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Original Research Article

Dual stimulation protocol for poor responders, promising approach to increase the success rate of in vitro fertilization cycles

Nagwan A. Bahgat[1,2]*

[1]Department of Obstetrics and Gynecology, Mansoura University Hospital, Mansoura Faculty of Medicine, Egypt
[2]Health Plus Fertility Center, Health Plus Group, Abu Dhabi, United Arab Emirates

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***Correspondence:**
Dr. Nagwan A. Bahgat,
E-mail: nagwanivm@yahoo.com

ABSTRACT

Background: It is well known that increasing the number of recruited oocytes affect positively the in vitro fertilization (IVF) cycle outcome. Dual stimulation protocol was invented to increase the number of recruited oocytes in the same cycle through double stimulation and oocyte collection.
Methods: This prospective randomized controlled study was done to compare the outcome of one conventional aspiration-embryo transfer cycle with double stimulation- aspiration then frozen transfer cycle. The results were divided into primary outcomes which included the number of oocytes collected, the maturation rate in both times of oocyte collection and in both groups and while the secondary outcome included the oocytes survival rate, fertilization rate, cleavage rate, available blastocyst, and finally pregnancy rate in both arms of the study.
Results: A total of 180 patients included. In the first group: 203 oocytes were collected with 151 mature oocytes (maturation rate 74%, fertilization rate of 81.5%), 61 good quality embryos and transferred in 27 cycles (49.6% transfer rate) and 4 got pregnant (14.8% pregnancy rate). In the second group: we got 381 oocytes in total through twice oocyte collection, with 289 mature oocytes (75.8% maturation rate). The second group underwent frozen oocytes transfer cycles. All oocytes were thawed: out of 289 frozen oocytes, 156 survived (53.9% survival rate), we obtained 6 pregnancies (26.1%).
Conclusions: Double stimulation protocol followed by double oocytes collection in the same cycle could result in more mature oocytes for patients with poor ovarian reserve who are willing for pregnancy or fertility preservation.

Keywords: Frozen cycles, IVF, Dual stimulation

INTRODUCTION

Poor responder patients considered a major challenge for the doctors treating them due to low oocyte yield and its consequences regarding the in vitro fertilization (IVF) cycle outcome. According to Bologna criteria we consider patients poor responders if at least two of the following three criteria had to be present to establish the definition: advanced maternal age (>40 years) or any other risk factor for poor ovarian response (POR); a previous POR (≤3 oocytes with a conventional stimulation protocol); and an abnormal ovarian reserve test (i.e. antral follicle count (AFC) less than 5-7 follicles or anti-Müllerian hormone (AMH) below 0.5-1.1 ng/ml).[1-3]

The difficulty of treatment included low number of oocytes and consequently low number of available embryos for transfer especially when blastocyst transfer is required. Recently more patients are in need for genetic testing of the embryos due to age factors, seminal parameters, recurrent miscarriages and genetic disease avoidance. With these requirements, there is increased demand for more oocytes and embryos available for biopsy which is difficult to be obtained in one stimulation cycle in those categories of patients.[4]

Several studies had been conducted to optimize the outcome of stimulation in this group of patients, including addition of growth hormone, dehydro-epiandrosterone, testosterone, advising patients for multivitamins and nutrients, and increasing the dose of stimulating drugs. But none of these additives proved its efficacy regarding the number of retrieved oocytes or the pregnancy rate.[5-7] Some clinician went through oocyte or embryo accumulation through several cycles which takes months in order to increase number of oocytes and embryos available for transfer especially if genetic testing is required which could possibly increases the pregnancy rate.[8] When we look at some poor responder cases at the time of oocyte aspiration, we noticed the presence of small to medium sized follicles which when aspirated will give immature oocytes, so we thought how to get benefit from these precious oocytes. By reviewing the literature we found that recent evidence indicates that follicular growth occurs in a wave-like fashion indicating that there are multiple follicular recruitment waves in the same menstrual cycle.[9] And previous studies had found that the existing antral follicles in the luteal phase could be stimulated and resulted in mature oocytes that fertilized and resulted in pregnancy.[10,11] From this point dual stimulation protocol was introduced by Kuang et al and called Shanghai protocol, aimed to double stimulate and double aspirate the ovaries in the same cycle in order to increase the number of oocytes retrieved and increase the chances for successful IVF in poor responders.[12,13] It also decreases the time needed to collect the desired number of oocytes for freezing in case of fertility preservation in cancer patients.[14] This protocol aimed to collect the medium sized follicles which if collected in the first time will results in immature oocytes and if left will turn to cysts, this is beside reduction of coast in case we decided to go for oocyte or embryo accumulation for preimplantation genetic testing.

