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ORIGINAL SCIENTIFIC ARTICLE

Ultrasound-based Decision Making on Stimulation Protocol for IVF Cycles

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ABSTRACT

The essential quality of a successful assisted reproductive technology cycle is selection of the correct stimulation protocol for individual patient. This in our study is done by combining ultrasound parameters with the basic biometric parameters [age and body mass index (BMI)] of individual patient to tailor the stimulation protocol for every patient. The parameters used on ultrasound were ovarian size (volume), number of antral follicles, ovarian stromal resistance index and peak systolic velocity (PSV). We developed a scoring system using the above-mentioned parameters. The score of the patient was used to calculate the dose of gonadotropins for ovarian stimulation in *in vitro* fertilization (IVF) cycle. In our experience, this scoring system has been proved to be very effective for optimum stimulation that would significantly reduce the incidence of ovarian hyperstimulation syndrome (OHSS) and also reduce the multiple pregnancy rates to acceptable levels.

Keywords: ART cycles, Stimulation protocol, Ultrasound scores.

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INTRODUCTION

The essential quality of a successful assisted reproductive technology (ART) cycle is selection of the correct stimulation protocol for individual patient. The aim is to select a dose of gonadotropin that would not cause ovarian hyperstimulation or may not even result in understimulation so that there are inadequate follicles grown up at the end of stimulation for an affordable *in vitro* fertilization (IVF) cycle. The morbidity and mortality associated with ovarian hyperstimulation syndrome (OHSS) makes it the most dreaded complication. Several studies have been done to decide stimulation protocols based on ultrasound parameters, age, biochemical tests, etc. Choosing the appropriate gonadotropin dosage to retrieve optimum number of oocytes involves multiple individual patient variables.[1] We have used ultrasound parameters chiefly with age and body mass index (BMI) to decide stimulation protocols in intrauterine insemination cycles.

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MATERIALS AND METHODS

This is a prospective study of 530 patients recruited over a period of 5 years from January 2010 to December 2015. This study was approved by the institutional ethical committee. After initial assessment and counseling, patients were educated and informed about the study. They were explained about the existing standard protocols and also about the study protocols. Advantages and disadvantages of both the protocols were discussed with every patient. They were allowed to take a decision as to whether they would be willing to participate in the study. A total of 495 patients agreed to be a part of the study.

Prestudy Work-up: Inclusion and Exclusion Criteria

Patients who consented to participate in the study were subjected to transabdominal scan to rule out any abdominal pathology. Transvaginal scan including Doppler and volume ultrasound was done to establish that the uterus was normal. Pathologies of the uterus that were suspected of affecting implantation were corrected. Hydrosalpinx if present was operated (tubectomy). A persistent ovarian cyst of larger than 3.5 cm was aspirated if it was simple. If it was a complex cyst (nonphysiological), it was treated by laparoscopic surgery.

Females with unexplained infertility, dysovulatory infertility (including polycystic ovarian syndrome), tubal factor infertility, mild to moderate male factor infertility, and mild endometriosis were included in the study. Only fresh and stimulated cycles were included for the study.

Those with moderate to severe endometriosis, those over 45 years of age and less than 20 years of age, ovum or embryo recipients, frozen embryo transfer cycles, and those with other endocrinal disorders (thyroid dysfunction, adrenal dysfunction) were excluded from the study.

TECHNIQUE

A baseline scan was done for all the patients on day 2 of the menstrual cycle after a detailed clinical history and height and weight of the patients were assessed to

calculate BMI. This scan was done with Voluson E8 Expert (GE Medical systems) BT12 and BT13, using volume transvaginal probe RIC 5-9. For each patient, B mode, color Doppler, power Doppler, spectral Doppler, three-dimensional (3D) ultrasound, and 3D power Doppler were done.

