Comparison between the efficacy of short and long GnRH-a protocols as a clinical outcome for viable pregnancies in women undergoing IVF/ICSI Cycles.

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Abstract

This retrospective case control study was executed to compare the efficacy of short and long acting Gonadotropin releasing hormone agonist (GnRH-a) on pregnancy outcome. A total of 540 cycles with a history of male factor were identified from 2013 to 2016. The patients were separated into two groups: long acting protocol (LAP) group consisting of 310 cycles which were induced by a combination of GnRH-a incorporated with Follicular stimulating hormone (FSH) and Human menopausal gonadotropin (hMG), while short acting protocol (SAP) group or flare up regimen comprises of 230 cycles introduced by a combination of GnRH-a along with FSH and hMG. Patients are divided into subsequent age groups ≤ 30, 31–35, 36–40 and > 40 years. In comparison with the SAP group, the duration of ovarian stimulation, calculated dose of GnRH-a, number of retrieved mature oocytes, good quality embryos for implantation and persistent pregnancies were all significantly found to be high ($p < 0.05$) in the LAP group as compared to the SAP group. The clinical pregnancy rates were comparatively high in the LAP group (33.12%; $P = 0.001$) than in the SAP group (28.23%). The findings of our study revealed that irrespective of patient's age, the long acting protocol was more reliable and fertile with reference to number of mature oocytes retrieved, time period for stimulation, total dose of GnRH-a during controlled ovarian hyperstimulation, high quality embryos, fertilization and cleavage rate as well as pregnancy outcome.

Introduction

Infertility is a common global problem that consistently increase over the past few years. According to the recommendations of National Institute for health and care excellence (NICE), the ongoing unresolved infertility after medication and other medical treatments have failed, might have treated through advanced procedures such as in-vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI)(1). However, the success of the procedure depends in part on retrieving an adequate mature ova to generate good quality embryos for uterine transfer, without introducing the patient to the risks of uncontrolled ovarian hyperstimulation.

Pituitary down regulation is the most important and critical step during assisted reproductive technology (ART) cycles that has been achieved through exogenous gonadotropin-releasing hormone agonists (GnRH-a) followed by controlled ovarian hyperstimulation to prevent premature luteinization and finally, ovulation that has been attained by avoiding endogenous LH surge(2). The low concentration of LH is capable to improve the oocyte maturation by enhancing the estrogen to androgen ratio; furthermore, it would improve the vascularization and thinking of internal lining of uterus that provides a safe environment for developing embryo in terms of receptivity and implantation(3).

Principally, three basic elements are important to acquire a controlled ovarian stimulation: A) Suppression of pituitary function, either by administering of GnRH agonist or antagonist and halt the premature spontaneous ovulation. B) Vigorous Growth of immature follicles are achieved through exogenous gonadotropins. C) Final growth of mature oocyte is attained 36-38 h prior to ovulation/egg retrieval(4).

There are two protocols frequently used to accomplish this goal: A so called long acting protocol regimen and short acting protocol regimen. In the long acting protocol regimen, GnRH-a is generally injected through a single depot dose in the mid of the luteal phase (two weeks prior to ovarian stimulation) of the previous cycle and continued up until development of multi-follicles is attained. GnRH-a will deeply down regulate the pituitary function and therefore, requires in higher amount and longer duration to get desired results(4).

Alternatively, in the short acting protocol regimen, GnRH-a is conducted at the commencement of menstrual cycle and still proceeds until the day of hCG. The most effective and commonly used method in ICSI/IVF cycles is the long acting protocol regimen that have been given excellent results in the last decade. However, short acting protocol regime gains its importance in poor respondents that have excessive pituitary desensitization and induced an early agonistic flare effect of the Gonadotropin releasing hormone(5).

Finally, at the end of stimulation phase, the most common drug used to mimic the natural LH surge is Human chorionic gonadotropin (hCG) that initiate the process of ovulation. It is still provocative that which method is the best treatment for all patients undergoing ICSI/IVF program(6). The data shared by one of the network meta-analysis that includes eight Cochrane reviews and consists of forty thousand subjects depicted more effectiveness of long protocol regime than in the short protocol regime in term of achieving controlled ovarian stimulation and viable embryos(7).

The present study was conducted to find out the effectiveness of two protocols in terms of clinical success rate, number of mature oocytes retrieved, controlled hyperstimulation, cleavage rate and number of live births.

