The Concept of Dual trigger is better then the more established use of hCG trigger in triggering oocyte maturation as well as helping in prevention of OHSS in GnRH antagonist IVF/ICSI Cycle a retrospective study

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Abstract: The study is conducted to identify whether the concept of application of "dual trigger is more useful than the more standard hCG Trigger in the prevention of OHSS and for acquiring and in determining other major aspects such as retrieval of more number of mature oocytes, followed by better fertilization rate, with more usable embryos and better pregnancy outcomes among the two methods.

"Dual trigger was first defined as the concept of a combination of drugs i.e GnRH agonist and a low dose hCG to help in triggering final oocyte maturation specially in preventing OHSS in high ovarian responders"

"hCG trigger has been since a long time used for triggering oocyte maturation because of its similarity to LH and half life and because it produces a action as similar to in an natural menstrual cycle.

1. Methods

Wastewater This study analyzed 80 records of women undergoing in-vitro fertilization in ARC International Fertility Center, Saveetha Medical College and Hospital, Chennai from December 2019 to March 2020.This is a retrospective study to assess whether dual trigger is better than conventional trigger and assess their overall risk of OHSS in patient prone in a GnRH an IVF/ICSI cycle. Data was collected retrospectively from patient database file and analyzed.

2. Results:

The median age was similar in both groups: 31.5 years interquartile range ie the IQR=35-26.3 in the Dual trigger group whereas the IQR in the hCG group was IQR= 31 years (34-25.0) .The study showed the following Indicating :

.The number of oocytes in Dual trigger it was--- (23.5±7.8) whereas in hCG group- it was ---(20.8±5.4)

The Number of Mature oocytes in Dual trigger was--- (19.1±11.7) whereas in hCG group it was ---- (14.1±4.3)

Maturity rate (%) was 81.6% in dual trigger group (19.1±11.7); and in hCG group it was 72.8%. Fertilization rate was 73.1% in Dual trigger group and 58.6% in hCG trigger group.

The clinical pregnancy rate among the groups ie in Dual trigger group was 33.01% and in hCG trigger group was 20.7%.

The data concerning with stimulation cycle and their characteristics is indicated as follows: Day 2 FSH (Dual trigger - 5.2±1.5 hCG 5.1±1.3; p-value- 0.693); Dosage of gonadotropin in (dual trigger - 1845±707 vs hCG 2095±906; p-value- 0.127); Duration of stimulation (Dual 10±1.2 vs hCG trigger 10±1.4; p-value- 0.876) and Cycle Cancellation rate (Dual trigger - 6.1 vs hCG 15.4; p-value- 0.003)

3. Conclusion:

It was found that there was no difference between follicular reduction prior to HCG and dual trigger treatment. However in terms of assessing OHSS reduction, gestation, and cancellation rates in both the IVF and ICSI cycles, Dual trigger showed to be much more effective compared with hCG treatment.

Note:

No significant difference was seen between the follicular reduction prior to hCG and **coasting,(coasting** is defined as a method in which the gonodotrophins given in that particular IVF/ICSI cycle is stopped and hCG to be given is delayed till the time the serum estardiol level comes to a level in which OHSS can be prevented).

Keywords: In-Vitro-fertilization, Dual trigger, GnRh a trigger, hCG, IVF/ICSI

4. Introduction:

The main essentials of achieving success in the process of In-Vitro-Fertilization are the development of Mature oocytes. HumanChrononic Gonadotrophin (hCG) has been the most commonly measured trigger used in ovulation induction cycles, but it has its own associated drawbacks as it is most commonly associated with ovarian hyper stimulation syndrome(OHSS)[1]. The use of Gonadotrophin-releasing hormone agonist trigger helps in achieving a much better oocyte maturation rate as well as lowers the rate of OHSS, but it too has its own drawbacks of higher Pregnancy loss with increased rates of miscarriage [2]. Co-administration of both hormones is found to increase both the ongoing rates of pregnancy as well as it increases the number of oocytes retrieved[3]. We aim to discuss if "The concept of application of Dual trigger is more useful than the more established usage of hCG trigger in triggering oocyte maturation as well as helping in prevention of OHSS in GnRH antagonist IVF/ICSI Cycle.

