse and is discharged into the pituitary portal system and bound to its receptors on gonadotroph cells in the anterior pituitary. Following, low and pulse release of follicular stimulating hormone (FSH) and luteinizing hormone (LH) happens which is necessary for the follicular growth and the ovarian secretion of estrogen. In the midcycle, in the presence of high levels of estrogen and lower levels of progesterone, sudden surge of gonadotropins especially LH takes place, which induces resumption of oocyte meiosis and ovulation after 36-40 hours [Hoff et al., 1983].

Assisted reproductive technology (ART) consisting in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) are based on the exact timing of ovulation, oocyte pick-up before ovulation and then insemination of oocyte [Casper et al., 2015].

Since the endogenous LH surge is usually absent in patients undergoing long agonist protocols, exogenous human chorionic gonadotrophin (HCG) is used to achieve final oocyte maturation and ovulation. The release of oocyte occurs usually 36-40 hours after triggering of ovulation similar to natural ovulation [Fauser et al., 2002, Melo et al., 2006].

HCG is employed because it possesses the same  $\alpha$  subunit and 85% of the amino acid residues of the  $\beta$  subunit of LH, and binds to the same LH/HCG receptors [Itskovitz et al., 1991, Balasch et al., 1994].

Unfortunately, given its significantly longer half-life (>24 h versus 60 min for LH), HCG is associated with a high risk of ovarian hyperstimulation syndrome (OHSS) as a result of its sustained luteotrophic effect, characterized by the development of multiple corpora lutea and supraphysiological concentrations of oestradiol and progesterone [Engmann et al., 2006, 2008].

In the last decades, GnRH antagonist has been introduced to the market to be used for pituitary desensitization in IVF/ICSI treatment cycles. GnRH antagonist protocol is shown to be an effective alternative to the standard long GnRH agonist protocol [Check et al., 1993].

The introduction of GnRH antagonist protocol has offered an alternative to HCG-induced ovulation triggering. The administration of GnRH agonist, which induces an endogenous rise in both LH and FSH concentrations (initial flare-up effect), has been shown to

effectively induce ovulation [Griesinger et al., 2006, 2007].

In addition, GnRH agonist trigger results in higher levels of LH (11.1 IU/L vs. 3.6 IU/L) and FSH (6.3 IU/L vs. 3.3 IU/L) in the follicular fluid at the time of oocyte retrieval compared with hCG trigger [Andersen et al., 2006].

This phenomenon occurs because the GnRH antagonist achieves pituitary down regulation by competitive inhibition with a relatively short action length, thereby maintaining the reaction ability of GnRH. The GnRH agonist is capable of displacing the antagonist from the receptor and inducing an initial activation (flare-up) which induces an endogenous LH and FSH surges, prior to receptor down-regulation [Fauser et al., 2002, P.Humaidan et al., 2005].

The combination of these two hormones surge is required for oocyte nuclear maturation and follicular rupture. Recent studies state that FSH has a specific function in the process of oocyte nuclear maturation [P.Humaidan et al., 2010, 2012]. FSH is now known to promote formation of LH receptors in luteinizing granulosa cells, keep gap junctions open between the oocyte and cumulus cells, and promote nuclear maturation and cumulus expansion [Atef et al., 2005, Yding Andersen et al., 1999].

The amplitude of the surges is similar to those seen in the normal menstrual cycle, thus reducing the risk of OHSS. Several randomized studies have shown that the incidence of OHSS is significantly decreased with GnRH agonist trigger [Melo et al., 2009, Galindo et al., 2009].

However, in contrast to the natural cycle, the LH surge consists of two phases. These are a short ascending limb (>4 h) and a long descending limb (>20 h). Thus,

final oocyte maturation trigger with GnRH agonist results in corpus luteum deficiency and a defective luteal phase, therefore, impaired endometrial receptivity [Segal et al., 1992].

