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RESEARCH ARTICLE

EFFECT OF DIFFERENT CONTROLLED OVARIAN HYPER-STIMULATION PROTOCOLS ON ENDOMETRIAL RECEPTIVITY PARAMETERS OF ICSI PROGRAMS.

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Abstract

Background: Infertility is defined by demographers as childlessness in a population of women at a reproductive age, currently, female fertility normally reaching the peak at age of 24 years and declines after the age of 30-32. Age, overweight, smoking, abnormal pituitary- ovarian hormones all are considered as risk factors of infertility. IVF technique was to overcome this condition by using patients' oocytes and sperms. A good number of high quality oocytes are required for maximizing the success rates with IVF cycles, since success rates correspond to the number of retrieved oocytes. Gonadotropic releasing hormones used for suppression of pituitary hormones with exogenous Gonadotropic hormones administration can achieve multifollicular stimulation in different protocols.

Objective: Comparing the effect of different controlled ovarian hyperstimulation protocols on the endometrial receptivity markers.

Methods: A total of eighty patients undergoing IVF/ICSI cycle were evaluated. One out of three types of controlled ovarian hyperstimulation (COH) protocols had been chosen for each patient according to her age, history and hormonal assay.

At day of embryo transfer blood sample was taken to measure the level of VEGF, also Doppler ultrasonography to measure the resistance index (RI), pulsatility index (PI), systolic velocity/diastolic velocity (V_S/V_d) was done.

Results: There were no significant difference between the three types of ovarian stimulation protocols, still the picture drawn suggests that GnRH antagonists are slightly less efficacious than GnRH agonists in long protocols.

Conclusion: Different types of controlled ovarian hyperstimulation protocols have no significant effects on endometrial receptivity parameters.

Introduction:-

In many countries infertility refers to" a couple that has failed to conceive after 12 months of regular sexual intercourse without the use of contraception"[(1)]. Almost three decades since the birth of Louise Brown (the first baby born after in vitro fertilization) the role of assisted conception treatment has expanded considerably. Modifications

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to the treatment, from superovulation strategies to generate more mature oocytes, through to advances in culture technology that allow embryos to thrive in the laboratory, have led to a steady increase in live births over the past 20 years—currently greater than 25% per cycle in the UK. [2] A good number of high quality oocytes are required for maximizing the success rates with IVF cycle, since success rates correspond to the number of retrieved oocytes, injectable Follicular Stimulating Hormone (FSH) is used to stimulate the development of multiple follicles, like Gonal-F, Follistim, Bravelle and Menopur. Induction of final maturation is done by using Human Chorionic Gonadotropin (HCG).

Gonadotropin-releasing hormone agonist (GnRH agonist) activates the GnRH receptor leading to increased secretion of FSH and LH. It was soon recognized that agonists, after their initial stimulating action - named a "flare" effect - eventually led to a paradoxical and sustained drop in gonadotropin secretion. While the Gonadotropin-releasing hormone antagonist (GnRH antagonists) compete with natural GnRH and bind to GnRH receptors in the pituitary gland; therefore, it minimizes or blocks GnRH action. It binds to those receptors competitively and reversibly, blocking the release of LH and FSH, results in suppression of ovarian estrogen release [3].

GnRH agonist and GnRH antagonist protocols have nearly the same efficiency regarding pregnancy rate in IVF cycles [4]. These protocols differ in some aspects. Regarding time per cycle, the cycle duration using GnRH antagonist protocol is substantially shorter than cycle duration using standard long GnRH agonist protocol, leading to a higher number of cycles in any given time period, which is valuable for patients with more limited time to get pregnant. Regarding antral follicle count, in GnRH antagonist protocol, the initial follicular recruitment and selection is done by endogenous endocrine factors before starting exogenous hyper stimulation, this leads to fewer growing follicles in comparison with the standard long GnRH agonist protocol; therefore, minimizing the risk of OHSS in patients expected to be high responders. Concerning subsequent final maturation induction, using GnRH agonist protocol requires subsequent usage of HCG, while using GnRH antagonist protocol takes the advantage for subsequently using GnRH agonist for final oocyte maturation. This leads to elimination of the risk of OHSS, while having a delivery rate after IVF of approximately 6% less [5] [6].

Materials and Methods:-

A prospective study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, from October 2016 to April 2017. Eighty women were included in this study.

Methods and study design:-

A total of eighty patients undergoing IVF/ICSI cycle were evaluated. Hormonal analysis (FSH, LH, E_2, Testosterone, Prolactin, TSH) for female partners at day 2 of the menstrual cycle. One out of three types of controlled ovarian hyperstimulation (COH) protocols had been chosen for each patient according to her age, history and hormonal assay.