Endometrial thickness was checked in each patient and was confirmed to be ≤4 mm. This confirmed low estrogen levels and no active follicle or residual corpus luteum. This was further confirmed on ovarian scan. Ovaries were scanned to assess their three orthogonal diameters and the volume was calculated (Fig. 1). The ovarian volume was calculated from these three diameters as ($X \times Y \times Z \times 0.523$), even though the scanner software also calculates it automatically from these three diameters. Number of antral follicles were calculated by eyeballing while scrolling the probe across the ovary. The same procedure was done for the opposite ovary also to assess its volume and number of antral follicles.

Followed by this, color Doppler was switched on. Subendometrial flow was assessed to confirm if it was very low or almost absent. Blood flow in the ovarian stroma was assessed with pulse repetition frequency (PRF) for color Doppler set at 0.3 kHz and wall filter set at the lowest. Blood vessels in the stroma were assessed for subjective assessment of abundance. Visually, the vessel with highest color brightness in the stroma was then assessed by spectral pulsed wave Doppler. These vessels should not be close to the follicle (Fig. 2). Spectral Doppler sample volume was placed on this vessel to assess the flow in this ovarian stromal vessel quantitatively (Fig. 3). Sample volume selected was 2 mm and the angle correction was done to calculate correct velocity. The PRF for spectral Doppler was set at 1.3 kHz and wall filter was set at 30 Hz. Quantitative assessment [resistance index (RI) and peak systolic velocity (PSV)] of the stromal flow was done for both the ovaries using spectral Doppler.

Three-dimensional ultrasound was used to acquire the volume of the ovary one at a time. Adequate region of interest and volume angle was selected for both ovaries individually. Adequacy of the acquired volume was assessed on A and B planes of the multiplanar image (Fig. 4). Computerized volume calculation (VOCAL) was applied on this acquired volume of ovary to calculate the

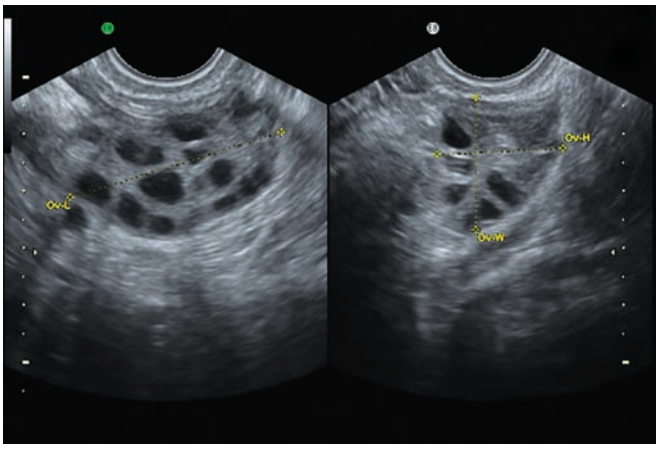

Fig. 1: B mode assessment of ovarian volume by three orthogonal diameters in two orthogonal planes