Materials And Methods

Subjects:

A total of 540 patients who underwent IVF/ICSI cycles from 2013 to 2016 and have a history of male factor infertility, tubal factor and ovulation disorders were enrolled at Lahore Institute of Fertility and Endocrinology, Hameed Latif Hospital. This retrospective study was approved by the Institutional Ethical Committee (IEC) in accordance with Helsinki Declarations.

Study design:

This was a retrospective single center, open label comparative study, with the objective to compare the efficacy of short and long acting gonadotropin releasing hormone agonist (GnRH-a) regime for controlled ovarian hyper-stimulation in subjects underwent IVF/ICSI cycles.

Inclusion criteria: Patients with age between 25-45 years, previously have not more than two IVF/ICSI cycle attempts, on day 2rd of menstrual cycle serum FSH level must be < 9 IU/L were included in this

study.

Exclusion criteria: Patients with any congenital anomaly, pelvic pathology, urogenital surgery, any sexual transmitted disease, habitual abortion, alcoholic addiction, any infectious disease, underwent hormonal replacement therapy during last three months, any uterine abnormalities and immunocompromised were excluded in this study.

Estimation of Clinical Parameters:

Clinical parameters such as height and weight were recoded to compute the body mass index (BMI) according to standard protocol(8).

Valuation of endocrine dimensions:

The blood samples were collected between 8 to 10 am from cubital vein and serum were separated instantly and stored at -20C^0 till the performance of hormonal assay including Follicular stimulating hormone (FSH), Luteinizing hormone (LH), estradiol (E2) and anti-mullerian duct hormone (AMH) on 2nd day of the menstrual cycle through electrochemiluminenscence Immunoassay according to the manufacturer's instructions (Elecsys® Roche Diagnostics, Indianapolis, USA).

Therapeutic Regimen:

GnRH-agonist long acting protocol:

A total of 310 patients were recruited in the long acting protocol (LAP) group induced by a combination of GnRH-a incorporated with Follicular stimulating hormone (FSH) and Human menopausal gonadotropin (hMG). Women with regular cycle on day 21 in the mid luteal phase started with administration of intramuscular single dose of 0.1-1.2 mg long acting decapeptyl® (Triptorelin acetate; Ferring) GnRH-a injection. A complete down regulation of pituitary had done when the serum LH level < 2 IU/ml and serum E2 level < 30 pg/mL was achieved. The transvaginal ultrasound scan (TVS) revealed a less than 5mm endometrium thickness which further confirmed complete pituitary suppression. At that stage exogenous gonadotropin rFSH (5.5µg; Gonal-F™, Merk Serono) and menotropins hMG (LG™ life sciences, Korea) administration was commenced depending upon body weight, age and follicular size of the patients, on cycle day 2 at doses ranging between 75-220 IU/day and 350-450 IU/day. Accordingly, further regular dosage of rFSH and hMG was calculated on the basis of ovarian stimulation that has been monitored through transvaginal ultrasound scan (TVS) and serum E2 levels. The folliculogenesis was consecutively observed through TVS and measuring the ratio of serum E2, LH and progesterone from the 8^{th} day to till the day of Human chorionic gonadotropin (hCG) injection (Pregnyl®, Organon), which is around 14 days post GnRH-a administration.

GnRH-agonist short protocol or Flare up regimen:

A total of 230 patients in the short acting protocol (SAP) group have got a 0.1 mg daily intramuscular dose of decapeptyl® (Triptorelin acetate; Ferring) started from day 3rd of the menstrual cycle and continued till the day of hCG. Controlled ovarian stimulation started from 2nd day of menstrual cycle at the dose of 100-220 IU of rFSH (5.5µg; Gonal-F™, Merk Serono) and 200-450 IU of hMG (LG™ life sciences, Korea) respectively. The daily dosage was adjusted in accordance with ovarian response.

Ovulation induction:

In both protocols recombinant hCG (6500-10,000 IU) was given intramuscularly to trigger the final maturation of follicles or when more than two follicles attained a diameter of 17 mm along with increased level of oestradiol 2000 pg/ml/mature follicle. While cycle cancelation has been done if there is poor ovarian response during stimulation i.e. no follicle of 15 mm will be seen on day 9, E2 level will be <5000 pg/ml on day 9, high risk of incidence of ovarian hyperstimulation syndrome (E2 level >7000-8000 pg/ml) etc. The time difference between the last gonadotropin injection and hCG regimen was no more than 24 hour. After 36 hour of hCG regimen transvaginal echo-guided ovarian puncture has been done and oocyte retrieval was performed. The assessment of oocyte quality has been performed after removing cells of the corona radiata which is based on directly under the inverted microscope. Oocyte maturity has been noted and mature oocytes of MII are microinjected. The optimal assessment of embryonic grading is based on the study of morphology such as cleavage rate, number of blastomeres, cytoplasmic appearance, extent of a-nucleated fragments, and regularity in the symmetry of blastomeres.

