

Non-Pituitary Down Regulation Protocol for Ovulation Induction in ART (IVF & ICSI)

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ABSTRACT

There significant improvement has been seen in the pregnancy outcome, in the introduction of many ovarian stimulation protocols. The use of pituitary down regulation protocol gives better result in the quality and number of oocytes which leads to more Embryos and more Embryos to be freezed for transfer to the patient uterus latter.

From literature we found that pregnancy outcome in minimal stimulation protocol gives almost the same result, especially in advanced patients age with less side effect. In our study on 1652 patients who admitted for ICSI and started their ovarian stimulation by HMG or FSH without pituitary down regulation protocol. This protocol gives the pregnancy outcome same as in the other protocols with less side effects and low coast.

Keywords

Ovarian stimulation, Pituitary, Fertilization, IVF, ICSI.

Introduction

ART (IVF/ ICSI) is a multistep process involving ovarian stimulation, ovulation induction, collection of oocytes, fertilization with sperm, and transfer of the fertilized oocytes to the uterus for implantation and maturation. Each stage must be carefully controlled via the administration of medications. At each stage, there are different protocols for the use of these drugs, and the most appropriate pharmacological regimen and therapeutic intervention are chosen after a thorough pretreatment evaluation and an accurate diagnosis [1]. Controlled ovarian stimulation is achieved with the use of gonadotropin-releasing hormone (GnRH) analogues and antagonist. Inhibitors of natural steroid hormones, such as clomiphene citrate, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) pituitary downregulation.

In 1980s, the use of GnRH agonists as downregulation resulted in increased pregnancy rates per cycle. This method has been improved over the years, with current pharmacological regimens and retrieval techniques yielding large numbers of oocytes [1]. GnRH agonists and, more recently, GnRH antagonists are used to initiate pituitary downregulation.

GnRH agonist and antagonist protocols utilize agonistic or antagonistic analogues of GnRH. GnRH analogues are decapeptides designed after human GnRH in order to interact with GnRH receptors. 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 [2-4]. GnRH agonists allows sustained stimulation of gonadotropin secretion, while GnRH antagonists act as mediators of chemical hypophysectomy [5]. Overall, both analogues are widely used in IVF to induce folliculogenesis via prevention of endogenous LH surge and timed oocyte retrieval [6,7]. 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 [6,8]. 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.

Minimal stimulation protocol

Clomiphen citrates is an estrogen receptor modulator and a competitive inhibitor of oestradiol, which has been used for fertility treatment since the last four decades [9]. The anti-estrogenic property of CC is the main drawback of this treatment. However, it was later discovered that the antiestrogenic property may cause suppression of the premature LH surge that is responsible for

maintaining folliculogenesis [10]. Minimal stimulation protocol utilizes CC in conjunction with human menopausal gonadotropin (HMG), is more effective compared to administering HMG alone [9-11].

Letrozole, an aromatase inhibitor is used alternative to clomiphene citrate for minimal stimulation protocol in some clomiphene resistant patients. These two protocols of pituitary down regulation give more numbers of eggs and more embryos.

In the same time multiple pregnancies increased for the mother and community. These tow protocol which are pituitary down regulation protocol lead to an increase in the consumption of gonadotrophin (HMG & FSH) and longer time to reach egg retrieval. The main aim of down regulation protocol is to avoid premature surge of LH and give good mature eggs.

In our study we are not using pituitary down regulation (agonist or antagonist) we are using ovarian stimulation protocol by HMG purified type (Diaclare HMG from BBT/Germany purified type).

We have almost same result if not better about the quality of Eggs, Embryos and pregnancy outcome. The difference in this new protocol (non pituitary down regulation) are a smaller number of gonadotropin use (HMG, FSH) less day to reach the Eggs retrieval and Embryo transfer with good pregnancy outcome.

Aim of the study

To prove the using of this new protocol will give good number of eggs with good quality and excellent embryos. The pregnancy outcome has no difference from other protocols.

This study should prove that consumption of gonadotropins is less and the time of ovulation induction is less so less cost effect benefit.

Patients and Method

Prospective study for all patients coming for (ART) (ICSI) from 1st of January 2016 till 31 December 2018 3 years. Age of patients 20-44 yrs old. The total number of patients: 1652 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 purified type.

Started at 3rd day of cycle with 300 IU/IM Diaclare 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). Repeat vaginal U/S on day 9 or 10 of cycle depends on follicle size (Diamete).

No antagonist or agonist was given in this protocol. When the leading follicle diameter 16-18mm for who has one follicle, and in three or more follicles who have multiple follicles 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 cryosample (we allow looking for normal forms and mobile sperms). 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 more than 30 years old. If the patients age less than 30yrs old 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 cather. 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

Prospective study from 1st January 2016 till 31December 2018 over 3years.

The total pick up = 1652 patients.

Total patients of these who had E.T = 1406.

The rate of fertilization in total patients were 72.5%.

Ages	20-24	25-29	30-34	35-39	40-44
Numb. of patients	358	450	510	250	84

Table 1: Age of patients / total number 1652.

1-4 eggs	5-10 eggs	11-15 eggs	16-20 eggs	>20 eggs
502	680	320	110	40

Table 2: Number of eggs collected.