IVF therapy requires the establishment of higher doses of follicle-stimulating hormone (FSH) to induce the growth of multiple ovarian follicles during the first half of the cycle. The hourly release of Gonadotrophin is due to the follicular phase of the menstrual cycle which binds to GnRH receptors on the gonodotropes due to which there is hourly release of secretion of Follicle-Stimulating hormone (FSH) and luteinizing hormone(LH),which regulate follicular growth. At mid-cycle, there is a rapid increase in estradiol levels that form the dominant follicles along with a small increase in the progesterone (P) levels which lead to a gonadotrophic surge. Due to the pulsatile increase of LH and FSH it initiates oocytes maturity and approximately 36-40 hours later it helps in triggering ovulation[4].

Because of its similarity LH and its long half life ,24 hrs prior administration of hCG has been traditionally employed to trigger ovulation at mid cycle. In 1973,Nakano et al showed that a bolus of GnRH agonist(GnRH a) given intravenously could induce LH surge.[5] Subsequently, it was found that the use of GnRHa trigger induces final maturation of Follicles through an endogenous surge of LH and FSH ,which closely resembles the normal mid cycle surge .The GnRH agonist trigger differs from the natural menstrual cycle in the way that it has a short ascending time >4 hours and a long descending time > 7 hour. .The

additional flare up FSH and Lh surge induced is believed to promote Oocyte meiosis, better oocyte retrieval, LH receptor formation along with better endometrial receptivity due to lower luteal phase steroid levels, along with release of proteolytic enzymes involved in ovulation[3].

The use of GnRHa trigger has shown to result in more number of retrieved mature oocytes as well as helping in eliminating the risk of OHSS as compared to the most commonly measured hCG trigger, but when used alone the GnRH a trigger results in a lower gestation rate and a extremely high gestational loss due to Luteal Phase Insufficency[6]. The study intends to correlate the overall outcomes from IVF cycle and to identify the ideal approach on the basis of fertilization rate, oocytes retrieved, clinical pregnancy rate achieved. These factors were consolidated and the derived outcomes were assessed yielding maximum results in each outcome variable of an IVF cycle.--.Initial demographic screening with regards to age distribution, BMI ranges, reason for infertility, followed by which determining the overall fertilization rate, clinical pregnancy outcomes and other primary outcomes concerning with number of oocytes and eggs that are usable, thereby conferring to a suitable approach that is applicable for minimizing the risk of OHSS susceptibility among the sample populations.

Ethical considerations:

The present study was approved by the Institutional Human Ethics Committee, Saveetha Medical College and Hospital ,Chennai

5. Material and Methods

IVF Protocol:

In –Vitro fertilization (IVF) is a type of medical procedure which involves retrieving eggs from a woman's ovary and fertilizing them with a men's sperm in a test tube or elsewhere outside the body to form an embryo.

Ovarian Stimulation: A baseline ultrasound, blood estradiol and progesterone test was done on second day of menstrual cycle .This was done so as to see whether the ovaries were suppressed to a baseline state. After ovarian suppression was confirmed , ovarian stimulation with **gonadotrophin fertility drugs** were commenced and continued throughout. The dose of gonadotrophins was based on age, weight, BMI , number of antral follicles and day 2 estradiol levels .(ultrasound was done on day 2 to check or detect any ovarian cycst)

A standard fixed antagonist protocol with recombinant FSH was started on day2 of the menstrual cycle in that ovarian stimulation. During the ovarian stimulation LH and Estradiol levels were measured on day 5 and day 8 of the cycle .Daily GnRH antagonist 0.25mg was started (ciscure) and was Continued right upto the day before the respective trigger was given so that a premature LH surge could be avoided.In recent times Dual trigger has been proposed for a potential treatment for women with history of high responders--- .On an average patients required 10 to 12 days of stimulation.Thus the results from our study showed that dual –triggered oocyte maturation proved as an efficient scheme to achieve optimal high quality embryo rates and reduce the risk of developing OHSS in exaggerated ovarian responders of GnRH antagonist cycles.

Ovulation Triggering: When the follicles had grown to a certain size the respective triggers were administered to the patients. The purpose of the dual trigger was to show that it helps in prevention of OHSS in patients at risk of OHSS ,as well as more number of mature oocytes are retrieved, with better fertilization rate ,more available good quality embryos and better pregnancy outcomes in a GnRH antagonist IVF/ICSI cycle as compared to the more standard usage of hCG trigger.

