

RESEARCH ARTICLE

Ultrasound-based Decision Making on Stimulation Protocol for Superovulated Intrauterine Insemination Cycles

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ABSTRACT

Deciding optimum stimulation protocols is one of the most important factors for the success of any assisted reproductive technologies. We have used ultrasound parameters chiefly with age and basal mass index (BMI) to decide stimulation protocols in intrauterine insemination (IUI) cycles. The parameters used on ultrasound were ovarian size (volume), number of antral follicles, ovarian stromal resistance index, and peak systolic velocity. A scoring system was developed according to the values of these parameters with age and BMI of the patient. This scoring system has been used to decide the dose of gonadotropins for superovulation in IUI cycles. It has been used for 1570 patients with cancellation of cycles due to poor response in only one patient and moderate ovarian hyperstimulation syndrome in only one patient. This shows that the scoring system has been very effective for deciding optimum stimulation protocols in patients with varying age, BMI, and ovarian reserve.

Keywords: Baseline scan, IUI cycles, Stimulation protocol.

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INTRODUCTION

Deciding optimum stimulation protocols is one of the most important factors for the success of any assisted reproductive technologies (ARTs). Suboptimal protocols can result into failures or complications in the form of ovarian hyperstimulation syndrome (OHSS) or cancellation of the cycle due to poor response. Ovarian hyperstimulation syndrome is the most dreaded one because

of its high morbidity and also unacceptable mortality. Therefore, several studies have been done to standardize stimulation protocols based on ultrasound, age, biochemical tests, etc. Choosing the appropriate gonadotrophin dosage to retrieve optimum number of oocytes involves multiple individual patient variables.¹ We have used ultrasound parameters chiefly with age and basal mass index (BMI) to decide stimulation protocols in intrauterine insemination (IUI) cycles.

MATERIALS AND METHODS

This is a prospective study of 1570 patients recruited over a period of 4 years from January 2010 to December 2014. This study was approved by institutional ethical committee. After initial assessment and counseling, all the patients who were taken for controlled ovarian hyperstimulation and IUI during this time frame were educated and informed about the study. They were explained about the existing standard protocols and also about the protocols designed based on ultrasound in this study, and the advantages and disadvantages of both. They were allowed to take a decision as to they would be willing to participate in the study of ultrasound-based decision on stimulation protocols or not. Total 1570 patients agreed to be a part of the study.

Prestudy Workup: Inclusion and Exclusion Criteria

Tubal assessment with either saline infusion salpingography or laparoscopy was done to exclude patients with bilateral tubal block. Patients with previous surgeries on their ovaries and those with cysts, dermoids, or endometriomas larger than 3 cm were also excluded from the study. Couples with severe male factor, who had opted for intracytoplasmic sperm injection (ICSI), and the female partners of these couples were also excluded from the study, though those who had opted for donor semen IUI were included in the study.

Patients included had dysovulatory or unexplained infertility including polycystic ovary syndrome (PCOS) and poor responders, mild to moderate male factor, grade 1, 2 endometriosis, and cervical factor infertility. Patients who were menstruating naturally and normally, meaning that all premenopausal patients, were all included in the study.

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Technique

A baseline scan was done for all these patients on day two or three of the menstrual cycle after a detailed clinical history and height and weight of the patient were assessed to calculate BMI. This scan was done with Voluson Voluson E8 Expert (GE Healthcare) BT 12 and BT 13, using volume transvaginal probe RIC 5-9. Modalities used for this scan were B mode, color Doppler, power Doppler, spectral Doppler, 3D ultrasound, and 3D power Doppler.

