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Modified non-pituitary down regulation protocol for ovulation induction in ART (IVF & ICSI)

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ABSTRACT

Infertility is a unique medical condition because it involves a couple rather than a single individual. Both the prevalence of infertility and the number of patients seeking the treatment of this disorder are increasing. Although it is not a physically debilitating disease; infertility may severely affect the couples psychological, sexual and social life.

From literature we found that agonist protocol has taken long time in treating the patient and not used for polycystic patient because of risk of hyperstimulation while antagonist protocol take less time and less drug cosumed and has advantages in polycystic ovaries patient,. In our study on 241 patients who admitted for ICSI and started their ovarian stimulation by HMG or FSH without pituitary down regulation protocol + single dose orgalutran (GnRH antagonist Ganirelix acetate) we find that pregnancy outcome same as in the other protocols with less side effects, good quality and excellent embryos, with low cost to the patient.

Introduction:

Infertility is one of the most concerning health problems facing all societies. It is estimated that about 70% of couples will be affected by infertility by 2025(1). The National Institute for Health and Care Excellence (NICE) recommends in-vitro fertilization (IVF)as the definitive treatment for prolonged unresolved infertility after other treatments have failed. IVF may be used to overcome female or male fertility problems (NICE 2013).

controlled ovarian stimulation comprises three basic elements.

Exogenous gonadotrophins to stimulate multi follicular development.

Cotreatment with either gonadotropins -releasing hormone (GnRH)agonist or antagonists to suppress pituitary function and prevent premature ovulation. Triggering of final oocyte maturation 36 to 38 hrs prior to oocyte retrieval (2).

Problems of ovarian stimulation include; poor responders, hyperstimulation, premature LH surge.

The main aim of introducing the agonists to IVF protocol was to prevent the premature LH surge.

synthetic agonist increases stability and binding affinity to its receptors 100-200 times higher. GnRH agonist first synthesized in 1972 (3).

It acts through Down regulation (Desensitization); When the GnRH receptors exposed to GnRH agonist for a prolonged period, the cell lose their ability to respond to the stimulus with their original sensitivity.

These analogues have certain amino acids substitutions in the gonadotropin amino acid sequence that increases the half-life and competencies of analogues compared to natural hormones (4-6).

GnRH agonists allows sustained stimulation of gonadotropin secretion, while GnRH antagonists act as mediators of chemical hypophysectomy (7).

Process is rapid and reversible.

GnRH is released in a pulsatile fashion but a Continuous supply of GnRH suppresses gonadotrophin secretion by desensitization of gonadotrophs.

Overall, both analogues are widely used in IVF to induce folliculogenesis via prevention of endogenous LH surge and timed oocyte retrieval (8,9).

Among the various GnRH agonist long protocols, namely ultrashort, short and long, the long GnRH agonist protocol has been used as the gold standard in IVF since its discovery in the 1980s (8,10). The recent development of GnRH antagonists has offered an alternative approach in IVF treatment with no significant difference in pregnancy outcome. The use of antagonist protocol to be safer in PCO patients.

In our study we are using ovarian stimulation protocol by HMG purified type (Diaclare HMG from BBT/Germany purified type) then add single one dose of GnRH Antagonist just 24 hours before HCG adminstration. We have almost same result according to the quality of Eggs, Embryos and pregnancy outcome. The difference in this new protocol (non-pituitary down regulation + one dose GnRH antagonist) are a smaller number of gonadotropin use (HMG, FSH), single dose antagonist, less day to reach the Eggs retrieval and Embryo transfer with good pregnancy outcome.

Aim of the study:

To prove that using of Orgalutran (GnRH Antagonist Ganirelix acetate) will reduce the risk of premature LH surge and give good number of eggs with good quality and excellent embryos, with less cost to the patient.

This addition to our previous Non-Pituitary Down Regulation protocol for ovulation induction in ART.

The pregnancy outcome has no difference from other protocols.

Patients and method:

Prospective study for patients coming for (ART) (ICSI) from 2.1.2023 to 26.12.2023.

Age of patients 20-39 yrs old.

The total number of patient 241 patients.

Site: Lamis IVF center Misurata Libya.

All patients had purified HMG or purified FSH for polycystic ovary (PCO) patients, all HMG and FSH are coming from BBT/ Germany named Diaclare highly purified type. Started at 3rd day of cycle with 300 IU/IM Diaclare highly purified HMG or FSH on daily dose for seven days.

