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ICSI outcome in PCOS women whom GnRH agonist was used as a Final Oocyte Maturation Trigger

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Abstract
Background: The introduction of gonadotropin releasing hormone (GnRH) agonist trigger for final maturation of oocytes in GnRH antagonist stimulated intra-cytoplasmic sperm injection (ICSI) cycles is widely used nowadays as it plays a major role in reducing ovarian hyper stimulation (OHSS) development in high responder patients e.g. polycystic ovary syndrome (PCOS). Polycystic ovary is one of the major causes of ovulatory dysfunction with an ICSI considered a suitable treatment modality in those patients with a high risk of multiple pregnancies and OHSS. Aim: The aim of this study is to compare ICSI outcome between GnRH agonist and hCG trigger in PCOS women stimulated by GnRH antagonist protocol. Materials and Methods: Forty one PCOS females were included. All were stimulated with GnRH antagonist protocol. When the total number and size of the developing follicles were accepted, oocyte maturation was triggered and they were divided in to two groups according to the type of the trigger: hCG trigger (n=18) and GnRH agonist (n=23). Following oocytes retrieval, microscopic assessment of oocytes maturity and embryos quality was done followed by calculation of pregnancy rate with a comparison of the results between both groups. Results: females whom their oocytes' maturation was triggered by GnRH agonist exhibited no significant difference regarding the total number of retrieved oocytes, oocytes' maturation rate and total number of good quality embryos in comparison with hCG-triggered at a p-value more than 0.05. While pregnancy rate in GnRH agonist was significantly less 22.72% vs 28.57% in hCG-triggered females, p-value=0.01. Conclusion: Triggering with GnRH agonist had a comparable ICSI outcome to hCG inform of oocytes and embryos quality. The exception is pregnancy rate which is significantly lower with GnRH agonist which indicates that GnRH agonist might have unfavorable effect on endometrial receptivity lowering implantation rate.
Keywords: GnRH agonist, hCG trigger, oocytes, embryos' qualities and pregnancy rate.

1. INTRODUCTION

Intra cytoplasmic sperm injection (ICSI) cycle needs an exogenous control of luteinizing hormone (LH) surge to permit a time for oocytes pick-up before endogenous ovulation takes place followed by insemination of the oocyte (Casper, 2015). As hCG has a biological activity, similar to LH, so it has been used to trigger the final maturation of oocytes(Casper,

ISSN 2515-8260 Volume 07, Issue 03, 2020

2015). The release of oocytes occurs usually 34-36 hours after triggering of ovulation similar to what happened during natural ovulation(Humaidan P. & Alsbjerg B., 2014). Due to its long lutheotrophic effect, the risk of developing OHSS being more with hCG trigger(Alyasin et al., 2016).

Gonadotropin-releasing hormone agonist is a peptide hormone which is secreted from the medio-basal part of the hypothalamus during the follicular phase of the menstrual cycle (Alyasin et al., 2016). It has been suggested that the use of GnRH agonist for the final oocyte maturation seems to have similar or sometimes better results compared to hCG trigger (Humaidan P. et al., 2013).However, unlike hCG trigger, it stimulates both follicle stimulating hormone (FSH) and LH surge(Reddy, Turan, Bedoschi, Moy, & Oktay, 2014). Mid-cycle FSH surge plays a specific role during oocyte maturation by causing a further expansion of cumulus cells that surround the oocyte and release of proteolytic enzymes which are necessary for ovulation to occur(Lamb et al., 2011). In addition, the increased level of LH following injection of GnRH agonist is faster following hCG trigger (Shapiro & Andersen, 2015). These characteristics of GnRH agonist triggering make the maturity of oocytes more physiological(Alyasin et al., 2016). Several studies showed that GnRH agonist trigger may lead to luteal phase defect which reduces the implantation potential of the embryos, lowers pregnancy rates and increases miscarriage rate when compared to conventional ICSI cycles triggered by hCG(Youssef M. et al., 2014).

GnRH agonist takes a shorter duration for increasing LH which avoids incidence of OHSS(Itskovitz et al., 1991). Ovarian hyperstimulation syndrome is a usual complication of ICSI cycles and females with PCOS whom undergone ICSI are at a greater risk(Susie Jacob, Thomas H. Tang, 2018) due to the presence of large number of antral follicles within the ovaries which are highly sensitive to exogenous stimulation by gonadotropins(Xing et al., 2015).

Thus, GnRH agonist is the preferred trigger in PCOS patients nowadays however, its adverse effect on oocytes' quality, embryos' quality and embryo implantation makes its usage is questionable(Kol et al., 2015).

So the aim of this study is to compare the effect of GnRH agonist trigger vshCG on ICSI outcome represented by total number of the produced oocytes, their maturity, embryos' quality and pregnancy rate in PCOS patients.

Study design

The study is a comparative, observational study and the samples were taken from the Fertility Center, Al- Sadr Medical City, Al- Najaf AL-Ashraf, Iraq.