Transvaginal scan was done in each patient to assess the endometrial thickness and uterine position in the pelvis. Ovaries were scanned to find out the longest axis and this section was saved in one half of the dual frame image. The probe was then rotated 90° to achieve the true transverse section of the ovary and that is stored on the second half of the dual frame. The longest diameter is measured on the long axis of the ovary. Another diameter (antero-posterior diameter) is taken on the same section, perpendicular to the longest section. The third diameter is measured on the transverse section of the ovary (side to side diameter), the longest measurement from one side to the other side, horizontally (Fig. 1). The ovarian volume is calculated from these three diameters as $(X \times Y \times Z \times 0.523)$, although the scanner software also calculates it automatically from these three diameters. Then, the probe is scrolled across the ovarian long axis slowly from one side to other and number of antral follicles (follicle smaller than or equal to 9 mm in diameter) are counted by eyeballing. The same procedure is done for the opposite ovary also to assess its volume and number of antral follicles.

Followed by this, color Doppler is switched on and the color box is placed on the ovary. The box size is large enough to include the whole ovary. The pulse repetition frequency (PRF) for color Doppler is set at 0.3 and wall filter is set at the lowest. This allows to visualize the flow in the ovary. On baseline scan, the stromal vascularity is to be assessed. So, the box size is now reduced to concentrate

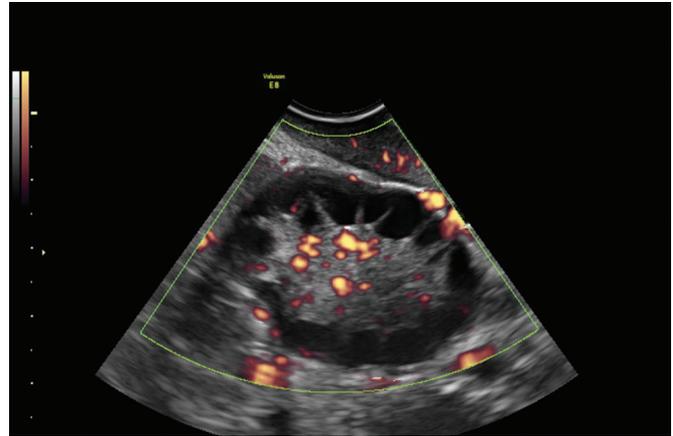


Fig. 2: Color Doppler of the ovary showing blood vessels in the stroma-ovarian stromal flow

on the stroma and to visually find out the vessel with highest color brightness in the stroma and not close to the follicle (Fig. 2). Spectral Doppler is now switched on and the sample volume is placed on this vessel to assess the flow in this ovarian stromal vessel quantitatively (Fig. 3). Sample volume selected is 2 mm and the angle correction is done. The PRF for spectral Doppler is set at 1.3 and wall filter is set at 30 Hz. Quantitative assessment of the stromal flow is done for both the ovaries using spectral Doppler.

Three-dimensional ultrasound is used to acquire the volume of the ovary one at a time. The volume box is large enough to include the whole ovary and the volume angle should also be set accordingly. Acquired volume is seen on multiplanar view and sections A and B are checked for inclusion of entire ovary on both. This means that acquired volume is adequate (Fig. 4). Computerized volume calculation (VOCAL) is applied on this acquired volume of ovary to calculate the exact volume of the ovary (Fig. 5). The angle of rotation step is set at 30° for a total rotation of 180°. With each rotation, the circumference of the ovary is traced, and at the end of six such rotations, the computer calculates the exact volume of the ovary.

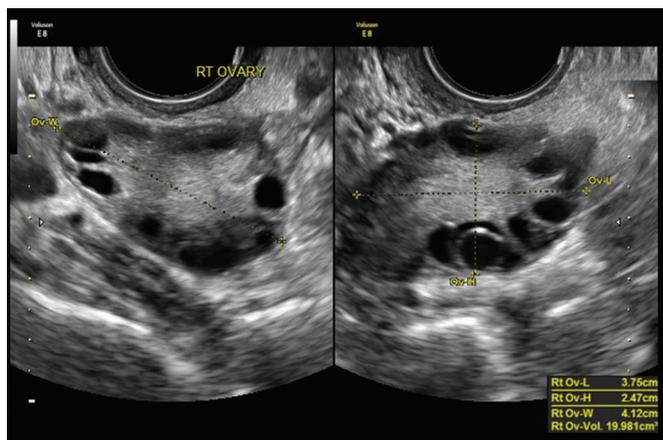


Fig. 1: B mode ultrasound showing longitudinal and transverse sections of ovary showing measurement of three orthogonal diameters to calculate ovarian volume