In our study we decided to offer our poor responder patients this dual stimulation protocol and to evaluate its efficacy in regard to number of oocyte, its maturation and rest of cycle outcome in comparison to normal stimulated cycle with one oocyte aspiration.

METHODS

This study was carried out as prospective randomized controlled study over 1 year duration between June 2016 till end of May 2017 in a large private IVF center in Abu Dhabi, United Arab Emirates (UAE) (Health Plus IVF center).

Patients included were randomly divided in to two groups, the first group will carry on stimulation and oocyte retrieval then fertilization and transfer in the same cycle as per our standard antagonist protocol. Second group will be scheduled for dual stimulation protocol where they will undergo double stimulation, double oocyte retrieval and oocyte vitrification in this cycle (embryo freezing is not allowed in UAE). Then thawing, fertilization, culturing till day 5 and embryo transfer in the following cycle.

The primary outcome included the number of oocytes collected, the maturation rate in both times of oocyte collection and while the secondary outcome included the oocytes survival rate, fertilization rate, cleavage rate, available blastocyst and finally pregnancy rate in both arms of the study.

Patients included in this study were infertile patients diagnosed as poor responders according to bologna criteria. 180 patients included in this study after explanation and consenting. 90 patients were allocated randomly in each group.

Inclusion criteria included infertile patients, poor responders, aged 40 years old or less.

Exclusion criteria included patients above 40 years old, sever male factor and the cases that require preimplantation genetic screening or diagnosis.

Patients in the first group attended the clinic on their second day of their period to have transvaginal ultrasound to count the antral follicles, exclude ovarian cysts and to ensure endometrial shedding then to start on stimulation with recombinant follicle stimulating hormone (rFSH) with the maximum dose, follow up after seven days to ensure response and follicular growth then to start on gonadotropin releasing hormone (GnRh) antagonist and plane for oocyte collection day after triggering with human chorionic gonadotropins (HCG) when the leading follicle reached 17-18 mm. Oocyte collection will be done under conscious sedation and ultrasound guidance followed by fertilization the collected oocytes to be fertilized using the intracytoplasmic sperm injection with Olympus IX73 Narishige Takanome micromanipulator, then all fertilized oocytes to be kept in the Esco Medical MIRI bench top incubator and incubated in 6202 Sage 1-Step media for blastocyst culture till day 5, transfer after blastocyst assessment will be in the same cycle.

Patients in the second group will follow the same protocol of the first group except that ovulation will be triggered with GnRH agonist and first oocyte retrieval done and oocytes collected to be vitrified, on the same day of trigger the medium sized follicles will be documented (follicles 14 mm or less) , then patient will rest for two days and restart stimulation with highly purified human menopausal gonadotropins FSH/LH with proper dose for five days. Ultrasound were used to determine the follicular size and in this phase no GnRh antagonist were given, then triggering with HCG, oocyte collection carried out again in the same cycle with vitrification of all oocytes. In the following cycle patients will attend the clinic on second day of period for scan and to start on estradiol valerate 2 mg twice daily for 1 week. Transvaginal scan to be done to ensure proper endometrial thickness of 8 mm or more then to start on vaginal progesterone for 5 days. This time thawing of the frozen oocytes will take place on the same day of starting progesterone, then fertilization, incubation till day 5 and embryo transfer will be performed.

Both groups will have standard embryo transfer, selection of embryos and their number will be based on their age, embryo grading and our standard protocol with maximum of 2 embryos. Clinical pregnancy to be considered when by transvaginal ultrasound intrauterine gestational sac and cardiac activity were detected.

Ethical approval

This study was approved by research and ethical committee for health pulse network.