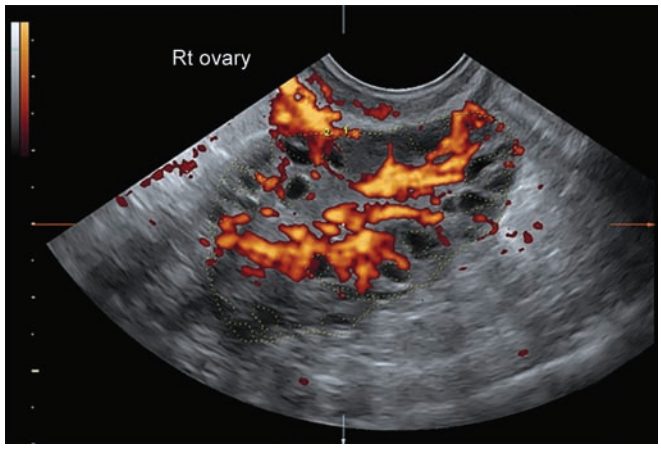

Fig. 2: Power Doppler showing stromal blood vessels in the ovary

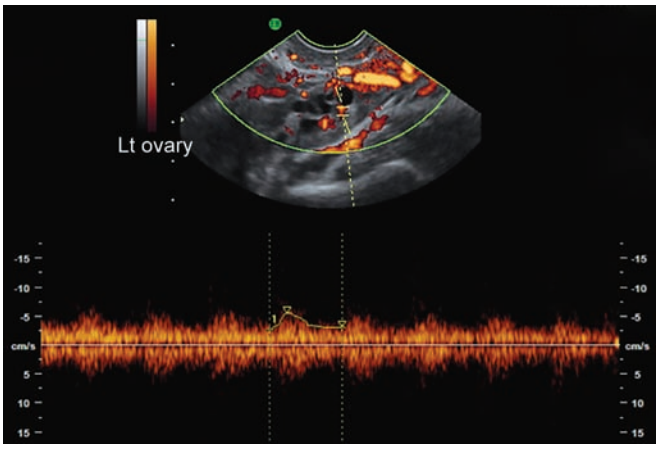

Fig. 3: Power Doppler with pulsed Doppler showing quantitative assessment of the ovarian stromal flow

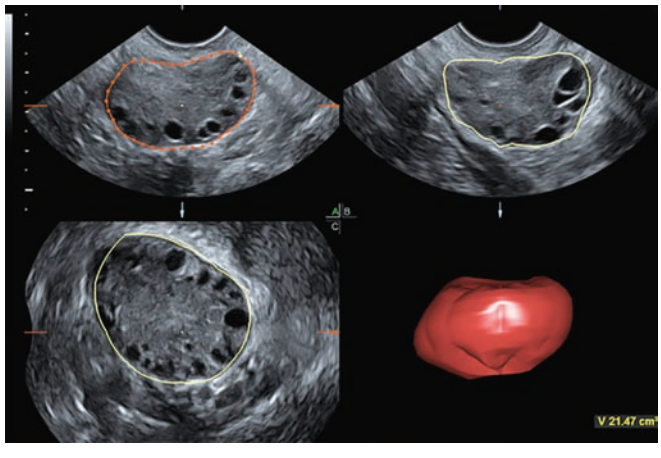

Fig. 4: Three-dimensional ultrasound with VOCAL to calculate the ovarian volume

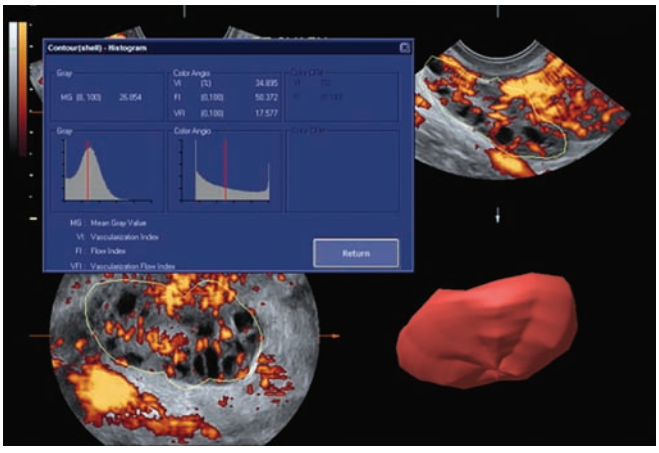

Fig. 5: Three-dimensional ultrasound with VOCAL and volume histogram for quantitative evaluation of the global ovarian stromal flow

