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Original Article

Impact of GnRH Agonists and GnRH Antagonists on Embryo Quality, Endometrial Thickness and Pregnancy Rate in In-vitro Fertilization

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Abstract

Objective: To compare the effects of GnRH agonists (long protocol) and GnRH antagonists (short protocol) on embryo quality, endometrial thickness (ET) and pregnancy rate in human in vitro fertilization (IVF).

Subjects & Methods: In this quasi experimental study 237 patients underwent short protocol and 175 long protocol of IVF. hCG was administrated when 2 or more follicles reached the size of 18mm. After 34-36 hours' oocytes were retrieved transvaginally. ET was carried out after 3-5 days under ultrasound guidance. Rising β-hCG concentration confirmed the diagnosis of pregnancy.

Results: There was no significant difference in proportion of primary and secondary sub fertility between the two groups. Regarding female age, embryo quality and endometrial thickness no significant difference was found between two groups. Number of attempts, no of oocytes, fertilization & cleavage rate, maturation of oocytes and embryos transferred were significantly associated with both long and short protocols.

Conclusion: Ongoing research in assisted reproductive technology has identified some issues which are important from the patient point of view such as ovarian hyper stimulation and safe successful pregnancy. This study shows that pregnancy rate is better with long protocol but that is not statistically significant. More studies should be designed with increase power for suitable comparison of long GnRH agonist protocols with short GnRH antagonist protocols.

Keywords: Embryo quality, Endometrial thickness, GnRH agonist (long), GnRH antagonist (short), Oocytes, Ovarian stimulation, Pregnancy rate, Uterine endometrium.

Introduction

In the last 50 years, development of assisted reproduction and unprecedented success has given hope to couples who were considered sub-fertile and constituted 10-15% of the general population. In the developed nations 1% of the children are thought to be conceived with the help of assisted reproductive technology (ART).[1] Since the birth of Louise Brown, first "test tube baby" more than five million babies have been born worldwide with the help of ART.[2] Joint research and collaboration between diversified scientific fields like biology, physiology, endocrinology, embryology, laboratory science and clinical medicine resulted in development of ART of today.[3] Howard and Jones in USA and Trounson from Australia pioneered COH (controlled ovarian hyperstimulation) by using gonadotropins derived from the urine and provided a useful tool for in vitro fertilization (IVF).[4-6] These injectable gonadotropins made it possible to expose ovarian follicles to higher hormonal levels and make a larger number of ovarian follicles to mature into oocytes which can be retrieved predictably in a large number from a single IVF cycle.[6]

In order to suppress internal secretion of pituitary gonadotropin, GnRH agonists are used which help in revolutionizing the process and procedure of stimulation of the ovaries and prevention of LH (luteinizing hormone) surge. It is named as super ovulation.[7,8] When greater number of good quality embryos is needed for transfer into the uterus, higher number of pre-ovulatory follicles is required to be recruited to yield better quality oocytes. This is done by hyper stimulation of the ovaries by external recombinant gonadotrophins.[9,10] Average number of follicles recruited in an ovarian stimulation cycle have risen to ten, twenty or more, which have led to the yield of enhanced number of oocytes per cycle.[11] Higher number of oocytes retrieved has been linked with greater chances of pregnancy.[12] Manipulation of menstrual physiology with the help of drugs and surgery is the key to success in ART.[13] GnRH agonists utilize agonistic analogues of gonadotropins which have some amino acids substitutions in their amino acid sequence which happen to make them more competent

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and enhance their half lives in comparison with the natural hormones. GnRH agonists provide continuous release of gonadotropin secretion.[14] GnRH antagonists cause sudden chemical suppression of the pituitary, thereby causing shutdown of LH (luteinizing hormone) and FSH (follicle-stimulating hormone) secretion.[15] GnRH analogues are given to the women undergoing ovarian hyper-stimulation to obviate LH surge which may cause the follicles to ovulate prematurely. This helps the leading follicles to reach the optimum size. This is followed by injection of ovulation trigger which is hCG (human chorionic gonadotropin). History of the menstrual cycle provides information about the day of ovulation of the natural cycle which helps the clinician to schedule the visits of the patient for hormone tests and ultrasound examination. Experienced clinician, after evaluating the situation of the patient, chooses the drugs, doses and time of ovulation induction from the available protocols.[16]