Whereas some studies have reported comparable clinical outcomes after GnRH agonist and HCG triggering [Acevedo et al., 2006, Eldar-Geva et al., 2007], most studies have reported a lower pregnancy rate following GnRH agonist triggering [Youssef et al., 2014, Orvieto et al., 2006]. For this reason, several schemes of luteal phase support have been used to increase the chance of pregnancy [Engmann et al., 2006, P.Humaidan et al., 2010], although outcomes still seemed to be inferior to those with the conventional HCG trigger [Engmann et al., 2008, Youssef et al., 2014].

Despite the issue regarding a significant reduction in pregnancy rates with GnRH agonist trigger compared with an HCG trigger, some studies did observe improvements in oocyte competence. Many studies have shown significant improvement in number of mature oocytes, fertilization or both [Kol et al., 2017, K.A. Green et al., 2018]. These improvements in IVF outcomes with a GnRH agonist trigger may be due to the difference in LH activity on final oocyte maturation compared with HCG and/or to the induction of the FSH surge, because FSH has been independently shown in vitro to have biologic importance [Atef et al., 2005, Yding Andersen et al., 1999].

In this study, we are assessing the effectiveness of GnRH agonist trigger to reduce OHSS and its effects on oocyte maturation, embryo quality, fertilization and clinical pregnancy rate.

## 2. Aim of the work

- **Research Hypothesis**

Null hypothesis; GnRH agonist trigger and HCG trigger have the same effect on OHSS rate.

- **Research question**

What is the effect of GnRH agonist trigger compared to HCG trigger on OHSS rate, oocyte maturation, fertilization and embryo quality?

- **Aim of the study**

The aim of this study is to compare the effectiveness of GnRH agonist trigger versus HCG trigger to reduce OHSS and also on oocyte maturation, fertilization, embryo quality and clinical pregnancy rate.

- **Outcomes**

- ☐ Primary outcome:

- OHSS rate (Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of assisted reproduction technology. 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].

- ☐ Secondary outcomes:

- Oocyte maturation rate (will be determined by M II oocytes 2 hours after opu characterized by visible of 1st polar body) [Catherine et al., 2012].

- Fertilization rate (defined as the ratio between the number of diploid zygotes (2 pronuclei) and the number of mature oocytes) [Catherine et al., 2012].

- Blastocyst rate (characterized by the appearance of two cell types, inner cell mass (ICM) and trophoectoderm (TE) and the fluid cavity) [Nahid et al., 2015].

- Chemical pregnancy rate (will be detected by measuring serum HCG at least 15 days after embryo replacement) [I.De Croo et al., 1998].
- Clinical pregnancy rate (will be determined by observation of a gestational sac with fetal heart beat by transvaginal ultrasound at 6 weeks of pregnancy) [I.De Croo et al., 1998].

### **3. METHODOLOGY: Patients and Methods**

- **Type of Study:**

A randomized controlled trial with blinding of the assessor will be conducted.

- **Study Setting:**

The study will be conducted at IVF unit in Ain-Shams University Maternity Hospital and some Private IVF centers.

- **Study Population:**

The study will be conducted on infertile women “primary or secondary infertility” undergoing ICSI cycle using GnRH antagonist protocol.

The patients will be divided into two groups, the 1<sup>st</sup> group will receive GnRH agonist trigger, freeze all embryos and transfer the next cycle, and the 2<sup>nd</sup> group will receive HCG trigger and transfer in the same cycle.

- **Sampling Method:**

Convenience sample of women undergoing ICSI cycles for treating primary or secondary infertility using GnRH antagonist protocol who will meet the inclusion criteria.

**Inclusion criteria**

- Female age between 18 and 40 years.
- Primary or secondary infertility.
- Baseline FSH and LH lower than 12 IU/l.
- Body mass index (BMI) between 18 and 40 kg/m<sup>2</sup>.

**Exclusion criteria**

- Ovarian endometriosis.
- Ovarian cysts before induction.
- Known endocrinal abnormalities (like hypo or hyperthyroidism, hyperprolactinemia, pituitary abnormalities).
- AMH  $\geq$  10 ng/ml.
- Duration of induction 6 days or less.
- Duration of induction more than 16 days.
- E2 drop during induction.
- Last E2 level before trigger more than 10000 pg/ml.
- Immature oocytes syndrome.