The two groups that were analyzed were group A: Dual trigger group ,group B hCG trigger group with a sample of 40 patients in each group. Trigger used in dual trigger was (inj.

Luperide 1 mg + inj. hCG 1500 IU)whereas the trigger used in hCG group was ovittrelle 250mg.

Oocyte retrieval:

This is a daycare procedure barring any complication, done under anesthesia. During the procedure fluid containing the follicles containing were aspirated with the help of a thin long needle long the ovum pick up needle (18 gauge)(cooks)through the vaginal fornix under ultrasound guidance. The ultrasound guidance enabled the visualization of the uterus, ovaries and the follicles containing the eggs as well as the tip of the needle which was introduced precisely into every ooctye and the follicular fluid was aspirated and successfully collected.

6. Results

Patient Demographics

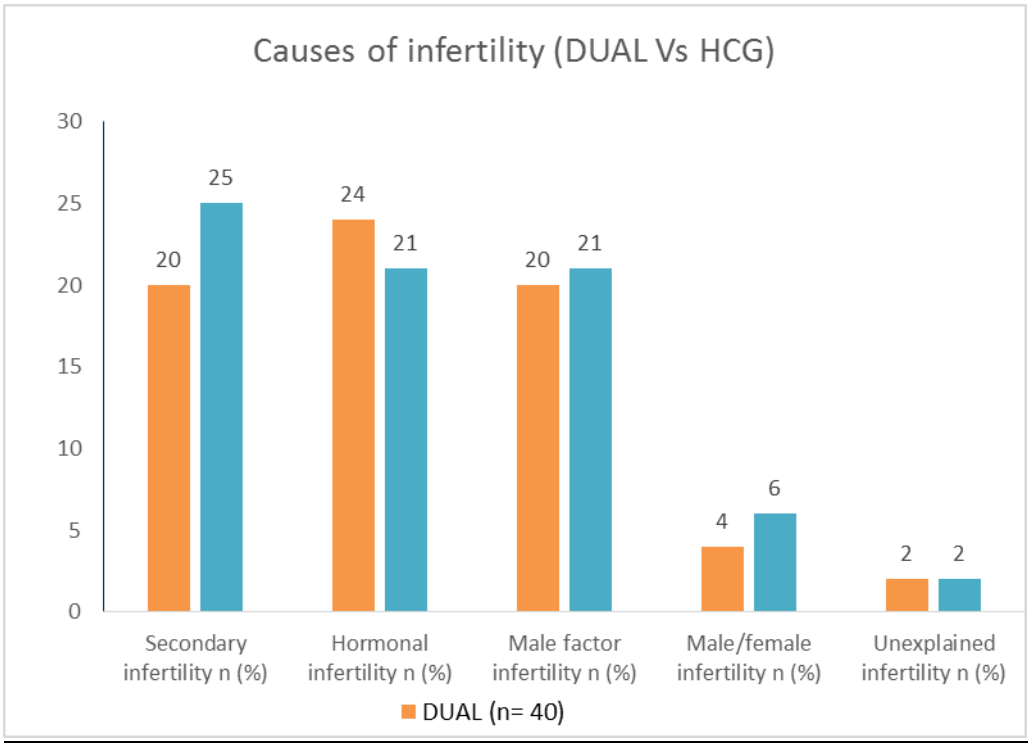
Patient demographics concerning with the average mean of age, BMI and the reason for infertility were determined from Dual trigger and HCG sample population who were undergoing IVF/ ICSI cycles. The demographic profile of both groups are shown in Table 1.

Table 1. Basic Vital Statics of patients undergoing IVF/ICSI cycles

Characteristics	Dual trigger (n= 340)	HCG GROUP (n= 40)	Total	P-Value
Age median years	31.5 (35-26.3)	31 (34-25)	31 (35-26)	0.517*
BMI median kg/m ^{2}	30.5 (36.3-24)	29 (32-25)	30 (33-25)	0.545*
Secondary infertility n (%)	20 (50)	25 (63.2)	22 (56.4)	0.523**
Hormonal infertility n (%)	24 (60)	21 (52.6)	44 (56.4)	0.751**
Male factor infertility n (%)	20 (50)	21 (52.6)	42 (51.3)	01.000**
Male/female infertility n (%)	4 (10)	6 (15.8)	10 (12.8)	0.661***
Unexplained infertility n (%)	2 (5)	2 (5)	4 (5)	1.000***