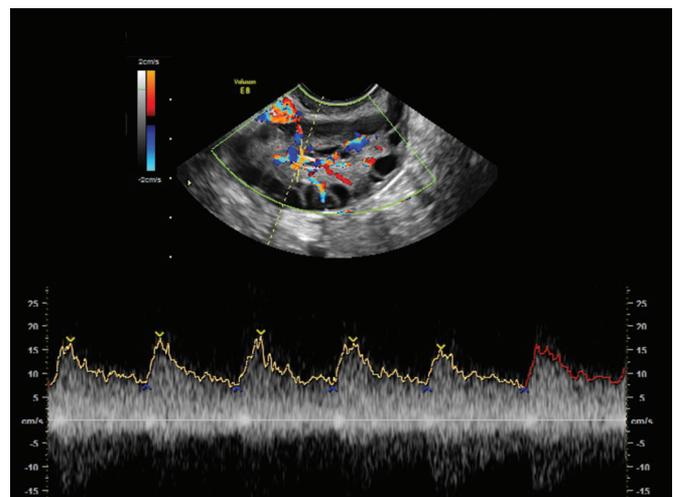


Fig. 3: Spectral Doppler of the ovarian stromal flow

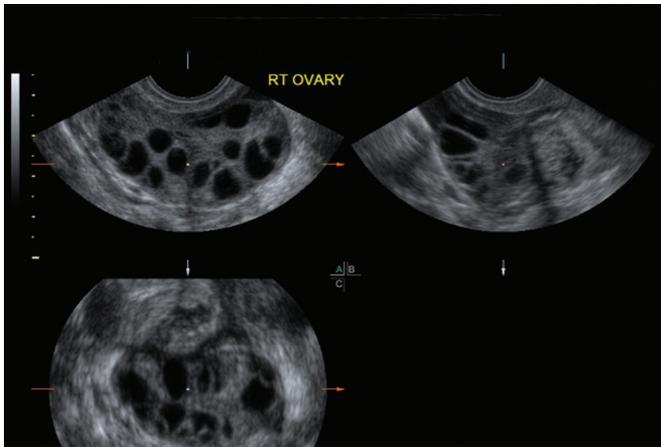


Fig. 4: Multiplanar view of volume of ovary of 3D acquired volume of ovary

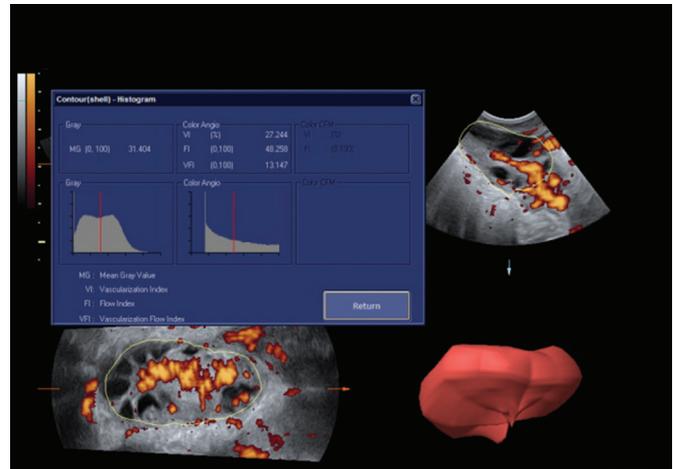


Fig. 5: Three-dimensional power Doppler acquired volume of the ovary and rendered with volume histogram