Pregnancy outcomes:

Fertilization was evaluated 18 to 20 hour after insemination. One or two embryos of grade I (smooth and regular blastomeres, devoid of fragmentation and embryo) or grade II (blastomeres are equal in size, minor fragmentation (< 20%) was transferred to the uterus after 3 to 5 days later using ultrasound guided catheter (Cook, Australia). The additional embryos were frozen based on the couple consent. Biochemical pregnancy was identified by a high level of β-hCG i.e., 50 mIU/mL, which was tested 14 days post embryo transferred. A radioimmunoassay kit was used to measure the serum concentration of β-hCG. Clinical pregnancy was confirmed by means of a gestational sac and heartbeat monitored during trans-vaginal ultrasound on 6 to 7 weeks later. Miscarriage or spontaneous abortion was demarcated as termination of pregnancy before 28 weeks. Luteal phase support was given Duphaston orally (10mg) or vaginal pessaries (Utrogestan 100mg) from the day of oocyte retrieval until clinical pregnancy was ruled out.

Statistical analysis:

Statistical analysis was done using statistical package SPSS (version 21; SPSS Inch., Chicago, IL, USA). Values are presented as Means ± SD or n/N (%). The means of two groups were compared through unpaired Student's t-test, while, categorical variables were calculated through χ^2–test. Fisher's exact test

was applied to relate multiple means from different groups. Significant statistical difference was considered $p<0.05$.

Results

A total of 540 IVF/ICSI cycles were met the inclusion criteria of present study. Factors which were contributed in male and female infertility are summarized in Table 1. While, demographic variables of patients are presented in Table 2. In each age groups, both SAP and LAP groups were similar in relation to BMI and onset of primary infertility. Basic endocrine levels were investigated and no significant differences were noticed between the SAP and the LAP groups Table 2. As shown in table 3 the overall dose of GnRH-a was significantly higher and the period of controlled hyperstimulation was significantly longer for all age groups ($p<0.05$) in the long protocol as compared to the short protocol. Whereas Antral follicles count and numbers of oocytes retrieved were significantly higher in the LAP group compared to the SAP group in all age ranges. However, MII oocytes were significantly higher in the LAP group in contrast with the SAP group (Age ≤30; 10.32 ± 3.25 versus 7.65 ± 3.21, Age 31-35; 9.69 ± 2.36 versus 6.14 ± 2.59) ($P<0.05$) respectively. With the progression of age, more gonadotropin was used but time duration remained unchanged. The E2 levels of each follicle on hCG trigger day were not significantly different in two age groups (≤30 and >40 years) between the short and long protocols (197.41 ± 51.2 pg/ml versus 198.01 ± 65.3 pg/ml and 147.98 ± 98.64 vs 148.36 ± 101.3 pg/ml ($P<0.05$) respectively. However, in other two sets of ages (31-35 and 36-40), the E2 levels in the SAP group were 166.23 ± 96.3 pg/ml and 152.31± 63.45 pg/ml, whereas, these levels were significantly increased in the LAP group 175.94 ± 21.7 pg/ml and 160.94 ± 98.5 pg/ml ($P<0.05$) respectively.

Comparatively, the serum LH levels on the day of Gn stimulation and hCG trigger day were significantly lower in the SAP group than in the LAP group (1.68 ± 1.01 U/L versus 1.96 ± 1.01 U/L $P = 0.003$ and 1.02 ± 0.16 U/L versus 2.14 ± 0.35 U/L $P = 0.001$). However, no significant differences have found in terms of progesterone concentration (P) and Progesterone/Estradiol (P/E2) ratio between the two groups listed in the Table 4. There was no significant difference in the fertilization and cleavage rate among both protocols (76.91% versus 75.98% $P = 0.721$ and 90.31% versus 91.04% $P = 0.632$). However, significant differences in the implantation and clinical pregnancy rate have found between the short and long protocols (31.56% versus 35.41% $P = 0.000$ and 28.23% versus 33.12% $P = 0.001$) respectively. Clinical pregnancy rates were significantly higher (17.02%, 8.06% and 3.22%) for age groups ≤30 , 31-35 and 41-45 years in the LAP group and being comparable to the SAP group (15.21%, 6.00% and 2.60% $P = <0.05$) respectively, as shown in Figure 1. The χ^2 – test revealed that, as age was increased the implantation rate, clinical pregnancy rate and number of good quality embryos were significantly decreased ($P = \leq 0.05$) in both protocols (Figure 2). However, there was no significant differences in early abortion, miscarriage and ectopic pregnancy rate in both protocol groups as presented in the Table 5. The Ovarian hyperstimulation syndrome (OHSS) rate was higher in the long protocol group (8.21%) than in the short protocol group (6.21%) respectively; $P = 0.002$.