From the age wise distribution, the mean age group was observed as 31- 31.5 among the sample population. The reason for infertility was observed among the sample population for both groups (see Figure 1)

Figure1: Distribution of patients on the basis of causes responsible for inferility among dual trigger and HCG groups



Primary Outcomes

The following table attributes on the major outcomes pertaining to fertilization rate, number of oozytes and cleavage of embryos among Dual trigger versus HCG groups. -

Table 3: Primary Outcomes/ Variables Involved

Outcomes/ Variables involved	Dual trigger (n= 40)	HCG GROUP (n= 40)
Number of oocytes	23.5±7.8	20.8±5.4
Number of Mature oocytes	19.1±11.7	14.1±4.3
Maturity rate (%)	81.6%	72.8%
Fertilization rate n(%)	73.1%	58.6%

Figure 4: Distribution of patients on the basis of fertilization and maturation rate

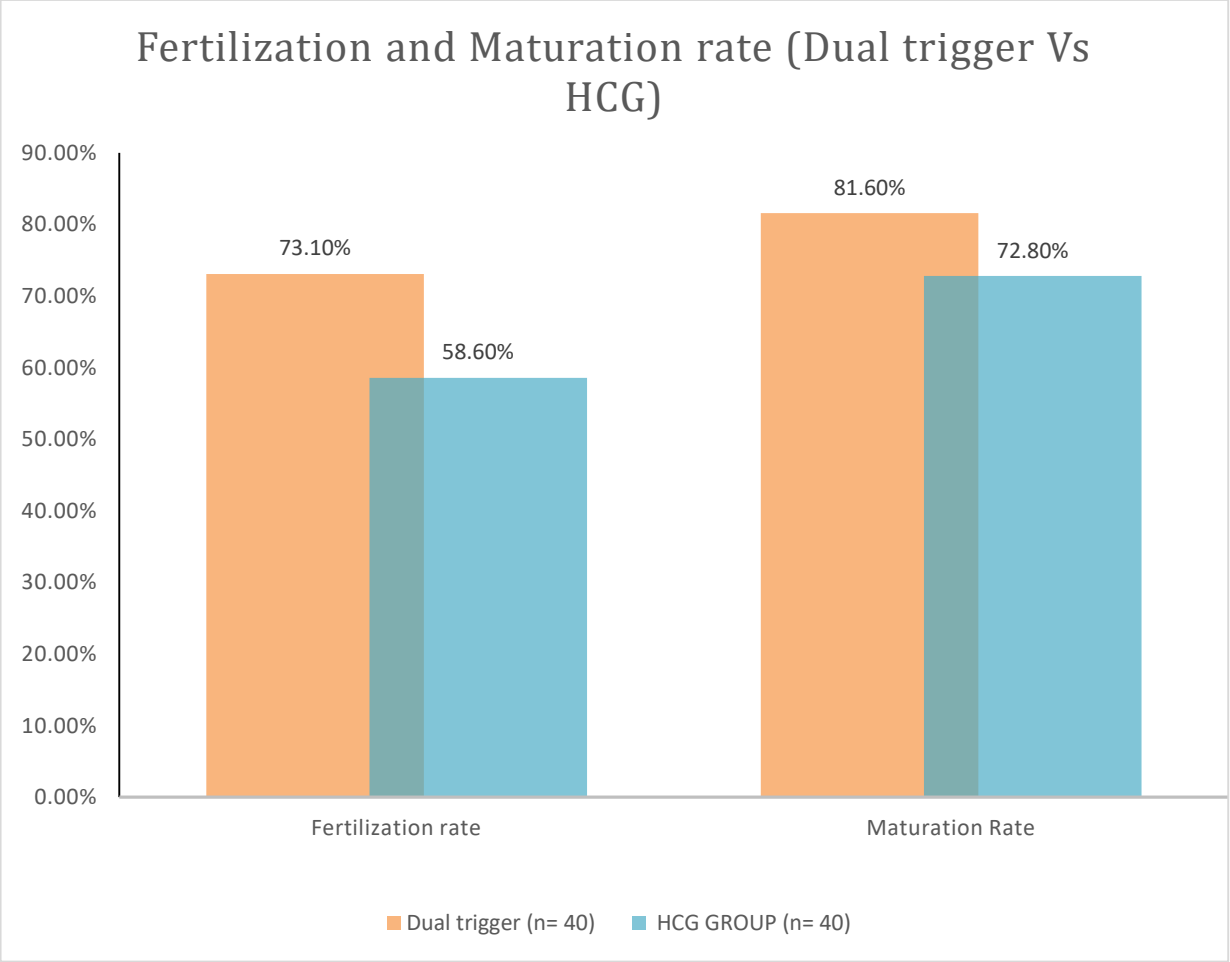
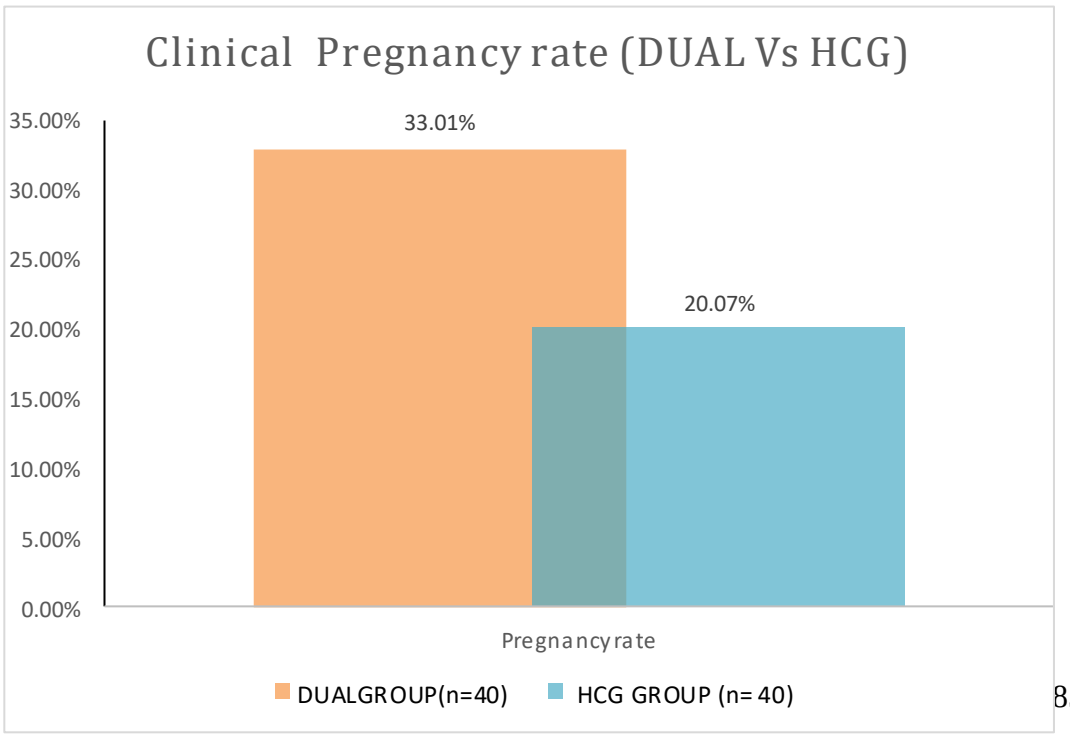