Immature Oocyte Syndrome (IOS) is usually defined by < 70 % of retrieved metaphase II (MII) oocytes in IVF cycle [P. Massart et al., 2013].

**• Sample Size:**

At least 41 patients per group (Total of 82 Patients) will be included in the study.

**• Sample Justification:**

The required sample size has been calculated using PASS 11 software.

The primary outcome measure is OHSS rate.



Results from previous study [Melo et al., 2009] showed that the OHSS rate in GnRH agonist trigger group was 0%, while for HCG group it was 16%.

Based on this, the required sample size is at least 41 patients per group (Total of 82 Patients) will be enough to detect significant difference with power 90% and significance level ( $\alpha$  error) 0.05.

- **Methods of randomization:**

To ensure that everyone has the chance of participation, after meeting the inclusion criteria the patients will be randomized 1:1 into one of the 2 groups. Randomization will be computer generated using computerized randomization program that runs a balancing algorithm. The balancing algorithm is balancing the following variables: female age (mean and frequency of age  $\geq 37$  years), smoking (frequency of yes/no), AMH ( $\leq 3$  ng/mL, 3-10 ng/mL,  $>10$  ng/mL) and mean BMI.

- **Allocation concealment:**

Sequentially numbered, opaque, sealed envelopes will be used.

The 1<sup>st</sup> group (GnRH agonist trigger) will be represented by a piece of paper with the word “GnRH agonist” on it.

The 2<sup>nd</sup> group (HCG trigger) will be represented by a piece of paper with the word “HCG” on it.

The 82 envelopes will be distributed into 2 groups each containing 41 envelopes.

• **Ethical Considerations:**

- The study will be presented for the approval from The Ethical Committee of the Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University.

- Written consent will be taken from all participates before recruitment in the study after explanation of the purpose and procedures of the study.

- Patients' personal data will be confidential and anonymous.