2. MATERIALS AND METHODS

Forty one sub-fertile females with PCOS whom they seek for infertility treatment in the IVF clinic were selected randomly to be included in the study through the period October, 2017to June, 2018. After taking an oral consent, they were divided according to the type of oocyte maturation trigger which was used: Group I; in which hCG was used (n=18) and Group II; In which GnRH agonist was used (n=23). All were included in ICSI program (as the center use ICSI in up to 99% of cases), following oocytes retrieval, microscopic

ISSN 2515-8260 Volume 07, Issue 03, 2020

assessment of oocytes maturity, embryos' quality and calculation of PR was done and the results were compared between both groups.

The age of the included females was 19-35 years old. They were diagnosed with PCOS depending upon Rotterdam criteria(B. C. J. M. Fauser, 2004) and attended the fertility clinic due to failure of getting a spontaneous pregnancy. Their male partners had either normal sperm parameters or mild oligo- astheno- and teratozo-spermia according to WHO,2010(Archer, 2018). Only women whom down regulated by GnRH antagonist were included ,to eliminate the confounding effect of GnRH agonist induction protocol on the outcome. Females with normal ovulation, endometriosis, uterine fibroid, their partners with sever impairment of semen quality and unexplained infertility were excluded from the study. The study was approved by ethical committee of Kerbala university/College of medicine. No.00000

Evaluation of the sub-fertile females was done depending on history, physical examination, body mass index (BMI) and fertility investigations (cycle day 2 hormones estrogen (E2), LH, FSH, Prolactin(by ELIZA test) and endometrial thickness (ET) by trans-vaginal ultrasound). Females of both groups had been down regulated using gonadotropin releasing hormone (GnRH) antagonist; Cetrotide 1 mg*1 for 7-10 days and hyper stimulated by recombinant FSH; Gonal-F 75 iu*2 for 7-10 days under a close supervision by serial trans-vaginal ultrasound (TVUS) ; real time ultrasound device (Philips 11*E), using "vaginal probe (5-7 MHZ)" and serum E2 assay. Ovulation trigger was done using either hCG; Pregnyl 5.000 iu*2 injection or GnRH agonist; Decapeptyle 0.1 mg*2 when the total number of the follicles and their size are accepted (8-14 follicles and their size more than 17 mm). Retrieval of oocytes was done by the gynecologist under general anesthesia 34-36 hours following the injection of the trigger. Assessment of oocytes maturity was done microscopically after denudation of the oocytes (enzymatic & mechanical which was done 4-6 hours following the retrieval). Only oocytes that resumed their first meiosis (MI) and started second meiosis (MII) are considered as mature oocytes and appropriate for ICSI(Dekel, 2018) . The semen was prepared concomitantly by direct swim-up from a pellet (Archer, 2018). Females of both groups were subjected to ICSI. Assessment of embryos was done at the 3rd day post injection and transfer of only fresh 8 cell stage embryos was the considered policy in the center. Embryos with even cell numbers, regular cells, and no or little amounts of fragmentation considered as good quality (grade I and II), while those with abnormal numbers irregularly appeared cells and with large amounts of fragmentations considered as bad quality (grade III and IV)(Gardner, 2018).

Pregnancy rate calculation was done by dividing the number of females who get a pregnancy which is diagnosed by a positive B-hCG in the serum 14 days following the injection on the number of females whom embryos were transferred to the uterus ×100%.

Data were analyzed statistically by Statistical Package for the Social Sciences (Version 24.0). In each group, either mean± SD for continuous data or total number and percentage for categorical data was calculated. An independent sample student t-test was used for comparing parameters between both groups. For categorical values Chi-square was used.Only at a P-value ≤ 0.05 the differences between the values were considered as significant statistically (the statistical analysis was done depending upon Danial,1999).

ISSN 2515-8260 Volume 07, Issue 03, 2020

3. RESULTS

Forty one PCOS females were included in the study and according to the type of trigger were divided in to: hCG trigger (n=18) and GnRH agonist (n=23) their demographic criteria are shown in Table (1). There were no statistically significant differences regarding these parameters between both groups except the type of subfertility which was significantly differed, p-value=0.002.

Table1. Demographic data of the hCG-triggered VS GnRH agonist-triggered groups

Parameters	**hCG(n=18) Mean ±SD**	**GnRH agonist (n=23) Mean ±SD**	**P-value**
Age (years)	27.22±4.3	27.7±3.8	0.66
BMI (Kg/m2)	27.9±5.3	29.6±3.6	0.23
Duration of subfertility (years)	6.9±2.9	6.8±3.3	0.91
Primary subfertility	14	15	0.002
Secondary subfertility	4	8	

***BMI : Body Mass Index.**

Table (2) compares hormonal profile, endometrial thickness (ET) at the second day of the cycle and total dose of gonadotropins used for stimulation in both groups. There was no significant statistical variation regarding the hormonal profile, ET and total dose in both groups, p-value =0.05.