Controlled Ovarian Hyperstimulation protocols:-

Long agonist protocol:-

It involved down-regulation with a GnRH agonist (Triptoreline: Decapeptyl® 0.1mg, Ipsen Pharma, Boulogne Billancourt, France) from the mid-luteal phase of the cycle prior to the treatment cycle, followed by ovarian stimulation from the second day of the menstrual cycle after assessment of pituitary down regulation, with FSH (Follitropine Alpha 75 IU: Gonal F®, Merk, Switzerland), or FSH and LH (Menogon® 75 IU, Ferring Pharmaceutical, FSH 75% + LH 75%)[7].

The antagonist protocol:-

It involved ovarian stimulation with FSH since day 2 of the menstrual cycle followed by the administration of a GnRH antagonist (Cetrorelix acetate for injection 0.25 mg: Cetrotide®, Merk, Switzerland), flexible method according to the size of the largest follicles when they reach 13-14 mm consequently. The initial dose of FSH was 150 - 300 IU daily according to patient underlying medical condition [3].

Short agonist protocol:-

Controlled ovulation induction was done by giving GnRH agonist (Triptoreline: Decapeptyl®0.1 mg, Ipsen Pharma, Boulogne Billancourt, France) once daily since day 1 or 2 of the menstrual cycle, then FSH with or without LH regularly given until day of hCG.

Triggering of ovulation:-
Ovulation was triggered by administering intramuscular hCG 10000 IU (Pregnyl®, Organon, Holland) or subcutaneous (Ovitrelle®, 250 microgram Merk, Switzerland), when at least 3 follicles larger than 17mm were found and the ovarian estradiol level was consistent or giving Decapeptyl® 0.2 mg in case of Antagonist protocol with high risk of OHSS.

Oocyte Retrieval and Grading:-
Oocytes retrieval was performed using a transvaginal probe 34-36 hours after the HCG injection just prior to the rupture of follicles, under general anesthesia, transvaginal ultrasound guided oocyte retrieval (TUGOR) using a very fine single lumen ovum aspiration needle through Wallace oocyte recovery system. The procedure usually took 20-30 minutes. After that, the patients were given antibiotics, analgesics, and luteal support.

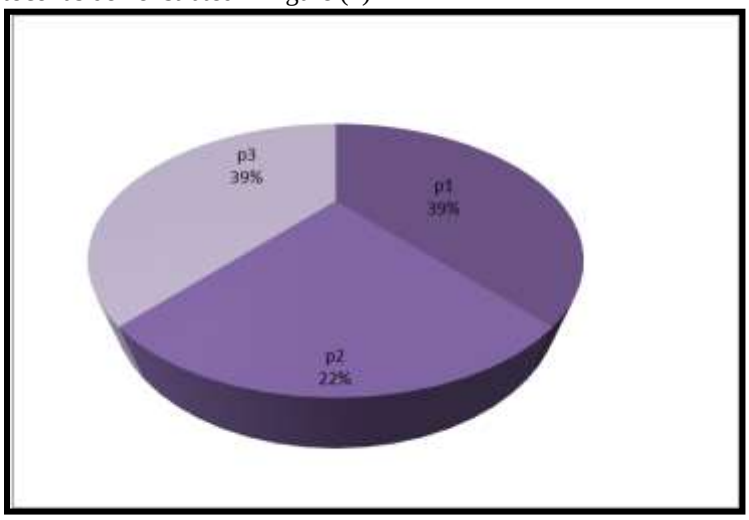
ICSI processes:-
At the IVF laboratory, aspirated follicles were examined in petri dish immediately. Flushing was done then kept 1-2hrs in the 37°C/ CO_2 incubator. all oocytes underwent denudation and grading at Laminar Flow Cabinet, the mature eggs were selected and held with a specialized pipette, a very delicate, sharp and hollow needle was used to immobilize and pick up a single sperm. After that, the needle was carefully inserted through the shell of the egg into its cytoplasm then it was kept in the CO_2 incubator waiting for result of cell division, with the aid of Nikon ICSI Microscope.

Embryo Transfer:-
When fertilization took place, the patient with a full bladder was placed in lithotomy position and the vagina was gently washed with normal saline. The fertilized embryo was then replaced into the uterine cavity under pelvic ultrasound guidance and by an intrauterine catheter. The selected number of embryos for transfer was determined according to the embryonic quality, an age of the patient, rank of the attempt and the clinical history. For the first attempt in women under 35 years, we transferred only 2-3 embryos, if embryo freezing was practicable and 2 embryos otherwise. For women over 35 years or younger women undergoing attempts ranked above two, we transferred 3 embryos and more if possible. A pregnancy test was done approximately 2 weeks after embryo transfer to check for successful implantation .At day of embryo transfer, blood sampling taken to measure serum VEGF, and 2D Doppler ultrasound done for measuring endometrial receptivity parameters; RI, PI, V_S/V_d as well as thickness.