Vaginal U/S on day 7 of cycle (day 5 of starting injection of HMG or FSH) done and repeated on

day 9 or 10 of cycle to measure folliclular size.

Orgalutran 0.25 mg sc injection was given when the leading follicles reaching 1617 mm in diameter (between 12 to 24 hours before HCG injection) in addition to the dose of Hp HMG.

When the leading follicle diameter 18mm 10,000 IU/ IM of highly purified HCG from Diaclare/ BBTis given.

At 34-35 hours from the injection of HCG, pick up of eggs were performed.

Immediate assessment of eggs in number and quality, all eggs classified by our Embryologist to GV, M1, and M2. In this study we were injecting eggs with sperm at stage M1, and M2 by this the ICSI procedure is completed the injected eggs are kept in the embryo incubator. Any egg with Grade 3 or 4 were not for sperm injection so we complete the ICSI procedure on G1 and G2. In our Andrology laboratory in IVF center we try to get the best sperm in the semen sample after complete preparation. The sperms can be fresh sample or from cryo sample.

The Embryo transfer should be at morula or blastocyst stage on day 4 or 5 of pick up time. The transfers Embryos have to be not more than three in number per patient, who aged 20-35 years old only one embryo transfer; sometimes two embryo transfer on patient request. All others we transfer only two Embryos.

We grade Embryos before transferred in our policy to G1 and G2 we don't transfer G3 or G4. We avoid transfer any Embryo with delay in division or stopped growing at any time so that it has to reach morula stage or Blastocyst. The Embryos loaded in the catheter for transfer by the embryologist and clinition complete the transfer to patient`s uterus without anesthesia, we are using labotech, cock or ketazato type of cathether. Our policy to give progesterone vaginal pessary as luteal support from the day of Embryo transfer for 10wks when there is pregnancy going on clinically.

The Results:

Table l

Age of patients/ total number

Ages	20-24	25-29	30-34	35-39
Numb. Of patients	19	41	71	110

Table ll

Number of eggs collected

1-4 eggs	5-10 eggs	11-15 eggs	16-20 eggs	>20 eggs
86	79	34	11	12

Table lll

The distributions in patients without eggs 3

Ages	20-24	25-29	30-34	35-39
Numb. Of patients With no eggs	1	1	7	10

Table lV

Type of eggs

Total numb. Of eggs	Good quality were injected by sperm	Poor eggs were not injected
1588	1282	306

Table V

Presence of polycystic ovaries in this study is

Total number	Polycystic ovaries patients
241	57

Table Vl

Polycystic ovaries distributions

Ages	20-24	25-29	30-34	35-39
Numb. Of Polycystic ovary patients	8	18	21	10

Table Vll

Embryo transfer +

Excellent embryo	442
No Embryo transfer	39

The summary of the results:

- Prospective study from 2.1.2023 till 26.12.2023.
- The total pick up 241 patients.
- Patients with no eggs 19 (7.88%).
- Good quality eggs 1282 (80.7%).
- Poor quality eggs 306 (19.2%).
- Total patients who had no E.T 39 (16.1%).
- Total no of excellent embryo 442 (91.8%).
- polycystic patients are around (24%)

Discussion:

The ideal ovarian stimulation regimen for IVF should have a low cancellation rate; minimize drug costs, risks, and side effects; require limited monitoring for practical convenience; and maximize cumulative live birth rate per oocyte retrieval procedure, that is, cumulative chances of one or more live births after exhausting all fresh and frozen embryos generated from a single oocyte retrieval. Singleton pregnancy delivered at term is the ultimate goal of ART. This we try to do in our study.

The first birth resulting from IVF derived from a single oocyte collected in a natural ovulatory cycle (11). Compared to stimulated IVF cycles, natural cycle IVF offers a number of attractive advantages.

Natural cycle IVF involves only monitoring the spontaneous cycle and retrieving a single oocyte before the midcycle LH surge occurs. It is physically less demanding, requires little or no medication, decreases costs, (12,13), and all but eliminates risks for multiple pregnancy and OHSS. The chief disadvantages of natural cycle IVF are high cancellation rates due to premature LH surges and ovulation and the comparatively low success rate of approximately 7% (14). because of this we didn't include the natural cycle in our study.

Clomiphene citrate was the first method of ovarian stimulation used in IVF, (15,16) but now has been almost entirely replaced by more effective stimulation regimens using human menopausal gonadotropins HMG or FSH, in combination with a GnRH agonist or antagonist (17).