So called long protocol consist of giving external recombinant gonadotropin along with GnRH agonists and causing suppression of pituitary FSH and LH. By this protocol cancellation rate is reduced and there is increase in recruitment of the follicles and getting larger number of oocytes. The incidence of premature surges of LH is also remarkably reduced. Ovarian stimulation, through the use of GnRH agonists, helps to improve pregnancy rate as a result of IVF.[10] In the short protocol GnRH antagonists are used in ART. They are highly potent and are effective in lower dosage.[17] Suppression of anterior pituitary is more rapid in the short protocol thereby preventing LH release. The suppression is also easily and rapidly reversible. Their mode of action is pharmacologically different from GnRH agonists.[14] GnRH antagonists are given in the mid-cycle which prevents an early LH surge.[18] No suppression occurs in the beginning of follicular phase which is an important time for recruitment of the follicles. Ovarian stimulation by GnRH antagonist protocol is not only short but also cost effective.[17] Perfect synchronization of female endocrines, endometrial physiology and embryonic factors form the basis of molecular communication between the uterine endometrium and upcoming embryo which helps in implantation and subsequent conception.[19] Changes in morphology of the endometrium and hormonal secretion ensure proper embryo transfer and implantation capable of progression to pregnancy.[20]

Operational Definitions

Embryo quality: Embryo quality **(**EQ) is based on division, age of embryo, day of embryo and degree of fragmentation or nucleation. And it is graded as grade 1, 2 or 3.

Endometrial thickness: Thickness of lining of uterus, more than 8mm is considered for ET.

Pregnancy Rate: Pregnancy Rate is the success rate for getting pregnant. It is the percentage of all attempts that leads to pregnancy.

Chemical pregnancy:β-hCG level <10 IU/L for the first time.

Clinical pregnancy:β-hCG level testing >10 IU/L with pregnancy on ultrasound examination.

Long protocol: Ovarian stimulation protocol using GnRH analogue in mid-leuteal phase of previous cycle is called long protocol (21st day of cycle down regulation).

Antagonist protocol: Ovarian stimulation protocols using GnRh antagonist is called antagonist protocol.

Subjects and Methods

In this quasi experimental study total number of 412 cases undergoing IVF were included who attended LIFE ART clinic at a private hospital in Lahore from 1st January to 30th June 2015.This study was approved by LIFE and HLH Ethical Committee. After taking approval from the institutional review board a specially designed questionnaire was used for data collection. The questionnaire was validated by LIFE research cell biostatistician. Patients who were not suitable for ET were excluded from data. Before signing the consent form, the couples were informed about the procedure and processes in detail. Anonymization and de-identification of the couples was carried out prior to the analysis of the data. Down-regulation was carried out in patients in the long protocol (n=175) group by starting the injection Decapepyle1.0 mg-1.3 mg from day 21 of the menstrual cycle. Serum level of E2 <30 pg/ml and LH <2 mIU/ml confirmed full suppression of pituitary gland. Recombinant FSH (Gonal-F, Puregon, Follitropin) were also given on regular daily basis. Ovarian response helped in adjusting the dosage of external FSH and LH.

When 2 or more follicles reached the size of 18 mm recombinant hCG (Pregnyl) was given. 34-36 hours after hCG administration oocytes were retrieved transvaginally. The patients on short protocol (n=237) GnRH antagonist (Cetrotide, Orgalutran) were started on 2nd day of the menstrual cycle and were continued till hCG administration. Embryo transfer took place after three to five days under ultrasound guidance. Fourteen days after embryo transfer, rising concentration of β-hCG confirmed pregnancy.

Data was entered on SPSS version 15.0 and analyzed. For continuous variables mean, SD and SE were calculated. For categorical variables frequencies and percentages were calculated. Chi-square test was used to check association for categorical variables and t-was used of continuous variables. Multiple logistic regression was used to determine the relationship between pregnancy and factors that influence the outcome.