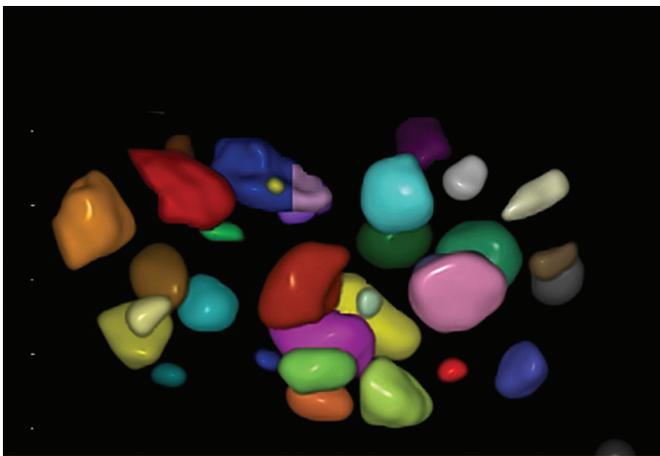


Fig. 6: Ovarian volume acquired by 3D ultrasound and rendered by Sono AVC

Another computer software called Sono AVC is then used for automated volume calculation 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 for including uncounted follicles and also to remove the wrongly counted follicles.

The ovarian volume assessed by 2D was used for the dose calculation. For calculation of antral follicle count, B mode scrolling was the method used generally. But when the follicle number is more than 10 and/or cannot be confidently calculated by just scrolling and eyeballing, 3D with Sono AVC is used to calculate average fixed cost (AFC).

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

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
Ovarian vol.	<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 as follows:

Score of 25	25 iu
Score of 20–24	37.5
15–20	75 iu
10–15	112.5 iu
6–10	150 iu

Gonadotropin stimulation of the patients was started on day 5 of the cycle and gonadotropin according to the dose decided by scoring was given every day. No oral ovulaogens were given. 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) or one mature follicle (18 mm in diameter) in each/either ovary or an increase in endometrial thickness to 5 mm was considered an adequate response. In patients with adequate response, stimulation with the same dose was continued till at least one follicle reached a diameter of 18 mm. The diameter of the follicle was taken as a mean of three orthogonal diameters measured in two orthogonal sections. Endometrial thickness was assessed when follicle was 18 mm in diameter or larger. Endometrial thickness was measured from outer margin of echogenic outer margin to outer margin of echogenic outer margin, not including the hypoechoic endometrio-myometrial junction. Follicle and endometrium were assessed by color Doppler and pulse Doppler for perifollicular and endometrial flow. When three-fourth of the follicular circumference was covered by blood vessels and at least one of the vessels showed resistance index (RI) of less than 0.48 and peak systolic velocity (PSV) of more than 10 cm/s, the follicle was considered to be mature for ovulation trigger.² Endometrium that

is at least 7 mm thick and shows blood vessels reaching zone 3 or zone 4^{3,4} with RI of less than 0.6 is 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 (PI) 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 6 mm in thickness. In patients with an inadequate response, the same protocol was continued for another 5 days. And the patient was rescanned to assess the response after that. Again depending on the presence of dominant or mature follicle, the same line of action as described above is followed. If there was no response 50% of the existing dose is increased for 5 more days and is reassessed by ultrasound. If there was an inadequate response, cycle was abandoned.

If instead there were more than four mature follicles on either side, it was considered a hyper-response and the cycle was abandoned.

In patients in whom mature follicle and good endometrium was achieved, ovulation trigger was given. In all patients, recombinant human chorionic gonadotropins (rhCG) 250 iu was given as a trigger. After trigger, IUI was done at between 34 and 36 hours. Postwash count for intrauterine semen sample was maintained between 6 and 10 million/ml for all patients. Luteal support was given to all patients in the form of vaginal pessaries of micronized natural progesterone 400 mg twice daily (b.d.) starting from day 2 of IUI till 2 weeks. Urine pregnancy test (UPT) and or beta-human chorionic gonadotropin (hCG) assessment in blood was done on day 17 of IUI for confirmation or otherwise of pregnancy.