Drug costs and monitoring requirements are moderately higher but still substantially less than in standard stimulation regimens involving higher-dose gonadotropin treatment after down-regulation with a long-acting GnRH agonist (18,19).

In one comparative trial, higher cancellation rates and lower pregnancy rates were observed in sequential clomiphene/gonadotropin compared to gonadotropin/GnRH agonist cycles (19).

Also, we try before this study with letrazole tablet altogether with gonadotropins we find no more difference with gonadotropins alone for older age.

The introduction of long-acting GnRH agonists in the late 1980s revolutionized the approach to ovarian stimulation in ART by providing the means to suppress endogenous pituitary gonadotropin secretion and thereby prevent a premature LH surge dur-

ing exogenous gonadotropin stimulation. Adjuvant treatment with a GnRH agonist eliminated the need for frequent serum LH measurements and assuaged fears of premature luteinization, which previously had required cancellation of approximately 20% of all IVF cycles before oocyte retrieval (20,21,22) Because fewer than 2% of cycles are complicated by a premature LH surge after down-regulation with a GnRH agonist (23) stimulation could continue until follicles were larger and more mature.

Its disadvantages are that GnRH agonist treatment sometimes blunts the response to gonadotropin stimulation and increases the dose and duration of gonadotropin therapy required to stimulate follicular development.

In 2015, 11% of stimulation cycles were cancelled before oocyte retrieval, most for lack of adequate response, and some for excessive response (24,25) When the ovaries become grossly enlarged, containing large numbers of follicles of all sizes, and serum estradiol concentrations are markedly elevated (>5,000 pg/mL), the risk for OHSS increases substantially (26,27,28).

In our protocol only, short time is needed for treatment and follow up.no cancellation cycle although 7.8% of our patient have no eggs picked up which could be because of LH surge.

The “short” or “flare” protocol is an alternative stimulation regimen designed to exploit both the brief initial agonistic phase of response to a GnRH agonist and the suppression that results from longer -term treatment (29,30)

GnRH antagonists offer several potential advantages over agonists. First, the duration of treatment for an antagonist is substantially shorter than for an agonist. Since its only purpose is to prevent a premature endogenous LH surge and its effects are immediate, antagonist treatment can be postponed until later in follicular development (after 5–6 days of gonadotropin stimulation), after estradiol levels are already elevated, thereby eliminating the estrogen deficiency symptoms that may emerge in women treated with an agonist (31).

Second, because any suppressive effects that agonists may exert on the ovarian response to gonadotropin stimulation also are eliminated, the total dose and duration of gonadotropin stimulation required is decreased (31,32).

In our protocol still, the duration of time is shorter and amount of drug used is less than this protocol.

The two GnRH antagonists available for clinical use, ganirelix and cetrorelix, are equally potent and effective. For both, the minimum effective dose to prevent a premature LH surge is 0.25 mg daily, administered subcutaneously (33,34) The treatment protocol may be fixed and begin after 5–6 days of gonadotropin stimulation (33,34,35) or tailored to the response of the individual, starting treatment when the lead follicle reaches approximately 13–14 mm in diameter(36,37) Alternatively, a single larger dose of cetrorelix (3.0 mg) will effectively prevent an LH surge for 96 hours. If given on day 6–7 of stimulation, the interval of effective suppression will encompass the day of hCG administration in most women (75–90%); the remainder may receive additional daily doses (0.25 mg) as needed, ending on the day of hCG treatment (38,39,40) The single-dose antagonist treatment regimen also can be withheld until the lead follicle reaches 13–14 mm in diameter (41).

In our protocol smaller, single dose of GnRH antagonist is given which means cost less to the patient.

In our study 45.6% of patients aged between 35-39

yrs and only 9% of them have no eggs.

80.7% of eggs collected are of good quality although our patient age ranges from 20 to 39 years old

Although 24% of our patient is polycystic ovaries no case of hyperstimulation is recorded.

Our protocol give all of the advantages of ART protocols in the same time has more advantage in cost benefit, less drugs used, less time consumed, for treatment.

Conclusion:

Our conclusion of this study has the advantage of low cost for the patient ,shorter time for pick up ,and give good result as in other protocols . There is no difference between it and agonist and & antagonist protocols in quality of eggs and in pregnancy rate. It also has the advantage of no case of hyperstimulation recorder although we used in polcystic ovaries patient These results have value for IVF&ICSI treatments.

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