Results

There was no significant difference in proportion of primary and secondary subfertility between two groups. There was also no significant difference between two groups in female

age, embryo quality, FSH (on day three) Number of attempt, no of oocytes, fertilization, cleavage rate, maturation of oocytes and embryos transfer were significantly associated with both long and short protocol (table 1). In long acting GnRH group 1, fertilization rate was 62.74% and cleavage rate was 94.46% while in short acting GnRH group 2, fertilization rate was 61.89% and cleavage rate was 92.61%. In agonist (long protocol) had positive pregnancy test and (72%) had negative pregnancy test while in antagonist (short protocol) (24.1%) had positive pregnancy test and (75.9%) had negative pregnancy test. (Table 2)

Rate of good quality embryos in long acting GnRH group was 28% and in short acting GnRH group was 24%. Primary subfertility had 26.4 % while secondary subfertility had 17.6% rate of good embryo quality (Table 3). Female age and endometrial thickness were significantly associated with pregnancy outcome. Pregnancy rate was improved as the endometrial thickness (p-value=0.05) increased. The estimated odds ratio (OR) of positive pregnancy with female age was (1.075), type of infertility (1.454), endometrial thickness (0.868), no of embryo transfer (0.986) and protocol (1.066) respectively. (Table 4)

Table 1: General characteristics of patients treated with the short and long protocol (GnRH) for ovarian stimulation

Variables	Long-acting(n=175)	Short-acting(n=237)	p-values
Female age (years) (mean±SD)	30.±4.	31.±5.1	0.071
Proportion of primary subfertility n(%)	161(92)	217(92)	0.873
Proportion of secondary subfertility, n(%)	14(8)	20(8)	
No of attempt (mean±SD)	1.1±0.5	1.2±0.529	0.034
No of oocytes (mean±SD)	11.±5.3	10.±5.232	0.004
Fertilization rate, n(%)	1120/1785 (62.74)	1259/2034 (61.89)	0.009
Cleavage rate, n(%)	1058/1120(94.46)	1166/1259 (92.61)	0.009
Matured (mean±SD)	10.2000±4.8848	8.5641±6.0084	0.017
Embryo transferred (mean±SD)	1.8092±0.7855	1.5511±0.6212	0.0001
On day 3			
FSH [mlU/ml], (mean±SD)	7.5534±6.9503	7.1815±2.9639	0.106)
On the day of HCG administration			
P4 [ng/ml], (mean±SD)	5.2232±2.1554	6.4222±3.9793	NS(0.414)
Endometrial thickness [mm], (mean±SD)	9.8573±1.6921	9.5574±1.6033	NS(0.635)

Table 2:Association of long vs short protocol with respect to pregnancy test

	Protocol		p-value
Pregnancy	Agonist(long) (n=175)	Antagonist(short) (n=237)	
Positive n(%)	49 (28.0)	57(24.1)	0.356
Negative n(%)	126(72)	180 (75.9)	

Table 3: Association of IVF outcome predictors and embryo quality embryo quality

Variables	Good Embryo quality(n)	Average Embryo quality(n)	Rate of good Embryoquality (%)%
Protocol			
Long,GnRH(a)	49	126	28
Short, GnRH(anta)	57	180	24
Type of subfertility			
Primary	100	278	26.4
Secondary	6	28	17.6

Table 4: Association between pregnancy and factors that influence the outcome.

Variable	B	SE	Wald	d.f	OR	95% CI	p-value
female age	0.073	0.024	9.175	1	1.075	1.026 - 1.127	0.002
subfertility(primary, secondary)	-0.374	0.476	0.618	1	1.454	0.572 - 3.697	0.432
endometrial thickness	-0.142	0.075	3.55	1	0.868	0.748 - 1.006	0.05
number of embryo transfer	-0.014	0.162	0.007	1	0.986	0.718 - 1.356	0.986
protocol (long, short)	0.063	0.238	0.071	1	1.066	0.669 - 1.698	0.79

Discussion

In our research primary and secondary subfertility, embryo quality, FSH (on day three), serum E2 and progesterone (P4) level on day of hCG injection and endometrial thickness had insignificant association with long and short protocol. Female age, number of attempt, no of oocytes, fertilization, cleavage rate, maturation of oocytes and embryos transfer were significantly associated with long and short protocol. Fertilization rate and cleavage rate was 63.79 % and 94.28% for long protocol; 64.53% and 92.47% for short protocol. Percentage of good quality embryos in the long protocol was 28% and short protocol was 24%. The pregnancy rate with long protocol was 28.0% whereas 24.1 % with short protocol. Age of the female and endometrial thickness showed a significant association with the pregnancy outcome in the logistic regression model.