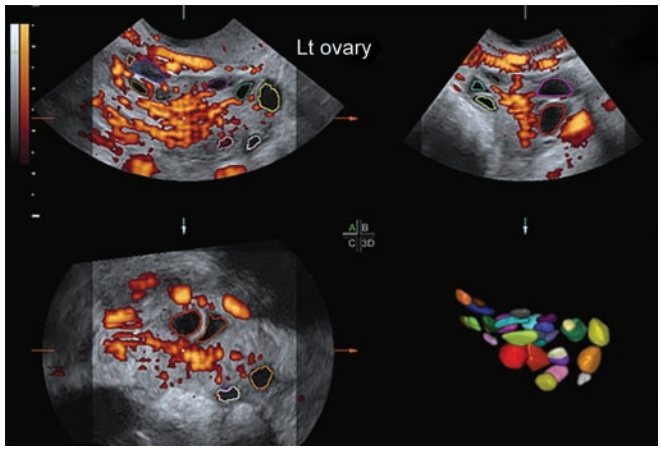

Fig. 6: Three-dimensional ultrasound volume of the ovary with Sono AVC, color coding the antral follicles

exact volume of the ovary (Fig. 5). The angle of rotation step was set at 30° for total rotation of 180°. With each rotation, the circumference of the ovary was traced and at the end of six such rotations, the computer calculated exact volume of the ovary. Another computer software called Sono AVC was then used for automated VOCAL of individual follicle (Fig. 6). This software color-codes each follicle and also measures all three diameters of the same and also displays mean diameter and volume of each follicle. Postprocessing may be required to include uncounted follicles and also to remove the wrongly counted follicles. Ovarian volume and stromal RI and PSV were averaged for both ovaries and antral follicle count (AFC) was total of follicular number in both ovaries.

A scoring system was developed based on clinical and ultrasound findings as follows Table 1:

Table 1: Scoring system for designing stimulation protocol

Score	*1*	*2*	*3*	*4*	*5*
Age	>40	35–40	30–35	25–30	<25
BMI	>30	30–28	28–25	25–22	<22
AFC	<5	5–10	10–15	15–20	>20
Ova volume	<3	3–5	5–7	7–10	>10
Stromal RI	>0.75	0.75–0.65	0.65–0.55	0.55–0.45	<0.45
Stromal PSV	<3	3–5	5–7	7–10	>10

The ovulation induction in all patients was done by recombinant follicle-stimulating hormone (rFSH) and the stimulation doses were based on the scores of individual patient based on the above-mentioned table. The doses according to the patient's score were decided (Table 2).

Table 2: Doses distribution according to patients score

Score of	
>23	75 IU
20–22	150 IU
16–20	225 IU
11–15	300 IU
6–10	375 IU
1–5	450 IU

For scores <10 and >20, fixed antagonist protocols were used and antagonist (cetrorelix 0.25 mg) was started on day 6 of stimulation. For other patients, long agonist protocol was used. Agonist (leuprolide acetate) 0.5 mg subcutaneous daily was given from day 21 of the previous cycle, and the dose was halved (0.25 mg) from the first day of menstruation. For the patient with scores lower than 15, recombinant luteinizing hormone (luteotropin alfa) (LH) 75 IU was added every day from day 6 of stimulation.

Stimulation of the patients was started on day 2 of the cycle. Each patient was scanned again on day 5 of the stimulation for follicular and endometrial growth. At least one dominant (larger than 10 mm in diameter) in each/either ovary or increase in endometrial thickness to 5 mm was considered an adequate response. Endometrial thickness was measured from outer margin of echogenic outer margin to outer margin of echogenic outer margin, not including the hypoechoic endometriomyometrial junction (Fig. 7). In patients with adequate response, stimulation with same dose was continued till at least one follicle of 18 mm and two of 16 mm were obtained. Endometrial thickness of 8 to 10 mm was considered optimum, but