Figure 5: Clinical Outcome ON Pregnancy rate Among Both Groups



While considering the overall incidence of early / moderate/ late OHSS appeared Lower within dual trigger (0) when compared with hCG showcasing 12.37% (p <0.001)

The outcomes concerning with stimulation cycle and its characteristics are indicated in the below table :

Table 6: Secondary outcomes concerning with stimulation cycle

Outcomes/ Variables involved	Dual trigger (n= 40)	hCG GROUP (n= 40)	p-value
Day 2 FSH	5.2±1.5	5.1±1.3	0.693
Dosage of gonadotropin	1845±707	2095±906	0.127
Duration of stimulation	10±1.2	10±1.4	0.876
Cycle cancelation rate	6.1	15.4	0.003

The study showed comparable significant deviation between the dual trigger group and versus the hCG group in terms of the average no of oocytes retrieved - 10.0+- 5.6 vs 8.7+- 5.0 P=0.2816. The M II oocytes conversion rate was seen more in the group of dual trigger as compared to the hCG trigger group 8.4+-5.0 vs 7.2+- 4.0,P=0.2588,there were significant deviation in the fertilization rate in the group of dual trigger as compared to the hCG trigger group -73.1% vs 58.6% , P<.015, clinical pregnancy rate in dual trigger group was found to be 33.0 as compared to the hCG group 20.7% with P=.0.037, incidence of early/moderate/late onset of OHSS was substantially under in dual trigger group :0 as compared to the hCG group 12.37, p <0.001.