Results:-

Ovarian stimulation:-

Type of ovarian stimulation protocol:-
According to the age, medical history and physical examination of patients, the ovarian stimulation protocols were chosen. Thirty nine percent of cases have underwent long agonist ovarian stimulation protocol, 22% short agonist and 39% antagonist protocol as demonstrated in figure (1)



P1= long agonist protocol, P2= short agonist protocol, P3= antagonist protocol
Figure (1):- Ratio of type of ovarian stimulating protocols used in the study

Comparison of ovarian stimulation protocols according to different demographic data:-

Regarding to the three types of ovarian stimulation protocols used in our study, there were no any significant difference among them concerning all demographic changes that including age, duration of infertility, BMI, types of infertility whether primary or secondary, and all hormonal assay done at day 2 of menstrual cycle (FSH, LH, E2, Testosterone and TSH) except PRL hormone which showed significant difference (0.031) among those protocols, although all were within normal range as shown in table (1)

Table (1):- Comparison between different ovarian stimulating protocols with Demographic parameters by ANOVA Test.

Variables	Long agonist protocol n=31	Short agonist protocol n=18	Antagonist protocol n=31	*P* Value
	M ±SD	M ±SD	M ±SD	
Age	31.31± 5.63	27.07± 6.31	29.03± 5.82	0.071
Duration of infertility	7.07± 4.71	5.55± 3.38	4.87± 2.82	O.O74
FSH	7.346± 2.336	6.829± 2.344	7.475± 2.675	0.667
LH	4.87± 2.27	4.66± 2.34	5.644± 3.52	0.422
E_2	37.4± 16.57	37.59± 13.7	32.76± 9.18	0.318
PRL	17.32± 6.26	16.94± 6.85	13.21± 6.28	0.031
Testosterone	0.79± 0.68	0.901± 0.468	2.01± 3.537	0.081
TSH	2.46± 0.64	2.13± 0.76	2.02± 0.908	0.082
BMI	28.24± 3.86	26.45± 3.24	26.62± 3.62	0.138
Primary infertility	21	16	21	
Secondary infertility	10	2	10	0.209

N=number, M=mean, SD=Standard Deviation, FSH=Follicle Stimulating Hormone, LH=Luteinized Hormone, E_2=Estradiol hormone, PRL=Prolactin hormone, TSH=Thyroid Stimulating Hormone

Comparison of ovarian stimulation protocol's details:-

While other variables including number of GnH ampoules, duration of infertility, E_2 level at day of HCG administration, number of oocytes retrieved and day of embryo transferred, all showed no significant difference whatever the protocol used as its obvious in table (2).

Table (2):- Comparison between different ovarian stimulating protocols with details of ovarian stimulation protocoles by ANOVA Test.

Stimulation-Protocol details	**Long agonist protocol n=31**	**Short agonist protocol n=18**	**Antagonist protocol n=31**	***P* value**
	SD M	**SD M**	**SD M**	
No. of GnH ampoules	5.58±21.77	5.23±19.44	5.48±19.32	0.166
E_2 at day of HCG administration (pg/ml)	529±1303.9	640±1173	651±1481	0.173
No. of retrieved oocytes	3.32±7.93	2.52±6.66	4.1±8.67	0.159
Day of Embryo Transfer	1.2±3.06	0.89±2.72	1.2±3.1	0.516

n=number, M=mean, SD=Standard Deviation, No.=number, GnH=Gonadotropic Hormone, E_2=Estradiol Hormone, HCG=Human Chorionic Gonadotrpin,

Comparison of different protocols with endometrial receptivity parameters

Regarding the different types of ovarian stimulating protocols used in the study, there were no significant differences between them, concerning the VEGF, RI, PI, V_s/V_d, endometrial thickness at the day of embryo transfer and pregnancy rate, *P*-value were (0.173, 0.626, 0.283, 0.184, 0.453, 0.838 and 0.198 respectively) as illustrated by table (3).

Table (3):- Comparison between different ovarian stimulating protocols with endometrial receptivity parameters at day of embryo transfer by ANOVA Test.