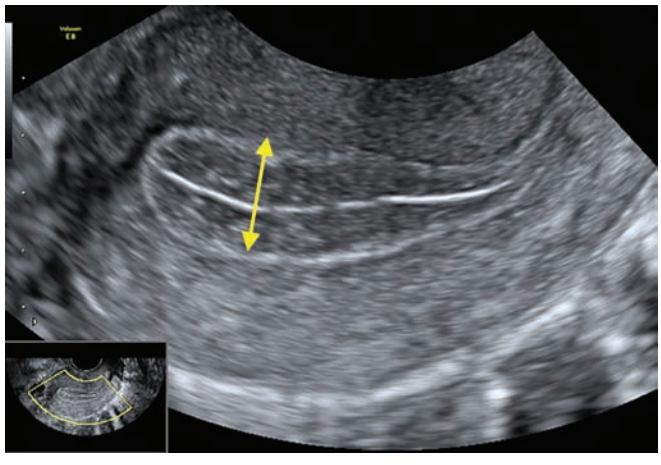

Fig. 7: B mode image of the endometrium with arrow demonstrating the endometrial thickness

7 to 11 mm was considered acceptable. Follicle and endometrium were assessed by color Doppler and pulse Doppler for perifollicular and endometrial flow. When three-fourths of the follicular circumference was covered by blood vessels and at least one of the vessels showed RI of less than 0.48 and PSV of more than 10 cm/second, the follicle was considered to be mature for ovulation trigger.[2] With multiple follicular development, stimulation was continued till at least three follicles in either ovary showed the above-defined Doppler parameters. Endometrium that was at least 7 mm thick and showed blood vessels reaching zone 3 or zone 4[3,4] with RI of less than 0.6 was considered an endometrium with good implantation potential. Before triggering ovulation uterine artery, Doppler was also done for each patient and was confirmed that the uterine artery pulsatility index was less than 3.2.[5]

Response was considered to be inadequate when on day 5 of stimulation, there was no dominant follicle in either ovary and/or the endometrium was also less than 5 mm in thickness. In patients with inadequate response, the same protocol was continued for another 2 days. Patient was rescanned to assess the response after that. If there was no response, 50% of the existing dose was increased for 2 more days and patient was reassessed by ultrasound. If there was inadequate response, cycle was abandoned. If instead there were more than 8 follicles of more than 14 mm in diameter or more than six medium-sized (12–14 mm) follicles in both ovaries, it was considered a hyperresponse and the cycle was abandoned.

In patients in whom mature follicles and good endometrium was achieved, ovulation trigger was given. In all patients, recombinant human chorionic gonadotropin (hCG) 250 IU was given as a trigger. After trigger, ovum pick-up was done at 34 hours following the standard method for the same.

Postwash count for semen sample was maintained between 4 and 8 million/mL for all patients. *In vitro* fertilization was opted for the patients in whom the postwash count was >5 million/mL and the ova retrieved were more than 7. When either the postwash sperm count was <5 million/mL and/or the number of ova retrieved were <4, intracytoplasmic sperm injection (ICSI) was preferred. Standard laboratory techniques were followed for both IVF and ICSI. Day 3 embryo transfer was done in all the patients following the standard technique for all. One/two embryos were transferred in all the patients. Luteal support was given to all patients in the form of vaginal pessaries of micronized natural progesterone 400 mg bd starting from the day of ovum pick-up till 2 weeks. Beta hCG assessment in blood was done on day 15 of embryo transfer for confirmation of pregnancy.

Efficacy of the stimulation protocol was assessed in terms of incidence of ovarian hyperstimulation, cycle cancellation rate for poor response, twin pregnancy or higher order multiple pregnancy as negative outcomes and conception rates, and ongoing pregnancy rates as positive outcomes.

RESULTS

Analysis of score pattern among patients Table 3:

Table 3: Patient distribution according to the scores

Score	*Number of patients*
>23	27
21–23	99
16–20	159
11–15	167
06–10	36
01–05	7

The results were tabulated under following heads Table 4:

Number (average) of dominant follicles on day 5 of stimulation (day 5 foll).

Number (average) of mature follicles at the time of trigger combined for both ovaries (Foll. at trigger).

Number (average) of middle-sized follicles at the time of trigger combined for both ovaries (mid. foll. at trigger).