Statistical analysis

The primary outcome included the number of oocytes collected in each group, maturation rate. While secondary outcome included result of thawing of oocytes, fertilization rate, available blastocyst, pregnancy rate in each group. All the outcome will be expressed in average and mean, and a Chi-squared test (χ^2) was performed for comparison of categorical variables. P values <0.05 were considered significant.

RESULTS

A total of 180 patients were included in this study. 90 patients in each group with age ranged from 22-40 years old with mean age of 39.3. Their body mass index (BMI) ranged from 21 till 35 with mean BMI of 27.6.106 patients with primary infertility and 74 with secondary infertility.

Table 1: Demographic criteria of patients.

Demographic criteria	All patients	First group	Second group
Age	22-40 (39.3)	24-40 (39.9)	22-40 (38.1)
BMI	21-35 (27.6)	21-35 (27.1)	21-35 (27.7)
Primary infertility	106	63	43
Secondary infertility	74	27	47

In the first group, out of 90 patients included in this arm 2 cycles were cancelled due to no response, in 5 cycles egg retrieval was performed but no egg was obtained.

A total of 203 oocytes collected in 83 cycles with 151 mature oocytes (maturation rate 74%), fertilized 123 oocytes (fertilization rate of 81.5%), 61 good quality embryo and transferred in 27 cycles (49.6% transfer rate) and 4 got pregnant (14.8% pregnancy rate) (Table 2).

In the second group: 90 cycles were started, 1 OPU was cancelled due to no response, 9 OPU resulted in no oocytes, in the remaining 80 cycles we collected 196 oocytes with 149 mature oocytes (76% maturation rate). All vitrified, then all cases started second stimulation after 2 days rest.

Table 2: Outcome of cycles in the first group.

Cycles	Number/mean	Rate (%)
Cycles cancelled	2	1.1
Cycles without oocytes	5	5.6
Oocytes collected	203 (2.5)	-
Mature oocytes	151 (1.8)	74
Fertilized oocytes	123 (1.5)	81.5
Blastocyst	61	49.65
Cycles with transfer	27	32.5
Pregnancy	4	14.8

On the second phase of the stimulation 7 OPU were cancelled due to no response. In the rest of the cases all retrieval done was with oocytes, so from 73 cycles we get 185 oocytes (means 94.4% increase in the number of oocytes) with 140 mature oocytes (75.7% maturation rate). So in total we got 381 oocyte in the second group with 289 mature oocytes (75.8% maturation rate).

Table 3: Primary outcome of cycles in the second group.

Outcome	First oocyte collection (%)	Second oocyte collection (%)	Total (%)
Cycle cancelled	1 (1.1)	7 (8.75)	8 (8.9)
No oocytes collected	9 (10)	0	9 (10)
Days of stimulation	12±2	7±2	-
Oocyte collected	196 (2.45)	185 (2.53)	381 (4.2)
Mature oocytes	149 (76)	140 (75.7)	289 (75.8)

Second group underwent frozen oocytes transfer cycles. All oocytes thawed: out of 289 frozen oocytes 156 survived (53.9% survival rate), 119 fertilized (76.3% fertilization rate) 56 good quality embryos and transferred in 23 cycles (31.5% transfer rate), and we were obtained 6 pregnancies (26.1%). No miscarriages detected in both groups. Two twins in the second group.

Table 4: Secondary outcome of cycles in second group.

Outcome	Number/mean	Rate (%)
Frozen oocytes	289 (3.9)	-
Oocytes survived after thawing	156 (2.1)	53.9
Fertilized oocytes	119 (1.6)	76.3
Blastocyst	56	47.1
Cycles with transfer	23	31.5
Pregnancy	6	26.1

Table 5: Primary outcome of cycles in both groups.

Outcome	First group (%)	Second group (%)	P value
Cycles cancelled	2 (1.1)	8 (8.9)	0.531168
Cycles without oocytes	5 (5.6)	9 (10)	0.919347
Oocytes collected	203 (2.5)	381 (4.2)	0.000026
Mature oocytes	151 (1.8)	289 (3.95)	0.000033

Table 6: Secondary outcome of cycles in both group.