Discussion

We have figure out 540 IVF/ICSI cycles using SAP and LAP regimen combined with FSH+HMG usage. The difference on ovarian response along with pregnancy outcome were determined. The application of SAP regimen for IVF/ICSI cycles has tremendously increased over the past decades. However, the trend to use them more often in cases of least prognosis has been moderately indistinct. The fact remains that, with reasonable patience, in routinely use, they have given a comparatively, lower pregnancy rate than would have been presumed with LAP group(4). Even so, for some clinicians, the SAP protocol would be the excellent treatment for patients with polycystic ovary syndrome (PCOS)(9). In this regard, the SAP/LAP agonist dilemma has been the matter of discussion for several years and the response is far from obvious.

However to improve ART outcomes, several approaches have been introduced such as, GnRH antagonist or agonist protocol, Gn dosage and time variations. Besides those the use of adjuvant therapies such as growth hormones, testosterone and oral contraceptives pills were routinely used to increase the production of oocytes(10). Nevertheless, there were still many reservations regarding the optimal protocol for controlled ovarian stimulation in *in vitro* fertilization.

Previously, it has been evident that LAP regimen in IVF may lead to ovarian desensitization(11). In our study, results showed that longer duration of stimulation and more consumption of exogenous gonadotropin trigger ovarian over suppression in the LAP group than in the SAP group in all four ranges. It is being used to improve the response of the ovary in terms of good quality oocyte and complete down regulation of premature surge of luteinizing hormone (LH). Likewise, previous studies describes that high level of follicular phase LH is responsible for lower pregnancy rates in women underwent IVF/ICSI cycle(12, 13). Our findings have shown a complete desensitization of pituitary (LH surge) in the LAP group enhances the number of mature oocytes and improves the pregnancy rate in comparison with the SAP group. This lower vulnerability of LH towards the developing oocyte might have positive effect to improve its quality and numbers. Another important aspect with respect to low level LH in the LAP group is positive association with endometrium receptivity by enhancing vascularization or thickness, which provides a comfortable bedding for embryo implantation. This was in line of previous study results suggested that low concentration of serum LH is suitable for embryo implantation in the LAP regimen by improving the endometrial receptiveness(14, 15).

Other previous studies reported that an appropriate concentration of LH is prime to achieve successful pregnancy outcome and patients with high (>5.00 mIU/L) and low (<1 mIU/L) concentration of LH have attained poor pregnancy rate in IVF/ICSI cycles(16, 17). An insufficient level of LH moderately decreases the E2 concentration in follicular fluid, produces immature oocytes, reduces fertilization rate along with odd pregnancies and might have responsible for extra pituitary side effects. However, E2 levels on the day of hCG stimulation were significantly higher (2879.51 ± 11.25 pg/L versus 3178.21 ± 12.36 pg/L; $p < 0.05$) in LAP group than in the SAP group, which is in agreement with previous studies(18, 19).

Our results also demonstrated that the LAP group patients (within each age range) significantly have increased antral follicles count, oocytes upsurge, mature MII oocytes number and high quality embryos as compared to the SAP group (<0.05). The same findings were found in the meta-analysis and in a retrospective study which revealed that LAP regimen in IVF responsible for increased number of antral follicle, mature oocytes retrieved, and high quality embryos, when compared to the SAP regimen (20, 21). In contrast, some studies have revealed that prolong down regulation might be increase the live birth rate but would reduce the number of oocyte retrieved and decreased good quality embryos in the LAP group in comparison with the SAP group(22, 23). Our study validated that no sever hyperstimulation syndrome has been reported, however the manifestation of mild to moderate hyperstimulation has been found in LAP group than in the SAP group. The cycle cancellation rate was significantly higher in the SAP group that is being comparable with the LAP group. Conversely, early abortion, miscarriage rate and ectopic pregnancy rate have no statistical difference between both regimens.