Age	20-24	25-29	30-34	35-39	40-44
Numb. of patients with no eggs	3	2	3	4	8

Table 3: The distributions in patients without eggs.

Out of 1652 twenty patients have no eggs /Total 20 patients gave no eggs 1.2%.

Total numb. of eggs	Good quality was injected by sperm	Poor eggs were not injected
10920	9828	1092
	90%	10%

Table 4: Type of eggs.

Total number	Polycystic ovaries patients
1652	272

Table 5: Presence of polycystic ovaries in this study is 17%.

Age	20-24	25-29	30-34	35-39	40-44
Numb. of polycystic ovary	50	90	95	33	4

Table 6: Polycystic ovaries distributions.

Excellent embryo G1 and G2	1402	78%
No Embryo transfer	250	22%

Table 7: Embryo transfer.

The summary of the results

Fertilization rate in this study 72.5%

20 patients only 1.2% has no eggs

The presence of polycystic ovary between the 1652 patients equal to 17%

Patients have no ET 250 patients 22%

Pregnancy outcome 35%

Early abortion only 20 patients 2%

These results are equal to other protocols in our center.

Discussion

The use of minimal stimulation protocol antagonist protocol, agonist protocol or our non-pituitary down regulation protocol on each patient is usually based on the physician's experience and decision.

Usually the decision on the benefits of each protocol option and on the patient's response gonadotrophin stimulation based on coming response high responders, intermediate and poor responders [12,13].

Poor ovarian response occurred in 9-24% of all IVF/ICCSI cycles and is defined as decreased ovarian response with sufficient stimulation [14]. Malmusi et al. described poor responder as patient with a low number of oocytes (less than 4), and no ovarian response with HMG or FSH greater than 300 IU [15].

Poor response has been shown to be associated with advanced maternal age, this effects eggs quality and numbers. This can be present in some young patient, but the causes are unclear [12,16]. Although many studies are conducted to identify which protocol is suitable for patients. There is no definite consensus on the matter since each protocol comes with both benefits and limitations. Our protocol in this study has the same benefits with less or no limitation and you can use it for all types of patients, who requires or ask for ART (ICSI) as can be good in young and advanced age.

The main side effects of GnRH antagonist long protocol include longer treatment duration, more ampoules of gonadotropin, ovarian cyst formation, and menopausal syndromes. However, the antagonist protocol can overcome these side effects, but its disadvantage is low follicular production [17]. Furthermore, the antagonist protocol has lower pregnancy and implantation rate

because of low LH level and impaired estrogen secretion [18].

Another study concluded that the antagonist protocol produced high oocyte numbers is poor response [19]. Others studies have other implicated the antagonist protocol in the prevention of moderate or severe ovarian hyperstimulation syndrome (OHSS) in PCO patient [20,21]. The antiestrogenic effects of CC suppress the premature LH surge while maintaining a positive influence on follicular development. The minimal stimulation protocol is a convenient protocol, which uses significantly fewer gonadotropin ampoules. The number of gonadotropin ampoules used in this protocol is significantly lower than agonist (5.7 vs. 25) [22]. This protocol has resulted in less mature oocytes; consequently, lower chance of obtaining viable frozen embryos. However, the pregnancy and transplanted rate appeared to be similar with the agonist protocol [10,23]. This protocol is cost-effective for women with advanced age or for those with poor ovarian reserve compared to agonist or antagonist protocols. Additional studies have yielded a similar result when comparing the minimal stimulation protocol to GnRH agonist (i.e., CC and gonadotropin protocol was not as effective as agonist in yielding more oocytes but the transplanted and pregnancy rate were comparable between these protocols) [24,25]. This protocol seemed to be a better option in some patients, such as those with poor ovarian response, when considering its cost-effectiveness and low risk of OHSS [26].

Some other limitations of using gonadotropins and CC in IVF included the higher prospect of multiple pregnancies, which was associated with preterm delivery, growth retardation and miscarriage. Although the correlation between ovarian stimulation and low birth weight is still debatable since it could be the confounding effect of the infertility background of the couple [27]. Exposure of oocytes to the high levels of gonadotropins in their developing phase leads to improper maturation of oocyte as well as incomplete meiotic division which results in chromosomal aneuploidy [28]. A study in a mouse model showed an increased rate of chromosomal aberrations in the female pronucleus in zygotes formed by ovarian stimulation [20]. A similar study has also found an increased rate of aneuploidy in the chromosomes and mosaicism in an in vitro fertilized embryo [29,30]. Baart, et al. also concluded that the high dose FSH protocol caused a higher rate of mitotic segregation errors leading to mosaicism and hence abnormal embryos compared to the minimal stimulation protocol with low dose FSH [30]. Moreover, congenital malformations like ventricular septal defect, cardiac defects and chromosomal abnormalities were found in patients undergoing IVF using CC [31].

Our protocol gives all of the advantage of the named three protocols, in the same time has more advantage in cost benefit and has less time consumed for the treatment.

In our study with a good number of patients of different age from 20-44 yrs, old gives good pregnancy outcome 35% without any side effect especially hyperstimulation syndrome in PCO patients, without any effect on LH level and estrogen level.

Conclusion

From this study we can say the non-pituitary down regulation protocol is a good alternative to be used in ART (IVF/ICSI) in our opinion this protocol will get its way between physicians who manage patient require ART (IVF/ICSI).

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