Positive beta hCG/urine pregnancy test (+hCG/UPT).

Ongoing pregnancy (of 8 weeks and more).

Cancelation of cycles (Cycle cancel) because of poor response. Poor response is when there are <3 mature follicles or no endometrial growth in spite of 10 days of stimulation as described earlier.

Ovarian hyperstimulation syndrome: According to above described criteria or the total ovarian volume of both ovaries together is >120 cc.[6] It is known that it is the medium-sized follicles that are culprit for OHSS.

Table 4: Distribution of pregnancies and complication rates among each

Score	*No. of pts*	*Day 5 foll. (av)*	*Foll. at trigger (av)*	*Mid. foll. at trigger (av)*	*+hCG/UPT*	*On preg.*	*Cycle cancel*	*OHSS*	*Twin preg.*	*Mult. preg.*
>23	27	12	15	8	10	08	00	02	03	01
21–23	99	11	12	5.7	36	34	00	02	14	02
16–20	159	9	10.3	5	67	64	00	00	08	01
11–15	167	7	7.8	4.3	74	71	02	00	02	00
5–10	36	5	4.7	3	12	10	03	00	00	00
1–5	7	2	1.5	1.5	01	01	03	00	00	00
Total	495				200	188	08	04	27	04

Twin pregnancies (Twin Preg.).

Higher order pregnancies (Mult. Preg.).

The above table indicates that the stimulation protocols were decided according to the below scoring system:

- With 40.4% conception rate per cycle
- With 37.9% ongoing pregnancy rates
- With zero moderate/severe OHSS
- 0.8% mild OHSS overall and 3.17% in the high-risk group.
- 5.45% twin pregnancy rate of treated patients and 13% of conceptions
- 0.8% of triplets of treated patients and <2% of conceptions
- Extremely low 1.6% cycle cancelation rate (chiefly in the extremely low score group).

DISCUSSION

In the unpublished pilot studies done earlier by us, though the number of antral follicles was presented as the most correlating parameter with the number of ova retrieved, when the doses were decided by AFC alone, there were significantly higher number of cycle cancellations due to very fast or delayed responses. Age and BMI as independent factors were not found to be reliable to decide the stimulation doses. Stromal flows (RI and PSV) when used to decide the stimulation protocols were the second most reliable parameters after AFC for decision on doses for stimulation. The drawback with flow parameters was higher hyperstimulation rates as the number of follicles growing could not be controlled. For these reasons, multiple parameters were included in this protocol design.

Several studies have been done since the year 2000, using biochemical, biometric, and ultrasound parameters in different combinations to decide optimal doses for stimulation for different patients undergoing IVF.

Ravhon et al[7] in 2000 used dynamic assessment of inhibin B and estradiol as predictors of ovarian response after downregulation with buserelin acetate. They have found these hormones to be highly correlating with the ovarian response in IVF patients. But, it required several blood tests at different times, which could be expensive and inconvenient to the patient. Moreover, the study sample was pretty small (n = 37).

In 2002, Kupesic and Kurjak[8] used 3D ultrasound for assessment of ovarian response in IVF cycles. This group combined AFC and ovarian stromal flow on the baseline scan to design the stimulation protocols. These parameters were shown to be most predictive of the ovarian response after pituitary downregulation in this study, followed by total ovarian volume, ovarian stromal area, and age. This study could predict favorable IVF outcome in 50% (11/22) of patients and poor outcome in 85% (29/34) of patients. Though the results were fairly convincing, the sample volume was only 56 patients.