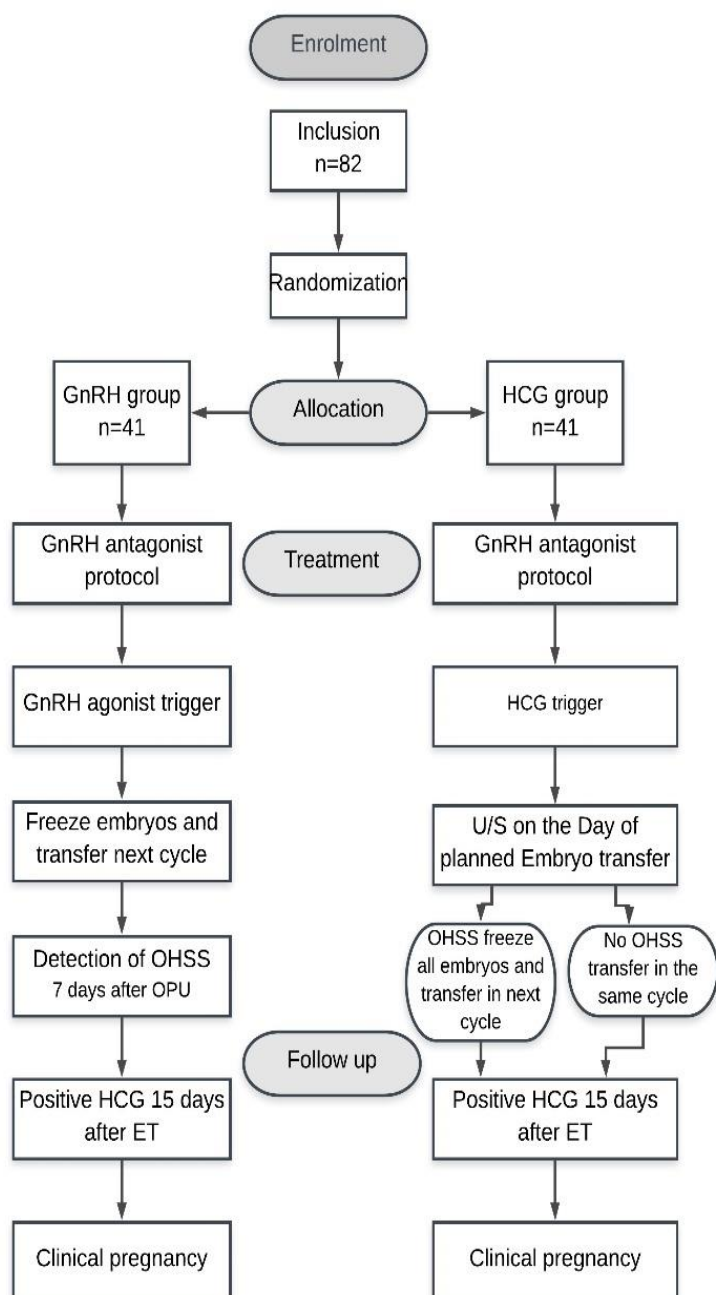
• **Study Procedures:**

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The patients will be divided into two groups, the 1<sup>st</sup> group will receive GnRH agonist trigger, freeze all embryos and transfer the next cycle, and the 2<sup>nd</sup> group will receive HCG trigger and transfer in the same cycle.

At least 41 patients per group (Total of 82 Patients) will be included in the study.

## Study flowchart



## Data collection form

<b>Paient name</b>											
<b>Telephone number</b>											
<b>Address</b>											
<b>Participant Number</b>											
<b>Study Group</b>											
<b>Demographic data</b>	Weight: _____ kg -Height: _____ cm -BMI: _____										
<b>Date of Birth</b>	D	D	M	M	M	Y	Y	Y	Or estimated age _____		
<b>History</b>	<b>Medical:</b>			<b>Surgical:</b>			<b>Smoking:</b> (Yes/No)		<b>Family history:</b> (Yes/No)		
<b>Final diagnosis</b>	<b>Infertility :</b> (Primary/secondary)					<b>If secondary:</b>					
						<b>Gravidity:</b>			<b>Parity:</b>		
<b>History of previous ICSI trials</b>	<b>Cycle no.</b>		<b>Protocol</b>			<b>Trigger</b>		<b>Outcom e</b>		<b>OHSS:</b> (Yes/No )	
	1										
	2										
	3										
<b>Date of written Consent</b>	D	D	M	M	M	Y	Y	Y	Y		
<b>Date of Enrolment</b>	D	D	M	M	M	Y	Y	Y	Y		
<b>Clinical Data</b>	<b>Hormonal profile</b>					<b>U/S Data</b>					
						<b>Lt ovary</b>		<b>Rt ovary</b>		<b>Endometri um</b>	

All the patients will be subjected to the following:

**1- History: -**

**• Personal history**

Including age, occupation and special habits of medical importance.

**• Past medical history**

Including history of chronic diseases “like DM, HTN and thyroid problems” and their treatment and history of past medical disorders.

**• Past surgical history**

Including history of surgical problems and previous surgeries and their relation to infertility

**• Menstrual history**

Including history of menarche, menstrual regularity and any abnormalities detected.

**• History of infertility**

Primary or secondary infertility, period of infertility and possible causes.

**• Obstetric history in case of secondary infertility**

Including number of pregnancies, methods of delivery and complications during pregnancy or delivery.

- **History of previous trials**

Including protocol used, induction medications and doses, outcomes and complications like OHSS.

**2- Clinical examination: -**

Including

- General examination, blood pressure, pulse and BMI
- Local examination, pelvic examination, PV examination, examination of cervix using cusco speculum.

**3- Investigations: -**

- **Day 2 U/S**

Showing antral follicular count and endometrial thickness.