Table2. A comparison between the mean level of cycle day 2 hormones, endometrial thickness and total dose of gonadotropins in the hCGVS GnRH agonist trigger groups

Parameter	**hCG(n=18) Mean±SD**	**GnRH agonist (n=23) Mean±SD**	**P-value**
Estrogen (Pg/ml)	37.7±16.5	44.2±25.9	0.35
Lutienizing Hormone (IU/L)	5.80±4.8	4.3±2.4	0.22
Follicle stimulating hormone (IU/L)	5.2±1.68	4.6±1.4	0.27
Prolactin (ng/l)	28.7±12.20	25.97±12.6	0.48
Endometrial thickness (mm)	3.60±0.93	3.8±1.1	0.52
Total dose of gonadotropin(IU)	1591.6±356.04	1614.10±506.0	0.87

Table (3) shows the mean total number of retrieved oocytes, their maturity and embryos' quality comparison between both groups. Although the mean total number of retrieved oocytes, the mean total number of mature ones and mean total number of good quality embryos were more in females which were triggered by GnRH agonist but insignificant statistically, p-value=0.42, 0.41 and 0.37 respectively.

ISSN 2515-8260 Volume 07, Issue 03, 2020

Table3. A comparison between hCG VS GnRH agonist regarding the mean total number of retrieved oocytes, their maturity and embryos' quality

Parameters	**hCG(n=18) Mean±SD**	**GnRH agonist(n=23) Mean±SD**	**P-value**
Total no. of oocytes	12.5±7.6	14.5±8.33	0.42
Total no. of mature (MII) oocytes	10.3±7.7	12.4±7.6	0.41
Total no. of immature (GV+MI) oocytes	2.7±3.4	2.00±2.3	0.47
Total no. of good quality embryos	6.2 ±4.6	7.5 ±4.3	0.37
Total no. of bad quality embryos	0.22 ±0.73	0.23± 0.42	0.97

***no: number, MII: Metaphase 2, MI: Metaphase 1 and GV: germinal Vesicle.**

As shown in Table(4), pregnancy rate was significantly less in the group at which GnRH agonist trigger was used, p-value=0.01.

Table4. Pregnancy rate comparison between hCG VS GnRH agonist trigger

Parameter	**hCG**	**GnRH agonist**	**Total**	**P-value**
Pregnant	4(28.57%)	5(22.72%)	9	0.01
Not pregnant	10	17	27	
Total	14	22	36	

4. DISCUSSION

The concept of using GnRH agonist as a final oocyte maturation trigger was come from its short half-life which allows more physiologic surge of ovulatory LH and FSH during the induction and leads to release more matured oocytes (Yen et al., 1968) and its' major benefit in lowering the incidence of ovarian hyper stimulation (OHSS) development by decreasing the number of corpus lutea due to restricted number of maturated follicles (Almasi-Hashiani et al., 2018). Unfortunately, this negatively affects implantation rate as the decrease in the number of corpora lutea leads to decrease progesterone release (an efficient amount of progesterone is necessary to stimulate uterine receptiveness for successful embryo implantation. So, the chance of getting a successful pregnancy is less when fresh embryo transfer is considered(Shapiro & Andersen, 2015).The current study showed that there was no significant difference in the mean total number of retrieved oocytes and their maturation rate between the women who triggered by GnRH agonist and women who triggered by hCG. However, some studies had showed that GnRh agonist trigger might improve oocytes' maturity(B. C. Fauser et al., 2002)(Griffin et al., 2012) many studies goes with what was showed by the current study by showing no significant difference between GnRH agonist and hCG trigger in term of the number of produced oocytes(Youssef M. et al., 2014)(Nadkarni et al., 2015).

The study also showed that the total number of good quality embryos in both groups were comparable. Similar results had been obtained and suggested that GnRH agonist does not have any effect on the quality of the resulting embryos(Alyasin et al., 2016).

The only drawback of GnRH agonist in this study is the pregnancy rate which was significantly less in comparison to hCG triggered cycles 22.72% vs 28.57%. The negative effect of GnRH agonist on pregnancy rate may result from the fact that GnRH agonist trigger, which is unlike to hCG trigger, reduces LH level through pituitary down regulation, so the

ISSN 2515-8260 Volume 07, Issue 03, 2020

amount of LH becomes inadequate for continuing the function of the corpus luteum (Shapiro & Andersen, 2015). The reduced activity of the corpus luteum leads to decrease progesterone secretion during luteal phase which is very important for embryo implantation (Yding Andersen & Vilbour Andersen, 2014).Therefore, using GnRH agonist as a trigger without accurate support of luteal phase leads to low pregnancy rate(Yding Andersen & Vilbour Andersen, 2014). However, it had been found by some studies that there was no significant difference regarding pregnancy rate between both triggers(Alyasin et al., 2016)(Youssef M. et al., 2014).

From our results, we can conclude that triggering with GnRH agonist had a negative impact on ICSI outcome represented by low implantation rate without compromising oocytes' and embryos' quality.So,its effect on endometrial receptivity need to be modified.

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