However, SAP group offers the advantages of better acquiescence and cost effectiveness for the poor responders by avoiding inappropriate desensitization of pituitary gland. Whereas, in our study LAP group seems to be more effective as compared to the SAP group in patients of progressive age group i.e. older than 40 years, in terms of salvaged mature oocytes, high grade embryos as well as high implantation and pregnancy odds.

Conclusion

In conclusion, our study demonstrated that irrespective of patient's age, the long acting protocol group was more reliable and fertile with reference to number of mature oocytes retrieved, time period for stimulation, total dose of GnRH-a during controlled ovarian hyperstimulation, embryo grading, fertilization and pregnancy outcome.

Declarations

Conflict of Interest:

The Authors declared no conflict of interest.

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Tables

Table 1: Contribution of factors in the male and female infertility of the two groups.

Variables	Short protocol group, N= 230	Long protocol group, N= 310	Over All, N= 540
Type of Infertility (%)			
Primary infertility	(178/230) 77%	(208/310) 67.09%	(386/540) 71.48%
Secondary infertility	(52/230) 23%	(102/310) 32.90%	(154/540) 28.51%
Etiology of infertility (%)			
Male Factors (%)			
Normozoospermia (>20) mill/ml	(67/230) 29.12%	(102/310) 32.9%	(169/540) 31.29%
Globozoospermia	(25/230) 10.8%	(36/310) 11.61%	(61/540) 11.29%
Asthenozoospermia	(19/230) 8.2%	(40/310) 12.9%	(59/540)10.92%
Oligozoospermia (>5-20) mill/ml	(70/230) 30.4%	(28/310) 9.03%	(98/540)18.14%
Severe Oligozoospermia (1-5) mill/ml	(25/230) 10.8%	(56/310) 18.06%	(81/540) 15%
Highly severe Oligozoospermia (<1) mill/ml	(24/230) 10.43	(48/310) 15.48%	(72/540) 13.33%
Female Factors (%)			
Normal	(42/230) 18.26%	(45/310) 14.51%	(87/540) 16.11%
Tubal Factor	(52/230) 22.6%	(58/310) 18.70%	(110/540) 20.37%
Endometriosis	(25/230) 10.8%	(20/310) 8.06%	(45/540) 8.33%
PCOS	(76/230) 33.04%	(78/310) 25.16%	(154/540) 28.51%
Fibroid (>2.0 cm)	(25/230) 10.86%	(89/310) 28.70%	(114/540) 21.11%
Unexplained	(10/230) 4.34%	(20/310) 4.83%	(30/540) 5.55%
Fertilization Method (%)			
ICSI	(185/230) 80.43%	(278/310) 89.67%	(463/540) 85.74%
IVF	(45/230) 19.56%	(32/310) 10.32%	(77/540) 14.25%

Note: PCOS = Polycystic Ovary Syndrome; mill/ml = Millions/milliliter; ICSI = Intracytoplasmic sperm injection; IVF = In Vitro Fertilization.

Table 2: Characteristics of patient receiving Controlled ovarian hyper-stimulation in Short and long protocol groups presented as mean ± SD.

Factors	Short protocol group, N= 230	Long protocol group, N= 310	*P*-value
No. of Cycles	230	310	-
Age (Years)			
≤30	42	84	-
31-35	58	119	-
36- 40	80	51	-
>40	50	56	-
BMI (Kg/m^2)			
Age (Years)			
≤30	22.15 ± 2.54	22.61 ± 1.35	NS
31-35	25.88 ± 2.13	24.99 ± 2.97	NS
36- 40	28.46 ± 2.19	28.92 ± 2.58	NS
>40	29.38 ± 2.98	29.14 ± 3.51	NS
Mean period of infertility (Years)			
≤30	2.43 ± 2.63	3.58 ± 1.96	NS
31-35	5.38 ± 1.63	4.99 ± 2.92	NS
36- 40	7.10 ± 4.15	7.29 ± 4.98	NS
>40	8.71 ± 6.32	9.12 ± 5.63	NS

Note: NS = Non-significant.

Table 3: Comparison of clinical data presented as mean ± SD of the two groups.