Endometrial receptivity Parameters	Longa agonist protocol n=31	Short agonist protocol n=18	Antagonist protocol n=31	*P* Value
	Mean ± SD	Mean ± SD	Mean ± SD	
VGEF (pg/ml)	354 ± 223.8	306.9 ± 229.2	362.8 ± 155.3	0.63
RI	0.5913 ± 0.1315	0.6439 ± 0.1576	0.6368 ±0.1148	0.28
PI	0.8416 ± 0.3467	0.9951 ± 0.3578	0.9715 ± 0.286	0.18
V_s/V_d	2.86 ± 2.327	3.861 ± 3.025	3.826 ± 4.316	0.45
Endometrial thickness (mm)	9.39 ± 1.05	9.322 ± 1.322	9.19 ± 1.579	0.89

N=number, M=mean, SD=Standard Deviation, VEGF=Vascular Endothelial Growth Factor, RI=Resistance Index, PI=Pulsatility Index, V_s=Peak systolic Velocity, $V_{d=}$diastolic Velocimetry.

Comparison of ovarian stimulation protocols and pregnancy rate:-

Although the long agonist protocol has higher pregnancy rate, but there are no significant differences between pregnant and non- pregnant ICSI groups of patients, concerning allocation of type of protocol, *p*-value (0.198) as shown in table (4).

Table (4):- Comparison of ovulation induction protocols of pregnant and non-pregnant ICSI groups of patients Chi test.

Protocols	Pregnant n=(36)	Non- pregnant n=(44)	*P* value
Long Agonist Protocol	19	12	
Short Agonist Protocol	5	13	
Antagonist Protocol	12	19	
Total	36	44	0.198

n=number

Discussion:-

During controlled ovarian hyper stimulation with three different protocols (long agonist, short agonist and antagonist) unphysiological approach to ovarian hyper stimulation for multifollicular growth establishment were done as clarified in figure (1), The efficacy of these techniques depends on a personalized protocol of controlled ovarian hyperstimulation (COH) and an adequate oocyte recruitment. The response of many patients to ovarian stimulation protocols used every time is not always as expected. Failure to respond adequately to standard protocols and to recruit adequate follicles is called 'poor response' [8] and result in lowering oocyte production, cycle cancellation and overall, decreased pregnancy rates.

Although there was no significant difference between pregnant and non-pregnant groups with the type of protocols, but long agonist protocol achieved best pregnancy rate, (19 out of 31) and this is agreed with the American studies that reported higher percentages of pregnancies per long protocol, in spite of the cost of extended, complex, and expensive stimulation protocols and increased chances for hyper stimulation and higher order multiple pregnancies due to the transfer of more than two embryos, which are considered as disadvantages of long protocol [9], and also agreed by a study done previously in Baghdad[6].

In the present study, the response of fewer oocytes after short agonist ovarian stimulation protocol observed in 18 patients was indeed associated with diminished pregnancy outcome, shown in table (3), that illuminates lowest level of E_2 at day of HCG administration, higher RI and PI and lowest level of VEGF at day of embryo transfer, then lower pregnancy outcome, table (3) and (4). A low response during short agonist ovarian stimulation is currently believed to represent ovarian aging and poor oocyte quality. Reduced ovarian reserve represents the most frequent etiological factor as proved by some authors [10][11]. Although it is highly correlated with maternal age, it is also

common in younger women and may be associated with prior ovarian surgery [12]. Recent studies have shown, that poor ovarian response is a first sign of ovarian ageing (early ovarian failure or early menopause)[13][14][15].

However, a low number of oocytes after such stimulation may constitute a normal response, resulting in low pregnancy rate, that's what concluded from randomized controlled trial study design done by some group when compared the efficacy of the long GnRH agonist vs. the short GnRH agonist vs. the GnRH antagonist regimens in poor responders undergoing IVF [16].Gonadotropin-releasing hormone (GnRH) antagonist and short agonist stimulation protocol has shorter duration of treatment and less gonadotropin use, table(2). GnRH long agonist protocol is better in folliculogenesis and pregnancy rate, which is the imperative goal of COH. Despite its costly and lengthy approach, GnRH agonist long protocol has delivered satisfactory results in most women[17].

As shown in table (2), long agonist protocol consumed higher number of GnH ampoules, and this is not favorable in many IVF centers, especially it takes longer days for stimulation.

At day of embryo transfer, 2D power Doppler done for all, cases underwent long agonist protocol achieved lowest RI, PI and V_S/V_d, so endometrial thickness and receptivity were optimum, table(3), that's why pregnancy outcome was higher, table(1), as endometrial thickness was proved to be reliable tool to decide on cycle cancellation or freezing of all embryos or cessation from further IVF treatment[18], although there was a little difference between the level of VEGF in the three types of protocols, this explained better quality of oocytes retrieved from our patients.

Conclusion:-

Different types of controlled ovarian hyperstimulation protocols have no significant effects on endometrial receptivity parameters.

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