RESULTS

Analysis of score pattern among patients:

Score	Number of patients
25	29
21–24	491
16–20	615
11–15	379
6–10	43
0–5	13

Score	No. of pts.	Day 5 foll	Foll at trigger	Mid foll at trigger	+hCG/UPT	On preg.	Cycle cancel	OHSS	Twin preg.	Mult preg.
25	21	6	8	5	10	9	0	0	3	0
21–24	491	6	7	5	172	166	0	1	10	2
16–20	615	5	7	4	198	191	0	0	7	0
11–15	379	3	5	3	123	113	2	0	2	0
6–10	43	2	3	3	12	11	1	0	0	0
0–5	13	1	1	1	2	2	3	0	0	0

The results were assessed under the following heads:

- Number of dominant or mature follicles per patient on day 5 of stimulation (day 5 foll)
- Number of mature follicles per patient at the time of trigger combined for both ovaries (foll at trigger)
- Number of middle-sized follicles per patient at the time of trigger combined for both ovaries (mid-foll at trigger)
- Positive beta hCG/UPT (+hCG/UPT)
- Ongoing pregnancy (of 8 weeks and more)
- Cancellation of cycles (cycle cancel) because of poor response. Poor response is when there is no dominant follicle (>10 mm) or no endometrial growth in spite of 10 days of stimulation as described earlier.
- Ovarian hyperstimulation syndrome: more than four mature follicles in both ovaries with at least four medium-sized follicles (12–16 mm) in either ovary or the total ovarian size of both ovaries together of > 180 cc (271+/- 87) (Oyesanya OA et al, Hum Reprod 1995; 10:3211-2). It is known that it is the medium-sized follicles that are culprit for OHSS.
- Twin pregnancies
- Higher order pregnancies (multiple pregnancy see Table below)

Considering individual parameter, no single parameter was found to be significantly more reliable than any other.

DISCUSSION

In the whole ART, whether it be IUI or *in vitro* fertilization (IVF), the most confusing and unconfident part is deciding the stimulation protocol. The most dreaded complication when gonadotropins stimulation is considered is OHSS and also to an extent multiple pregnancies. On the contrary, it is also a fear of cancelling the cycle because of poor response and then facing the patient. There have been several publications on how to decide stimulation protocols since year 2000.

One of the earliest studies by Ravhon et al⁶ has used dynamic assessment of inhibin B and estradiol after buserelin acetate as predictors of ovarian response and have found these to be highly correlating with the ovarian response in IVF patients. This required several blood

tests at different times and the study sample was pretty small ($n=37$). These were the two major drawbacks of this study.

In 2002, Kupesic and Kurjak⁷ used three-dimensional ultrasound for assessment of ovarian response in IVF cycles. The antral follicle count and ovarian stromal flow parameters on the baseline scan 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. Although fairly convincing, the sample volume was only 56 patients.

Popovic-Todorovic et al⁸ in 2003 combined age, BMI, cycle length and smoking status, and ultrasound features of the ovaries also to design a dosage nomogram of rFSH for IVF/ICSI patients. This was a prospective study and also had a larger sample volume than the previous two studies ($n=145$). 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 highly significant predictor of number of follicles and oocytes retrieved. Using this nomogram 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 proving the reliability of ultrasound parameters and age and BMI for decision on stimulation doses. 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 ($p=NS$). Eighty-six per cent 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.

A study by Ng et al⁹ showed basal FSH to be the most reliable parameter for assessment of ovarian response followed by AFC and BMI. In this study, AFC was predictive of number of follicles (S. estradiol level) achieved on the day of hCG and BMI was predictive of gonadotrophins dosage. According to this study, Doppler was not thought to correlate with the ovarian response, though here only 2D power Doppler was used. This modality assesses only few of the all vessels in the ovarian

stroma as compared with 3D power Doppler that gives an idea about the global vascularity of the ovary.