In 2003, Popovic-Todorovic et al[9] combined age, BMI, cycle length, smoking status, and ultrasound features of the ovaries to design a dosage nomogram of rFSH for IVF/ICSI patients. According to this study, total number of antral follicles and ovarian stromal blood flow were the two most significant predictors of ovarian response, and ovarian volume was a highly significant predictor of number of follicles and oocytes retrieved. The positive aspects of this study were its prospective design and a larger sample volume than the previous two studies (n = 145). The nomogram developed by this group for dose calculation was evaluated by the same group in another study. The results of this study were in absolute favor of individualizing dose according to the dosage nomogram. It proved the reliability of ultrasound parameters, age, and BMI for decision on gonadotropin doses for ovarian stimulation in ART cycles. In the study group, 101 patients (77.1%) had an appropriate response (defined as 5–14 oocytes), compared with 86 (65.6%) in the control group ($p < 0.05$). Fewer than five oocytes were retrieved in two patients (1.5%) in the study group, compared with 14 patients (10.7%) in the control group ($p < 0.05$). By comparison, >14 oocytes were retrieved from 27 patients (20.6%) in the study group and from 26 (19.8%) control patients (p = not significant). Eighty-six percent of the individual dose patients did not require any dose adjustment on day 8, compared with 45% of the standard dose patients ($p < 0.01$). The ongoing pregnancy rate per initiated cycle was 36.6% in the study group and 24.4% in the control group ($p < 0.01$). One patient (0.8%) in the study group and four patients (3.1%) in the control group were hospitalized due to OHSS. The results thus were quite assuring.

In 2005, Ng et al[10] showed basal FSH to be the most reliable parameter for assessment of ovarian response followed by AFC and BMI where AFC was predictive of number of follicles (serum estradiol level) on the day of hCG and BMI was predictive of gonadotropins dosage. According to this study, Doppler was not thought to correlate with the ovarian response, though this study used only power Doppler and not 3D power Doppler. This modality assesses only few of all vessels in the ovarian stroma as compared with 3D power Doppler, which gives idea about the global vascularity of the ovary. But a study from Netherlands has shown around the same time that AFC is a better marker than FSH for selection of older patients with acceptable pregnancy offer.[11]

In 2006, a Chinese group[12] presented a study on the role of inhibin B in predicting ovarian response in IVF cycles. They assessed inhibin B before starting stimulation and on day 5 of stimulation along with serum estradiol and progesterone levels and found inhibin B on day 5 to be predictive of ovarian response.

Another Chinese group[13] in 2009 also found dynamic inhibin B levels, predictive of ovarian response to stimulation in IVF cycles as compared with estradiol, FSH, and LH when assessed before starting stimulation, on day of stimulation, day 3, and day 5.

te Velde[14] has shown that AFC and ovarian volume provide direct measurements of ovarian reserve, while anti-Müllerian hormone (AMH), inhibin B, and estradiol are released from growing follicles and so their levels reflect the size of developing follicle cohort. Follicle-stimulating hormone is controlled by negative feedback of inhibin B and so high FSH represents small cohort size.

A landmark study by Oliveness et al,[15] – the CONSORT study – used basal FSH, BMI, age, and AFC for individualizing FSH dose for ovarian stimulation. This was a large (n = 1,378) prospective multicentric study. This study concluded that the most predictive factors for ovarian response to gonadotropin stimulation were basal FSH, BMI, age, and number of antral follicles. A dose calculator was developed using these factors as predictors and was evaluated in a prospective clinical trial.

An ultrasound-based study on prediction of ovarian response in 2007 by Merce et al[16] evaluated ovarian volume, AFC, and 3D power Doppler indices vascularization index, flow index, and vascularity flow index for their reliability to calculate the number of follicles grown and oocyte retrieved embryos transferred. This study clearly showed the relevance of ovarian volume and AFC to the number of follicles matured and oocytes retrieved. It also mentioned that 3D power Doppler indices made the assessment of ovarian response to stimulation protocols easier.

Study by Freiesleben et al[17] in 2008 for designing the stimulation protocol for IVF and ICSI patients concluded that AFC and age could predict the low response better, whereas to predict hyperresponse AFC and cycle length were better parameters.