Primary and secondary subfertility had insignificant association with long and short protocol of ovarian stimulation in IVF/ ICSI.

Main aim of assisted conception is to improve pregnancy rate in couples with subfertility. In vitro fertilization and embryo transfer in the subfertile females leading to clinical pregnancy became possible because of intelligent assessment of needs of individual patient and a personalized tailoring of COH and successful recruitment of adequate number of oocytes.[21]

In many cases the response of the females to COH is not adequate and failure of treatment is imminent. These females are called poor responders who need an individualized ovarian stimulation protocol.[22] Pregnancy rate after undergoing IVF got better because of using GnRH agonists for COH. Untimely early LH surges were also lessened due to GnRH agonists.[11] GnRH antagonists have lesser side effects and also prevent early LH surges.[14] When GnRH antagonist suppresses the pituitary secretion of gonadotropins, it can be reversed rapidly.[23] Short GnRH antagonist protocol is flexible, effective and easier to use and may become an alternative to the long protocol.[14] Greenblatt, Meriano and Casper looked into type of COH protocols (both long and short) in 34 consecutive ICSI cycles and their effect on oocyte maturity, rate of fertilization and cleavage. All these three parameters appeared to be better with the long protocol. More oocytes became mature (metaphase II) as well as more cleaving embryos were seen with long GnRH protocol with a higher fertilization rate in comparison with short GnRH antagonist protocol.[24] Successful IVF depends on maturation of adequate number of oocytes. Chances of Successful fertilization and subsequent clinical pregnancy are decreased when the retrieved oocytes are meiotically incompetent.[25] Al-Inany and Aboulghar published a systematic review in 2002 in which long and short protocols were compared for efficacy in assisted reproduction by COH. Short protocol was shown to have resulted in lesser number of clinical pregnancies (OR 0.79; 95% CI 0.63-0.99). As far as premature LH surges' prevention is concerned OR was found to be 1.76 [95% confidence interval (CI) 0.75-4.16].[24]

Malmusi et al., found that pregnancy rate was better with long protocol whereas implantation was similar in long and short protocol. Retrieval of higher number of matured oocytes and greater number of good quality embryos were also shown in long protocol.[26] A prospective study was done on the data from 4 Egyptian universities' "integrated fertility centers" by Youssef et al. in 2008. Women over the age of 40 were included who had a normal hormonal profile and no pelvic abnormality. Women who received long protocol were 285 whereas 246 women received short protocol. Standard ICSI procedure was performed. Pregnancy rate achieved by long protocol was 26.6 % and by short protocol was 10.2% (P <0.001).[27] Mao et al. published retrospective analysis of comparison between long and short protocol in 2014, regarding thickness of the endometrium, quality of embryo and pregnancy rate. No significant differences were noted between long and short protocols for embryo quality, (63.16% vs. 66.26%, p> 0.05). Pregnancy rate was higher with long protocol as compared to short protocol (59.60% vs. 43.42%, p < 0.05) Type of subfertility and endometrial thickness had significant association with pregnancy success as shown by logistic regression analysis.[28] Cheung et al. in 2005 published a randomized controlled trial comparing GnRH antagonist and long GnRH agonist protocol in women who underwent assisted reproduction (IVF) but were identified as poor responders. They were not able to find any significant differences in many important parameters like cancellation rates, stimulation duration, gonadotropins consumed, and average numbers mature follicles, oocytes, and number of embryos acquired. The antagonist group had higher number of embryos transferred, means and standard deviations being 2.32, 0.58 versus 1.50, 0.83 (P = 0.01). Implantation rates were similar but pregnancy rates were higher for the antagonist group, though the difference was insignificant statistically.[29] Ovarian response is categorized on the basis of levels of serum FSH and E2, number of oocytes, dose of gonadotropin and cycle cancellation rate. Females with lower number of oocytes (>4) were labeled as poor responders and FSH higher than 300 IU was taken as no ovarian response. [15]

Conclusion

In this study, pregnancy rate is better with long protocol but that was not statistically significant. More studies should be designed to compare long GnRH agonist protocols with short GnRH antagonist protocols with increase sample size for suitable comparison.

Conflict of Interest

This study has no conflict of interest a declared by any author.

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Authorship Contribution:
Author1:Conception, plaining and final review of article.
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Author4:Active participation in research and critical review of article