	Short protocol group, N= 230	Long protocol group, N= 310	*P*-value
Overall Gn dosage (IU)			
≤30	1530.03 ± 573.25	2089.52 ± 569.53	<0.05
31-35	1748.27.1 ± 632.45	2328.98 ± 692.36	<0.05
36- 40	2029.21 ± 706.56	2619.35 ± 705.98	<0.05
>40	2315 ± 596.74	3036.37 ± 633.21	<0.05
Total Duration of stimulation (days)			
≤30	8.49 ± 1.63	11.52 ± 1.96	<0.05
31-35	8.73 ± 1.98	11.31 ± 1.33	<0.05
36- 40	8.81 ± 1.54	11.78 ± 1.55	<0.05
>40	8.91 ± 1.23	11.02 ± 1.23	<0.05
Antral Follicle count			
≤30	13.36 ± 2.61	15.32 ± 3.69	<0.05
31-35	10.36 ± 1.23	12.86 ± 2.10	<0.05
36- 40	7.56 ± 2.13	10.36 ± 2.63	<0.05
>40	3.53 ± 1.25	5.53 ± 1.05	<0.05
E2 level of each follicle on hCG trigger day (pg/ml)			
≤30	197.41 ± 51.2	198.01 ± 65.3	NS
31-35	166.23 ± 96.3	175.94 ± 21.7	<0.05
36- 40	152.31± 63.45	160.94 ± 98.5	<0.05
>40	147.98 ± 98.64	148.36 ± 101.3	NS
No. of oocytes retrieved			
≤30	8.31 ± 5.21	14.03 ± 3.25	<0.05
31-35	7.21 ± 5.96	11.36 ± 2.36	<0.05
36- 40	5.21 ± 3.21	9.56 ± 3.64	<0.05
>40	4.54 ± 3.99	6.32 ± 1.03	<0.05
MII oocytes retrieved			
≤30	7.65 ± 3.21	10.32 ± 3.25	<0.05

31-35	6.14 ± 2.59	9.69 ± 2.36	<0.05
36- 40	5.10 ± 2.15	5.72 ± 2.16	NS
>40	3.31 ± 3.21	3.36 ± 2.31	NS
Good quality embryos			
≤30	4.02 ± 2.01	6.38 ± 3.98	<0.05
31-35	3.21 ± 1.95	5.13 ± 3.51	<0.05
36- 40	2.11 ± 1.64	4.02 ± 2.91	<0.05
>40	1.01 ± 1.48	2.01 ± 1.03	NS

Note: NS = Non-significant; hCG = Human chorionic gonadotropic.

Table 4: Evaluation of serum endocrine levels of the two groups presented as mean ± SD.

Variables	Short protocol group, N= 230	Long protocol group, N= 310	*P*-value
Basic serum endocrine level			
2rd day FSH (IU/L)	8.17 ± 2.51	8.59 ± 2.32	0.34*
2rd day LH (IU/L)	5.01 ± 1.33	5.19 ± 1.98	0.31*
2rd day E2 (pg/L)	50.48 ± 18.96	54.84 ± 49.68	0.81*
AMH (ng/mL)	4.99 ± 1.81	5.87 ± 2.10	0.39*
Serum endocrine level on Gn stimulation day			
FSH (mIU/L)	2.36 ± 1.89	3.99 ± 2.01	0.0001*
LH (mIU/L)	1.68 ± 1.01	1.96 ± 1.01	0.003*
E2 (pg/L)	20.31 ± 11.01	25.01 ± 10.35	0.000*
Serum endocrine level on hCG decision day			
Progesterone (ng/L)	0.89 ± 0.58	0.88 ± 0.49	0.36*
LH (mIU/L)	1.02 ± 0.16	2.14 ± 0.35	0.001*
E2 (pg/L)	2879.51 ± 11.25	3178.21 ± 12.36	0.000*
Progesterone/Estradiol	0.41 ± 1.99	0.50 ± 2.01	0.19*
Endometrium thickness on the day of the hCG (mm)	9.16 ± 1.36	13.03 ± 2.36	0.001*

Note: * = Student's t-test; FSH = Follicle stimulating hormone; Gn = Gonadotropin; LH = Luteinizing hormone; E2 = Estradiol; hCG = Human chorionic gonadotropic; AMH = Anti-Mullerian hormone.

Table 5: Evaluation of clinical outcomes of short and long protocol groups.