In 2006, a Chinese group¹⁰ 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 day 5 to be predictive of ovarian response.

Another Chinese group¹¹ in 2009 also found dynamic inhibin B levels, predictive of ovarian response to stimulation in IVF cycles as compared with estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) when assessed before starting stimulation, on day of stimulation, day 3, and day 5.

Another landmark study by Olivenness et al,¹² the consolidated standard of reporting trails (CONSORT) study – used basal FSH, BMI, age, and AFC for individualizing FSH dose for ovarian stimulation. This was a large ($n=1378$), prospective, multicenter study. This study concluded that the most predictive factors for ovarian response to gonadotrophins 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 al¹³ evaluated ovarian volume, AFC, and 3D power Doppler indices vascularization index (VI), flow index (FI), and vascularity flow index (VFI) for their reliability to calculate the number of follicles grown, 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.

The only study that worked on the dose calculations for IUI patients was the one by Freiesleben et al¹⁴ in 2008 with 159 patients. They evaluated age, spontaneous cycle length, body weight, BMI, smoking status, total ovarian volume, AFC, total Doppler score of ovarian stromal blood flow, baseline FSH, and estradiol as possible predictive factors of ovarian response. This study concluded that body weight and antral follicle count may be used to achieve appropriate ovarian response for IUI in ovulatory patients. A study by the same investigators for IVF and ICSI patients concluded that AFC and age could predict the low response better, whereas to predict hyper response, AFC and cycle length were better parameters.

The latest study in 2014 by Hashish and Shaer¹⁵ developed an equation for calculation of gonadotrophins for ICSI cycles. The equation included age, FSH, BMI, and E2 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 gonadotropins stimulation protocol for IUI cycles in this study – age, BMI, AFC, ovarian volume, stromal RI, and PSV – are based on all these 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, AFC, stromal RI, and stromal PSV were the parameters. In group 1, out of 25 patients, two patients showed development of multiple follicles on day 5 of stimulation and hCG was withheld and two patients showed delayed development of the follicles only after increasing the dose of gonadotrophin twice. In group 2, two patients showed multiple follicles, and in three patients, no follicles developed to maturity in two patients and stimulation was abandoned on day 28 of the cycle. Evidently, some response-deciding factors were missed to be considered 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 the increasing age, the number of antral follicles decrease and in turn the ovarian reserve.

Average fixed cost showed the best correlation with women's age and declines linearly at a rate of 3.8% per year.¹⁶ AFC declines at a rate of 4.8% per year before the age of 37 years and at a rate of 11.7% after 37 years.¹⁷ Both AMH and AFC have reflections of primordial follicles and both are stable between cycles.¹⁸ Linear correlation is seen between AFC and AMH and both help to predict the extremes of response. Therefore, we have included AFC as one of the parameters for 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 above-mentioned studies 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 cancellation rates >50%,¹⁹ and therefore ovarian volume was also included in decision making. Average fixed cost and ovarian volume provide direct measurements of ovarian reserve.²⁰

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.²¹ Ovarian stromal PSV after pituitary suppression is predictive of ovarian responsiveness and outcome of IVF treatment.²² Kupesic and Kurjak²³ have shown correlation in the ovarian stromal FI and number of mature oocytes retrieved in IVF cycles and pregnancy rates.

Considering normal response in a normal reserve-normal response ovaries, 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 references mentioned above and vice versa, and therefore, accordingly the standard dose has been increased or decreased step by step with each factor.

With only six cycles cancelled due to poor response and only once of moderate OHSS, with no cases of severe OHSS out of total 1570 patients, the dose calculation with the described scoring system definitely proves itself to be reliable. Larger studies on the same may potentiate its reliability.

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 IUI cycles, as the OHSS rates and cycle cancellation rates due to poor response are negligible. This study we believe may prove to be an important guide for safe use of gonadotropins for ovulation induction in IUI cycles and can therefore significantly improve the conception rates with IUI.

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