Original Article

Effectiveness of Anti-Mullerian Hormone-tailored Protocol Compared to Conventional Protocol in Women Undergoing *In vitro* Fertilization: A Randomized Controlled Trial

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Abstract

Background: Assessment of ovarian reserve before an *in vitro* fertilization cycle (IVF) is one among the many factors that predicts a successful cycle. Individualized protocol based on ovarian reserve is designed to optimize the pregnancy outcome without compromising the patient safety. Although authors have shown that anti-Mullerian hormone-tailored (AMH) protocols have reduced the treatment burden and improved pregnancy rates, a few others have questioned its efficacy. **Aims:** The aim of this study was to decide whether the AMH-tailored protocol or the conventional protocol better decides IVF outcomes. **Setting and Design:** Prospective randomized controlled trial conducted at a tertiary level university hospital. **Materials and Methods:** Patients undergoing their first IVF cycle who fulfilled the inclusion criteria were recruited and randomized to each group. Serum follicle-stimulating hormone was done for the patients on day 2 or 3 of a prior menstrual cycle, and serum AMH was done in the preceding cycle. **Statistical Analysis:** Analysis was performed using SPSS software version 16. **Results and Conclusion:** There were 100 patients in each group. A total of 83 patients underwent embryo transfer in the conventional group and 78 patients in the AMH group. The clinical pregnancy rates per initiated cycle (36.4% vs. 33.3%) and per embryo transfer (45.1% vs. 41.3%) were similar in both the groups. There was no statistical difference in the number of cycles cancelled due to poor response or the risk of ovarian hyperstimulation syndrome in both the groups. Hence, this study showed the similar effectiveness of AMH-tailored protocol and conventional protocol in women undergoing IVF.

Keywords: *Anti-Mullerian hormone, follicle stimulating hormone, in vitro fertilization*

Introduction

Utilization of assisted reproductive techniques (ARTs) for infertility has advanced significantly since its inception. Controlled ovarian stimulation plays an important role in ART and to provide the best treatment to every single woman, protocol and the dose of gonadotropins is to be tailored according to unique characteristics of the patient. Most clinicians want to succeed in the index cycle as studies have shown that dropout rates are around 40% after the failure of the first cycle.[1] Assessment of ovarian reserve before an *in vitro* fertilization (IVF) cycle is one among the many factors that predicts a successful cycle. There are various ovarian reserve markers such as age, antimullerian hormone (AMH), follicle stimulating hormone (FSH), and antral follicle count which help us in counseling patients and individualizing treatment strategy. Age has been a firmly established

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predictor[2,3] of pregnancy chance following ART. In women circulating AMH has been shown to be solely produced by the ovaries and its level essentially reflects the follicular ovarian pool. There has been an evolution of various assays for the determination of AMH such as Diagnostics Systems Lab, Immunotech, etc. over several years. However, with the recent consolidation of these two companies by Beck-man Coulter there is finally a single commercially available assay– the AMH Generation II assay (AMH Gen II assay) that has led to the easy interpretation of results.[4] One of the largest data series by Howels *et al*. have shown basal FSH, age, body mass index (BMI), and antral follicles during screening are among the important variables which are used to decide the FSH dose for an optimal outcome.[5] Few studies state that AMH-tailored stimulation strategy has significantly reduced complications such as ovarian hyperstimulation syndrome (OHSS) and financial burden while increasing the pregnancy rates,[6,7] whereas others have questioned its efficacy in predicting poor ovarian response.[8-11] Thus, the aim of this randomized controlled trial (RCT) was to decide whether the AMH-tailored protocol or the conventional protocol based on FSH and age better decides ART outcomes, ovarian response, and cycle cancellation.

Materials and Methods

Women undergoing their first cycle of IVF for the following indications were invited to participate in the trial: (1) male factor, (2) unexplained infertility, (3) minimal/mild endometriosis as defined by the American Fertility Society classification, and (4) anovulation or a combination of these factors. Inclusion criteria were women between 21 and 39 years of age with a BMI <30 kg/m2 and both ovaries adequately visualized on ultrasonography. Patients with hypogonadotropic hypogonadism, moderate or severe endometriosis, patients undergoing IVF for fertility preservation and patients with a serum FSH >15 IU/ml were excluded from the study.

This prospective RCT was conducted at a tertiary level university hospital from the year 2011 to 2013. Eligible women were informed about the trial and provided with an information sheet. Informed consent was obtained from women willing to participate in the trial, after which they were randomized into two groups as follows: conventional protocol group and AMH-tailored protocol group.

Randomization was done using computer-generated block randomization and sealed opaque envelops was used for allocation which was opened after recruitment. The duration of the study was 2 years. Ethical clearance was obtained from the ethical committee of the institution, and the study was registered in the clinical trial registry of India CTRI/2012/11/003139.