Variables	Short Protocol group, N= 230	Long Protocol group, N= 310	*P*-value
Fertilization rate (%)	76.91	75.98	0.721*
Cleavage rate (%)	90.31	91.04	0.632*
Implantation rate (%)	31.56	35.41	0.000*
Clinical pregnancy (%)	28.23	33.12	0.001*
Cycle cancellation (%)	15.23	8.43	0.001*
Ovarian hyperstimulation syndrome (%)	3.21	6.21	0.002*
High quality embryo (%)	62.01	65.54	0.001*
Early abortion (%)	5.523	5.43	0.791*
Miscarriage rate (%)	3.21	3.34	0.541*
Ectopic pregnancy rate (%)	3.01	3.21	0.249*

Note: Data are presented as n/N (%); * = χ^2- test

Figures

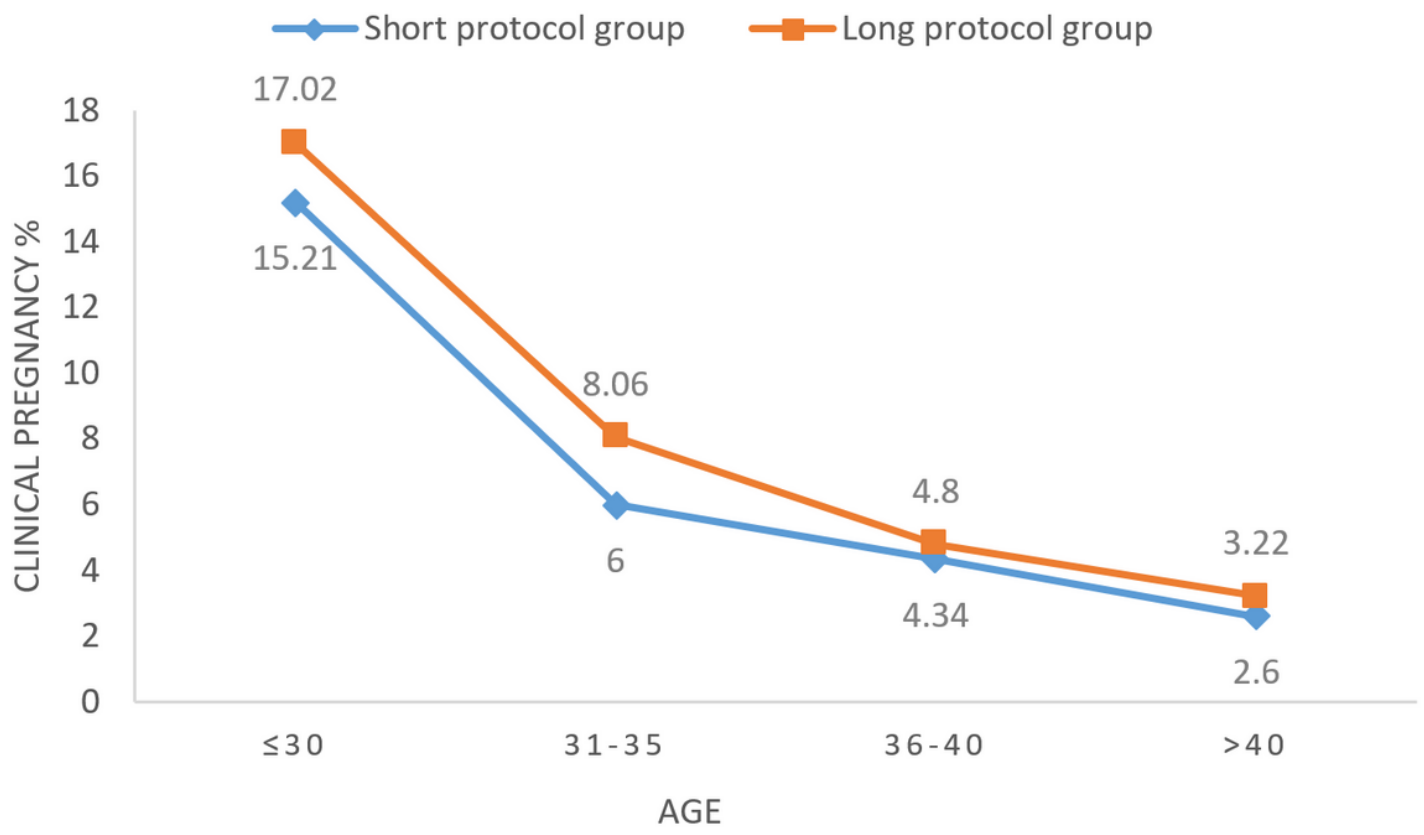

Figure 1

A comparison between clinical pregnancy rates and age of the patients. In the long acting protocol group the clinical pregnancy rates of the four age groups were 17.02%, 8.06%, 4.8% and 3.22%respectively, which were significantly higher as compared to the short acting protocol group (15.21%, 6.0%, 4.34% and 2.6% respectively: <0.05. However, pregnancy rates decreased as age increased in both protocols.