The latest study in 2014 by Hashish and Shaeer[18] developed an equation for calculation of gonadotropins for ICSI cycles. The equation included age, FSH, BMI, and estradiol after downregulation. This equation showed concordance probability index of 60%. The drawback of this evaluation is that it is too complicated.

The parameters that we have used to calculate the dose for gonadotropin stimulation protocol for ART cycles in this study are age, BMI, AFC, ovarian volume, stromal RI, and PSV based on all the studies mentioned above and also on the results of the pilot studies done earlier. These studies were done by taking a group of 25 patients each. For group 1, we had taken AFC, BMI, and age as parameters for deciding the stimulation protocol. In group 2, it was AFC, stromal RI, and stromal PSV. In group 1, out of 25 patients, 7 patients showed development of multiple follicles on day 5 of stimulation and hCG was withhold and 4 patients showed delayed development of the follicles only after increasing the dose of gonadotropin twice. In group 2, four patients showed multiple follicles and in five patients no follicles developed to maturity till day 11 of stimulation and stimulation was abandoned. Evidently, some response deciding factors were missing consideration in both these groups. Therefore, we decided to combine parameters of both the groups to finally calculate the dose for this study.

It is a known fact that with increasing age, the number of antral follicles decreases and in turn also the ovarian reserve.

Antral follicle count showed the best correlation with women's age and declines linearly at a rate of 3.8% per year.[19] Antral follicle count declines at a rate of 4.8% per year before the age of 37 years and declines at a rate of 11.7% after 37 years.[20] Anti-Müllerian hormone and AFC both have reflections of primordial follicles and both are stable between cycles.[21] Linear correlation is seen between AFC and AMH, and both help to predict the extremes of response.[22,23]

Therefore, we have included AFC as one of the parameters for assessing ovarian reserve and omitted AMH. Age is known to be one of the most important factors that reduce not only the ovarian reserve but also the oocyte quality. It has been shown in several studies mentioned above that BMI may be an important determinant of the ovarian response. This is because flow in the ovarian stroma is less in obese patients as compared with controls.

Ovarian volume <3 cc was significantly predictive of higher IVF cancelation rates >50%[14] and therefore, ovarian volume was also included in decision making. Antral follicle count and ovarian volume provide direct measurements of ovarian reserve.[24]

Inclusion of stromal blood flows as one of the decision-making parameters was based on the following evidences. It has been shown that measurement of ovarian stromal flow in early follicular phase is related to subsequent ovarian response in IVF treatment.[25] Ovarian stromal PSV after pituitary suppression is predictive of ovarian responsiveness and outcome of IVF treatment.[26] Kupesic and Kurjak[8] have shown correlation in the ovarian stromal flow index and number of mature oocytes retrieved in IVF cycles and pregnancy rates.

For an ovary that shows normal parameters for ovarian reserve and response, the standard stimulation protocols are selected (long agonist protocol with rFSH 225 IU/day). The dose of gonadotropins has been modified depending on each factor that would improve or deteriorate the response of ovary to gonadotropins.

Increasing age, less number of antral follicles, smaller ovarian volume and obesity, and less ovarian flow may

demand higher doses as per references mentioned above and vice versa, and therefore, the standard dose has been increased or decreased step by step with each factor accordingly, depending on the scores of individual patient. With very low scores, it is known that the follicles grown would be very few in number (1–3) and are also likely to be of low quality and less likely to give good quality embryos. Moreover, these patients also consume a large dose of stimulation drugs, and therefore, ideally, these patients should be advised ovum donation. But still few patients with very low scores have been included in the study because there are patients who would wish to use their own genetic material at all costs.

CONCLUSION

As per the results of our study, we believe that the scoring system devised in this study for deciding the stimulation protocol is highly reliable for safe planning of stimulation protocols in ART cycles as the OHSS rates and cycle cancelation rates due to poor response are negligible. Multiple pregnancy rates are also acceptable. This study, we believe, may prove to be an important guide for safe use of gonadotropins for ovulation induction in ART cycles.

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