7. Discussion

Ovarian hyper stimulation syndrome is the least prevalent but a very serious and life thereaning complication of ovulation induction which manifests itself as a variety of clinical ,chemical and laboratory signs and symptoms .The occurrence of Severe OHSS although less is a grave and life-threatening complication and occurs in ,2 to 6% of IVF cycles resulting in hospitalization in about 1.9% of cases. It is mainly divided into two subtypes: Early (typically occurring between 5-7 days of ovarian stimulation due to the response of hCG given in that stimulation.), Late (occurring due to the increased hCG produced by the placental conception.) It is associated with massive ovarian enlargement <12 cm shift ,ascites : OHSS is usually associated with the presence of ascites, which is a major sign of the Capillary leak phenomenon seen in OHSS. Here there is increased in the intra peritoneal pressure which exceeds the normal capacity of the intra luminal pressure of the abdominal inferior vena cava which in turn compresses the inferior vena cava leading to a reduction in the preload , hence cardiac output is decreased which in turns leads to renal impairment and respiratory failure, liver dysfunction, electrolyte imbalance resulting in significant morbidity and rarely, mortality due to thromboembolic disease, adult respiratory distress syndrome, and hepatorenal failure[7,8] In our study demographic characteristics

representing the median age was similar in both groups: 31.5 years interquartile range IQR=35-26.3(in the Dual trigger group and 31 years 34-25.0 in the hCG group; also . Their BMIs were also similar with Dual group=30.5, IQR=36.3-24.0 kg/m 2 ; hCG group=29, IQR=32-25 kg/m ^{2}.

The adminstration of hCG has been used as a trigger to induce follicular maturation in almost all cycles of IVF stimulation due to its similarity to LH and its long half life , which helps in the maturation of follicles ,its lutenization and finally ovulation[8]. However, the hCG molecule has a high biological activity, which is about 6-7 times higher than the endogenous LH, with a half-life exceeding 24 h, whereas the LH has a half life of 60 min. hCG has a greater affinity to LH Receptors as compared to LH, and hence exerts a more longer luteotrophic activity for 8-9 days, along with multiple follicular development with increased levels of serum E2 and P4 throughout the luteal phase,[3] all of which increase the risk of developing OHSS.[6] The risk of developing OHSS is almost equal for both urinary and recombinant hCG[10].

Various strategies have been used for preventing or decreasing the risks of OHSS: (1) Individualized controlled stimulation: The foremost and most important way to prevent OHSS is to individualize the Stimulation protocol to be used after an assessment of the patient and by identifying their risk factors. Choosing the right protocol: (a) Reducing the exposure to gonadotropins by choosing protocols, in which the use of FSH is postponed till the mid to late follicular phase.[1] (b) Use of chronic low-dose protocol in PCOS. (c) Withholding or at times cancelling:Witholding the use of exogenous gonadotrophins over the administration of hCG till the level of serum estradiol declines.(d)Use of GnRH antagonist protocols: The main use of GnRH antagonist protocol in high-risk patients is the potential use of GnRHa trigger. GnRh agonist trigger minimizes the risk of OHSS in an GnRH antagonist cyclewhich has already been downregulated. Because of the mode of action of antagonist, the action on the pituitary decrease and it becomes receptive to GnRH agonist. GnRH agonist reduces the risk of OHSS by quick luteolysis,its more physiological FSH and LH surge gives better oocyte rates, better embryo. (3) Reduced dose of hCG trigger: As Equivalent with the usual dose of of hCG for trigger i.e 10000 I.U, doses of 5000 IU have been used without impairing the clinical outcome.[12] but its relative use to reduce the risk of OHSS still remains an doubt.[13]