- **Day 2 hormonal profile**

Hormonal profile “FSH, LH, E2, AMH, Prolactin, TSH and any other lab investigations.

**4- ICSI cycle: -**

- **Ovarian stimulation**

Stimulation will be done used GnRH antagonist protocol.

Fixed antagonist protocol will be used (on the 6<sup>th</sup> day of ovarian stimulation GnRH antagonist in the form of Cetrotide 0.25 mg will be introduced till the end of stimulation)

Stimulation will be started day 2 or 3 of cycle using:

- Standard 150 IU of highly purified FSH (Fostimon) for patients <37 years old.
- 75 IU FSH will be added to the starting dose in case of:
  - Age > 37 years old.
  - BMI > 30 kg/m<sup>2</sup> (PCO excluded).
- PCO 112.5 IU.

[Tronson et al., 2000].

### • Folliculometry

Behavior of induced follicles will be recorded by transvaginal U/S.

1<sup>st</sup> U/S will be done on day 6 on FSH stimulation.

Then every other day until at least 3 of the follicles reach a volume of 18-24 ml then the trigger will be given.

The cycle will be canceled if the responding follicles are less than 3 follicles.

### • Triggering method

Either GnRH agonist trigger or HCG trigger.

The 1<sup>st</sup> group will receive GnRH agonist trigger in the form of Decapeptyl 0.2 mg Subcutaneous [Pabuccu et al., 2015].

The 2<sup>nd</sup> group will receive HCG trigger in the form of Choriomon 5000-1000 IU intramuscular [Ioanna et al., 2009].

### • Last E2 before trigger

Will be recorded on the day of trigger before triggering.

- **OPU**

U/S guided Vaginal oocyte retrieval will be done 36 hours after triggering.

Single lumen aspiration needle will be used applying pressure of 120 mmHg negative aspiration pressure [Kumaran et al., 2015].

- **Oocyte Preparation (oocyte denudation).**

The cumulus and corona cells will be removed using enzymatic digestion after 2 hours following oocyte retrieval then incubated in IVF medium at 37°C till ICSI was performed [Catherine et al., 2012].

On the same day of oocyte retrieval, husband's sperm also collected.

- **ICSI Procedure.**

Intracytoplasmic sperm injection will be performed as soon as possible after denudation. Only morphologically normal-appearing mature oocytes with a visible first polar body by the time of ICSI procedure will be microinjected. [Catherine et al., 2012].

- **Assessment of Fertilization and Embryo Development.**

Assessment of fertilization will be done 16-18 h after ICSI by checking the number of polar bodies and pronuclei. The fertilization rate is defined as the ratio between the number of diploid zygotes and the number of mature oocytes. [Catherine et al., 2012].



- **Embryo scoring**

Non-invasive embryo examination is based on simple methods of observation focused on morphology and dynamics of embryo development.

The analysis is performed under contrast-phase microscope with Hoffmann modulation contrast (HMC) or difference-interference contrast (DIC), enabling more precise assessment without fixing and staining.

Generally, the following parameters influence most often the selection of the good quality embryos:

- pronuclear morphology;
- polar body structure and placement;
- appearance of cytoplasm (pitting, vacuoles and halo effects) and zona pellucida;
- early cleavage;
- number of blastomeres in particular days of culture;
- size, symmetry and fragmentation of blastomeres;
- compaction and expansion of blastomeres;
- multinucleation – more than one nucleus in each blastomere.

**Grading of blastocysts at day 5**

- Inner cell mass
  - A. *Numerous and tightly packed cells*
  - B. *Several and loosely packed cells*
  - C. *Few cells*
- Trophoectoderm
  - A. *Many cells organized in epithelium*
  - B. *Several cells organized in loose epithelium*

### *C. Few cells*

[Bączkowski et al., 2004]

#### •At Day 5:

- 1<sup>st</sup> group (GnRH agonist group) all embryos will be frozen in day 5 or 6 and embryo transfer (ET) will be done next cycle.
- 2<sup>nd</sup> group (HCG group) ET will be done in the same cycle.