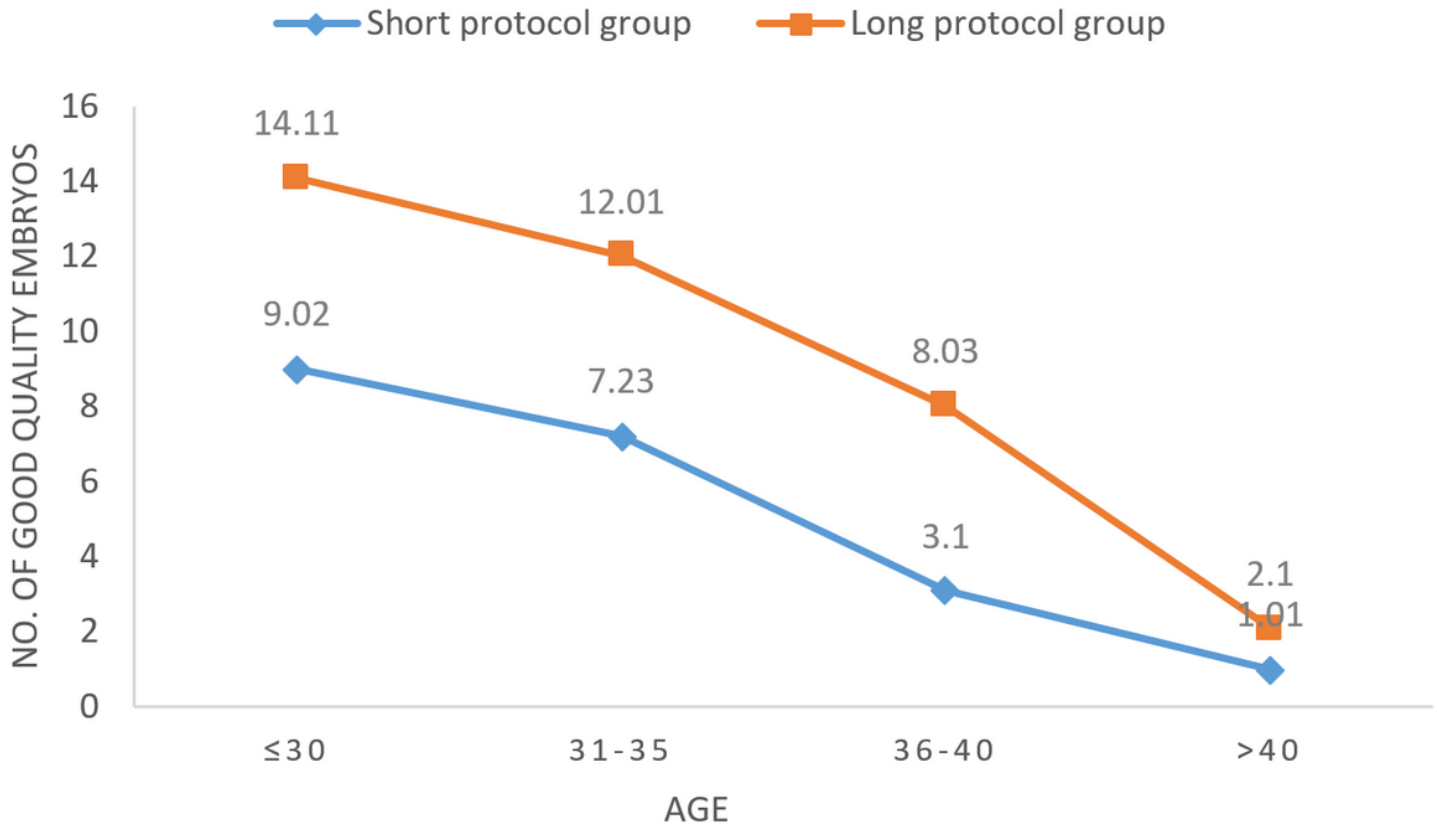

Figure 2

A comparison between good quality embryos and age of the patients. The number of good quality embryos were significantly high in the long acting protocol group in comparison with short acting group for all age groups and significantly (p<0.05), decreased as age increased.