Use of alternative agents as trigger –

(a) GnRHa: GnRh agonist trigger minimizes the risk of OHSS in an GnRH antagonist cycle which has already been down regulated. Because of the mode of action of antagonist, the action on the pituitary decrease and it becomes receptive to GnRH agonist if and when the standard doses of regimine are used. GnRH agonist reduces the risk of OHSS by quick luteolysis its more physiological FSH and LH surge gives better oocyte rates, better embryo. It has a similar effect as that of a natural menstrual cycle but differs from the natural menstrual cycle by having a short ascending time > 4 hours and a long descending time>20 hours. In GnRh agonist the flare up of LH usually lasts for 4 hours and that of FSH lasts for 20 hours with ovulation occurring 24--- 36 hours later(In gonadotropin only or antagonist-stimulated cycles)

.It is well established that after administration of hCG, there is an increased risk of developing OHSS compared to when a GnRHa agonist is used for triggering final oocyte maturation. This is largely owing to the long half-life of hCG and its subsequent potent luteotrophic effect. (b) Freezing all oocytes or embryos is another option to reduce the risk of OHSS in patients at risk as OHSS occurs due to the increasing levels of hCG expressed by placental conception cycle. Hence the embryo transfer should be done in the consequent cycle so as to prevent OHSS in patients at risk

An attractive approach to avoiding OHSS is to substitute hCG with GnRHa has been successfully used in this manner and have led the way thus far in reducing the incidence of OHSS. However, owing to the short half-life of the endogenous LH peak with GnRHa triggering, most of the studies performed in patients showed reduced implantation and pregnancy rates. This thus requires a need to identify novel oocyte maturation triggers that have an effective safety profile in terms of reducing the risk of OHSS, compared to what is currently available, but that also maintain on- going pregnancy success rates.

Comparing the causes of infertility for the study among both the groups showed: **Secondary infertility** n (%) **Dual trigger** 20 (50); HCG group 25 (63.2); Total 22 (56.4) p-value -0.523**; Hormonal infertility n (%) **Dual trigger** 24 (60); HCG group 21 (52.6); Total 44 (56.4) ; Male factor infertility n (%) (Dual trigger20 (50); HCG group 21 (52.6); Total 42 (51.3); p-value - 1.000**); Unexplained infertility n (%) (Dual trigger2 (5); HCG group 2 (5); Total 4 (5); p-value - 1.000***); Male/female infertility n (%)- (Dual trigger4 (10); HCG group 6 (15.8); Total 10 (12.8) ; p-value - 0.661***)

This data demonstrates a positive correlation between the mean ovarian volume, the ascitic volume and presence of OHSS symptoms. An explanation for this could be that the more active the corpora lutea are, due to more prolonged LH receptor stimulation either via hCG or GnRHa-induced LH, the larger they remain and the more vasoactive substances are released from the ovary. This alters capillary permeability to a greater extent, causing larger fluid shifts and accumulation of fluid in the third space compartment. While considering stimulation cycle and their characteristics is indicated as follows: **Day 2 FSH (-** 5.2±1.5 in **Dual trigger** 5.1±1.3; *p-value*- 0.693); **Dosage of gonadotropin (Dual** 1845±707; **hCG** 2095±906; *p-value*- 0.127); Duration of stimulation **(Dual-** 10±1.2; **hCG trigger** 10±1.4; *p-value*- 0.876) and Cycle Cancellation rate (**Dual-** 6.1; **hCG**15.4; *p-value*- 0.003).

8. Conclusion:

It was found that there was no significant difference between follicular reduction prior to HCG and Dual trigger, pertaining in terms of gestation, in terms of reduction of OHSS, and cycle cancellation rates in both the IVF and ICSI cycles. However comparatively Dual trigger showed significant outcomes from the observed sample population, for instance there was a increase in fertilization rate compared to in hCG trigger (Dual trigger 73.1% vs. 58.6% p = 0.015 (HCG); and also the Clinical pregnancy rate (Dual trigger) 33.01% vs 20.7% p=0.037 (hcg) and from the Cycle cancellation rate was (Dual trigger) 6.1 vs 15.4 p =0.003

. However comparatively The study of Dual trigger shows that from the observed sample population, for instance there was an increase in fertilization rate compared to in hCG trigger group.

(Dual trigger 73.1% vs. 58.6% p = 0.015 (HCG); and also the Clinical pregnancy rate (Dual trigger) 33.01% vs 20.7% p=0.037 (hcg) and from the Cycle cancellation rate was (Dual trigger) 6.1 vs 15.4 p =0.003.

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