#### •Embryo transfer:

2-3 Day 5 embryos will be transferred if available.

Each patient will be laid in lithotomy position with full bladder. Uterus, endometrial cavity, cervix, and cervical canal will be visualized using transabdominal US. Under sterile condition, Cusco's speculum will be inserted to expose the cervix. The catheter is introduced through the cervix. Once the position of the catheter will be confirmed the catheter will loaded with embryos and advanced through the outer sheath under sonographic guidance toward the endometrial cavity till the tip of the catheter reach 10-20 mm from the fundus. Then the embryos will be pushed inside the endometrial cavity, and slowly inner catheter will be withdrawn.

Each patient will be kept in this position after freeing of her legs for at least half an hour-1 hour. [Singh et al., 2017].

#### •Luteal phase support

Each patient will receive progesterone form the day of OPU in the form of prontogest 400 mg vaginal

pessaries twice daily continued until the 1<sup>st</sup> antenatal care U/S. [Yanushpolsky et al., 2015]. In addition to Aspocid 75mg once daily with folic acid 500 mcg once daily.

- **Embryo freezing**

- For GnRH agonist trigger group

Embryos will be cultured to day 5 for cryopreservation at the blastocyst stage. Only good quality embryos (Grade A and B blastocysts) were cryopreserved using a slow freeze technique in freezing media, appropriate for embryo stage

Blastocysts will be loaded in groups of 1-4 inside a straw and frozen in a programmable freezer. [Pavone et al., 2011].

- **Endometrial preparation for FET for.**

Estradiol 2 will be started on day 2 or 3 of cycle with oral dose 6 mg divided on 3 doses in addition to Aspocid 75mg once daily with folic acid 500 mcg once daily.

U/S on day 9 or 10 of cycle to assess endometrial thickness, then every other day until endometrial thickness of 8 mm or more is reached.

When endometrial thickness reaches 8 mm or more FET is planned.

Progesterone will start 5 days before the planned FET day [Glujovsky et al., 2010].

- **Embryo Thawing**

At the time of thaw, blastocysts will be removed from liquid nitrogen and held in the air at room temperature for 30

seconds, followed by immersion into a water-bath at 30° C for 40-50 seconds. Cryoprotectants will be removed by sequential dilution in Blastocyst Thawing solutions containing decreasing concentrations of cryoprotectants. Blastocysts will be thawed the day of transfer and allowed to expand 2 hours prior to transfer [Pavone et al., 2011].

- **Embryo transfer**

The same procedure described before.

- **Luteal phase support**

The same as described before except that the progesterone administration will start 5 days before the planned FET day.

- **Pregnancy test**

Quantitative serum B-HCG will be done 15 days after ET. Then repeated after 48 hours.

- **1<sup>st</sup> antenatal care visit for positive B-HCG**

One month after ET (6 weeks GA).

- U/S will be done to detect the following:

- Number of Gestational sacs.
- Fetal cardiac pulsations.

- **Assessment of patients for OHSS**

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of assisted reproduction technology.

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].

- Mild OHSS

Grade 1 - Abdominal distention and discomfort

Grade 2 - Grade 1 disease plus nausea, vomiting and/or diarrhea plus ovarian enlargement from 5 to 12 cm

- Moderate OHSS

Grade 3 - Features of mild OHSS plus ultrasonographic evidence of ascites

- Severe OHSS

Grade 4 - Features of moderate OHSS plus clinical evidence of ascites and/or hydrothorax and breathing difficulties

Grade 5 - All of the above plus a change in the blood volume, increased blood viscosity due to hemoconcentration, coagulation abnormalities and diminished renal perfusion and function [Kumar et al., 2011].

GnRH group will return after 7 days after OPU for evaluation.

HCG Group will be seen before ET on the day of planned ET.

- IF no OHSS proceed with ET.
- IF OHSS diagnosed we will freeze all embryos and transfer the next cycle.

U/S will be done To assess the size of ovaries and the

presence or absence of free fluid in pelvis and abdomen.