In the conventional protocol group, the study patients were advised testing of basal FSH on the 2nd or 3rd day of the menstrual cycle before ART. The protocol used in the conventional group was either long or antagonist for normoresponders and hyper responders and short flare for poor responders. Gonadotropin dose was decided according to age and FSH value. The initial starting dose was 100/150 IU for patients with polycystic ovaries irrespective of age and for those younger than 30 years. For patients between 30 and 35 years of age, the dose was 225/250 IU and those above 35 years ranged between 300 and 375 IU. Patients with serum FSH value ≥15 IU were excluded from the study. In the AMH-tailored protocol, patients were advised AMH in the preceding cycle. AMH assay used was the commercial GENERATION II assay kit with values presented in the concentration of nanogram/ml. Interassay and Intraassay coefficients of variation were 5.3% and 5.4%, respectively. The AMH value was allocated into 4 bands with differing ovarian reserve according to previous studies as shown in Table 1.

In the long agonist protocol, down-regulation with GnRH agonist was initiated on day 21 of an oral contraceptive pill (OCP) pretreatment cycle. After 2 weeks, downregulation was confirmed with serum estradiol and progesterone concentration and ultrasound for endometrial thickness. Ovarian stimulation with recombinant gonadotropins was commenced after the confirmation of downregulation. In the antagonist protocol stimulation with gonadotropins was initiated on the 2nd or 3rd day of an OCP pretreatmnet cycle and GnRH antagonist (0.25 mg/day) was started as flexible protocol when at least 3 follicles reached around 12–13 mm in size.

In the short flare protocol, GnRH agonist was started on day 1 of an OCP pretreatment cycle, and recombinant gonadotropins were started on the day 3 of the cycle.

Ovulation trigger was induced with 5000 IU of human chorionic gonadotropin when at least 3 follicles of

Table 1: Protocol based on Anti-Mullerian hormone value

AMH value (ng/ml)	Protocol	Gonadotropin dose (IU)
<0.5	Antagonist/short agonist flare	375
>0.5-1.1	Antagonist	300-375
>1.1-4.8	Long agonist/antagonist	150-225
>4.8	Antagonist	150

AMH=Anti-Mullerian hormone

17 mm were seen on transvaginal ultrasound, and transvaginal oocyte retrieval was done 35–36 h later. Embryos were graded by the embryologist according to the number, size of the cells and degree of fragmentation and high-quality embryos were transferred. Embryo transfer was done on day 3 or day 5. If there were more than 4 high-quality embryos available on day 3 then the transfer was extended to day 5 or else it was carried out on day 3. The number of embryos to be transferred was decided depending on the age of the patient and quality and stage of embryos. Maximum number of embryos transferred on day 3 was three and day 5 was two.

The sample size was 100 patients in each arm with a total of 200 patients.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac confirmed by ultrasound. Ovarian hyperstimulation syndrome was diagnosed on the basis of the American Society for Reproductive Medicine guidelines and managed accordingly.[12] The primary endpoint was the clinical pregnancy rate per cycle. Secondary outcomes were a mean number of mature oocytes obtained, the total dose of gonadotropins utilized, total oocytes fertilized, cycles cancelled, elective embryo cryopreservation, embryo transfers per initiated cycle, and the incidence of OHSS.

Statistical analysis

The analysis was performed in SPSS software version 16 (IBM Corp., USA). The baseline characteristics of the two groups of patients were compared using an independent *t*-test, and Chi-square test was used for categorical variables.

Results

The two groups were relatively well matched with age and other baseline characteristics as shown in Table 2. A few patients dropped out after enrolment in the study due to reasons such as spontaneous conception in the waiting period, unexpected medical reasons and this accounted for 4 patients in the AMH protocol group and one patient in the conventional protocol group. These patients were excluded from the analysis, as the outcome was calculated according to initiated cycles and embryo transfer done. There were 83 women who underwent embryo transfer in the conventional protocol group and 78 women in the AMH-tailored protocol group [Figure 1].

Table 2: Baseline characteristics

Baseline characteristics	Conventional protocol	AMH protocol	*P*
Age, mean (SD)	31.28 (4.8)	31.98 (4.8)	0.813
FSH, mean (SD)	6.26 (2.09)	-	-
AMH, mean (SD)	-	6.02 (5.42)	-
Stimulation protocol (%)			
Long agonist	23 (23.5)	20 (20.8)	
Antagonist	75 (75.2)	73 (76)	
Short agonist	1 (1.3)	3 (3.2)	
Type of infertility (%)			
Primary	80 (81.8)	81 (84.5)	0.64
Secondary	19 (19.1)	15 (15.4)	
Women who had embryo transfer (%)	83 (83.8)	78 (81.3)	0.227

FSH=Follicle stimulating hormone, AMH=Anti-Mullerian hormone, SD=Standard deviation

The primary outcome evaluated was clinical pregnancy per initiated cycle and per embryo transfer. The clinical pregnancy per initiated cycle and per embryo transfer was slightly higher in the conventional protocol group compared to the AMH protocol, but, it was not statistically significant [Table 3].

The secondary outcomes such as the mean number of mature oocytes obtained, the total dose of gonadotropins utilized, total oocytes fertilized, and cycles cancelled, elective embryo cryopreservation, embryo transfers per initiated cycle and the incidence of OHSS did not show any statistical difference between the two groups [Table 4].