Outcome	First group (%)	Second group (%)	P value
Survived oocytes	-	156 (2.1) (53.9)	-
Fertilized oocytes	123 (1.5) (81.5)	119 (1.6) (76.3)	0.642919
Blastocyst	61 (49.7)	56 (47)	0.861097
Transfer cycles	27 (32.5)	23 (31.5)	0.921893
Pregnancy	4 (14.8)	6 (26.1)	0.418665
Miscarriages	0	0	-

DISCUSSION

This study was conducted to evaluate the efficacy of dual stimulation protocol in the treatment of poor responders and if it may result in improvement of the outcome of IVF cycles in this group of patients, also we aimed to reduce wasting of medium sized follicles with their precious oocytes and to reduce time and coast of those patients to get pregnant or to freeze their oocytes before treatment as in cancer patients. By analyzing the results of our study, we found that there is great and significant improvement of the primary outcome in favor of dual stimulation protocol that the number of recruited oocytes had increased by 94% on the second oocyte collection and the total oocytes collected was almost double the oocytes collected in the cycles of the first group not only the number but also the mature oocytes collected in the second group was almost double the mature oocytes collected in the first group. These results were with agreement with study done by Kuang et al in 2014, but not in study done by Zhang et al who found that the maturation rate of oocytes collected from stimulation in the luteal phase is less than maturation rate of oocytes collected in the follicular phase.[15] In that study they started the stimulation with minimal stimulation in contrary to our study we started with the maximum dose of rFSH and on the luteal phase stimulation we used highly purifies human menopausal gonadotrophins at maximum dose to enhance the number of obtained oocytes and lower the incidence of cycle cancellation due to no response, although we used the maximum dose of medications in dual stimulation group we did not notice any complication from the medication or the double oocyte collection regarding anesthesia ,surgery or postoperative pain and infection. The other difference from the original Shanghai protocol was that we used GnRH antagonist in the first phase of stimulation to prevent premature ovulation while they used ibuprofen only and we get the same results, in the second phase of stimulation we did not use any medication to stop the premature ovulation and we didn't have any premature ovulation also. In the original protocol used progesterone to delay the menstruation but we did not use any medication to delay menstruation. We started the stimulation after 2 days to decrease the days of stimulation in the second phase of stimulation with the almost the same results they got in the original protocol.

In our study we noticed that the incidence of cycle cancellation and cycles without oocytes was the same as conventional stimulation in the first group which eliminated the worry about more exposure to hormones without benefit in case of cancellation.

On the other hand, we noticed that the secondary outcome of dual stimulation was remarkably reduced and showed no significant rise in relation to conventional simulation and single oocyte collection. We attributed this drop in the outcome to oocyte vitrification and the result of thawing as we are not allowed to freeze embryos by UAE law, we got only around 50% survival of all oocytes vitrified in the second group which was very low survival rate in comparison to study done by Keskintep et al and study of Cobo et al, which brought to our mined questioning the quality of oocytes obtained from luteal phase stimulation but this was answered by the study done in the Shanghai protocol by Kuang et al were they had euploid embryos that result in pregnancy and lives births later on, and the difference was that they could freeze embryos which definitely had better survival rate, in spite of having the secondary outcome in the second group decreased, yet the fertilization, blastocyst rate and pregnancy rates was not significantly affected in relation to first group, this is in agreement with study done by Cecillia et al in who found no significant increase in the number of blastocyst and euploid embryos in both groups.[16-18]

In our study we got pregnancy rate higher in the group of double stimulation than the group of one collection but it was non-significant increase, this increase is attributed to the increase of the number of oocytes retrieved and it may be more significant if we had embryo freezing. Also, the increase in pregnancy rate may be due to frozen oocyte transfer which gives us better way to prepare the endometrium.

CONCLUSION

Double stimulation protocol followed by double oocytes collection in the same cycle could results in more mature oocytes, save time and coast for patients of poor ovarian reserve who are welling for pregnancy or fertility preservation. Different protocols for double stimulation

could be used. No increased complications from medication or surgical procedure reported. The limitation of our study is that a larger studies are needed to evaluate the outcome of this way of treatment in poor ovarian reserve patients.

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