Patients will be informed to report if any symptoms appeared before the planned visit or after ET in HCG Group.

**Treatment of discovered OHSS patients.**

-The treatment will depend on degree of hyperstimulation:

**Mild hyperstimulation**

Treatment for OHSS is supportive, as needed. Mild ovarian hyperstimulation can develop into moderate or severe disease, especially if conception ensues. Therefore, women with mild disease should be observed for enlarging abdominal girth, acute weight gain, and abdominal discomfort on an ambulatory basis for at least 2 weeks or until menstrual bleeding occurs.

**Moderate hyperstimulation**

Treatment of moderate OHSS consists of observation, bed rest, provision of adequate fluids and sonographic monitoring of the size of cysts. Serum electrolyte concentrations, hematocrits and creatinine levels should also be evaluated.

Intake or output less than 1000 mL/d or a discrepancy in fluid balance greater than 1000 mL/d is a cause for concern. The beginning of the resolution of OHSS is apparent when the cysts shrink, as seen on two consecutive ultrasonographic examinations, and when clinical symptoms recede. In contrast, early detection of progression to the severe form of the syndrome is marked by continuous weight gain (>2 lb/d), increased severity of existing symptoms, or appearance of new symptoms (eg, vomiting, diarrhea or

dyspnea).

### Severe hyperstimulation

One should transfer the patient to a different center if no one who is experienced in managing severe OHSS is available at the present location.

Severe OHSS is not common, but it is dangerous. Severe and critical forms of OHSS are potentially lethal disorders, and history taking and physical examination are paramount at the time of admission. In most clinical situations, patients require bed rest. Daily physical examination should consist of measuring the patient's weight and abdominal girth. Fluid balance must be assessed every 4 hours.

Medical treatment of severe hyperstimulation is directed at maintaining intravascular blood volume. Simultaneous goals are correcting the disturbed fluid and electrolyte balance, relieving secondary complications of ascites and hydrothorax and preventing thromboembolic phenomena.

The main interventions are fluid management and correction of hypovolemia. These measures consist of initial fast intravenous administration of normal saline. Dextrose 5% in normal saline or normal saline is infused at a rate of 125- 150 mL/h with 4-hour tabulations of urine production. If urine production is restored or improved, a maintenance protocol is started. The patient should be closely monitored for clinical signs of overhydration. If urine output is unsatisfactory, hyperosmolar intravenous therapy is indicated with an infusion of 200 mL of 25% human albumin. The use of diuretics in patients with low urine production and hypovolemia is counterproductive and dangerous.

To prevent thrombosis, subcutaneous heparin 5000-7500 U/d is begun on the first day of admission. It is stopped

after adequate mobilization is achieved.

To manage ascites, ultrasonographic-guided paracentesis is indicated if the patient has severe discomfort or pain or if she has pulmonary or renal compromise.

#### Critical hyperstimulation

Critical OHSS may include renal failure, hepatic damage, thromboembolic phenomena, ARDS and multiorgan failure. Its management and treatment requires intensive care in a critical care unit.

[Kumar et al., 2011]

#### • **Statistical Analysis:**

The collected data will be revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20.0.1 for windows; SPSS Inc, Chicago, IL, 2001).

Normally distributed numerical data will be presented as mean and SD, and skewed data as median and interquartile range. Qualitative data will be presented as number and percentage.

Comparisons between treatment groups will be performed primarily according to the intention-to-treat (ITT) principle

Inferential analysis of quantitative variables will be done using independent t-test in cases of two independent groups with parametric data and Mann Whitney U In cases of two independent groups with non-parametric data.

Inferential analysis of qualitative data will be done using



Chi square test for independent groups.

Relative risk (RR) will be calculated from 2X2 table to detect the ratio between the risk of the outcome in the patients group to the risk of outcome in the control group.

The level of significance will be taken at P-value <0.05, otherwise it will be considered no significant. The P-value is statistical measure for the probability that the results observed in a study could have occurred by chance.

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