Discussion

The study was designed to compare the clinical outcomes between the AMH-tailored protocol and conventional

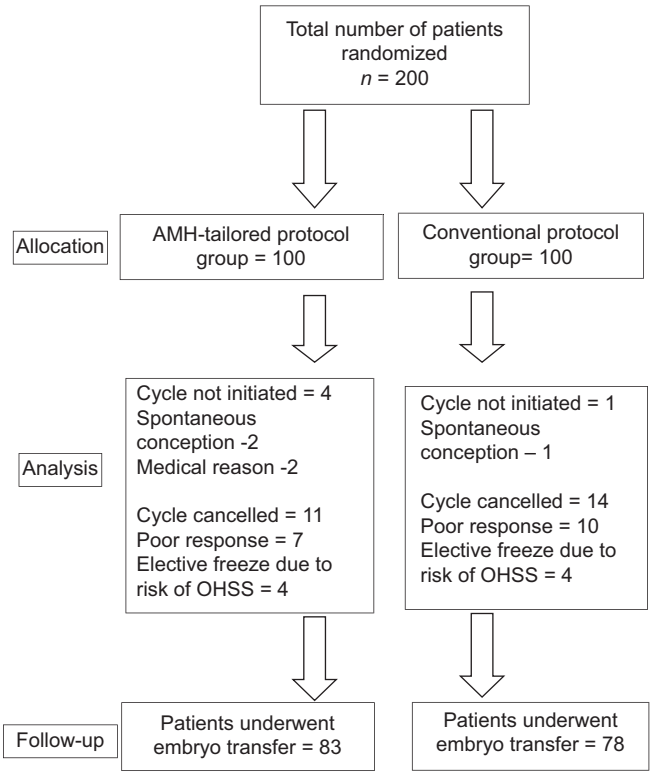

Figure 1: Flowchart

Table 3: Primary outcome

	Conventional protocol (%)	AMH protocol (%)	*P*
Clinical pregnancy per initiated cycle	36/96 (37.5)	32/99 (32.3)	0.197
Clinical pregnancy per embryo transfer	36/83 (43.3)	32/78 (41)	0.186
Clinical pregnancy per patient randomized (ITT)	36/100 (36)	32/100 (32)	0.55

ITT=Intention to treat analysis, AMH=Anti-Mullerian hormone

Table 4: Secondary outcomes

	Conventional protocol	AMH protocol	*P*
Mean no of mature oocytes, mean (SD)	6.64 (6.04)	6.02 (5.4)	0.57
Total dose of gonadotropins used, mean (SD)	2222 IU (1251 IU)	2182 IU (947 IU)	0.5
Total oocytes fertilized, mean (SD)	4.63 (4.2)	4.53 (4.2)	0.91
Cycles cancelled			
Poor response	7	10	0.47
Elective freezing	4	4	0.96
Embryo transfer per initiated cycle (%)	83 (83.8)	78 (81.3)	0.22
OHSS (%)	2 (2)	4 (4.2)	0.75

AMH=Anti-Mullerian hormone, SD=Standard deviation, OHSS=Ovarian hyperstimulation syndrome

protocol for ART in our unit. We found that there was no statistically significant difference in the primary outcome which was the clinical pregnancy rate per embryo transfer and per initiated cycle between the two protocols. There are innumerable debates in favor and disfavor of various ovarian reserve tests and its predictive ability in the success of ART.

The evidence of AMH being a novel marker for poor response and the excess response is favored by La Marca *et al.* and Carles *et al.*[13,14] However, the same has been disfavored by Broekmans *et al.*[8] and Broer *et al*.[10,11] This supports our present data which also showed a similar clinical pregnancy rate when protocol was decided according to FSH and age compared to AMH-tailored protocol though there was slightly higher clinical pregnancy rate in the conventional protocol arm (45.1% vs. 41.3% and 36.4% vs. 33.3%) which was not statistically significant. Another retrospective study which investigated the concordance between AMH and FSH in four different groups of patients showed similar clinical pregnancy rate and live-birth rate in all the groups. The group that had a normal AMH and was expected to produce more oocytes than those groups with a low AMH did not do so. They stated that high FSH levels still has a value in predicting poor ovarian reserve.[15]

Retrospective study by Yates *et al*. has shown that when the protocol and gonadotropin dose was tailored on basis of AMH value resulted in better clinical outcome in terms of pregnancy and live-birth rate and at the same time reducing the cost of treatment and risk of OHSS when compared to the conventional protocol. In their study, the improved pregnancy rate could also be because they used antagonist protocol for the majority of patients in the AMH-tailored protocol group and antagonist protocol has shown improved outcomes at both low and high extremes of ovarian reserve.[6] Nelson *et al*. concluded in their large prospective cohort study that a single measurement of AMH can be used to categorize patients and has an influence on the treatment burden and clinical outcome. They also stated that antagonist protocol has a better outcome at extremes of ovarian reserve.[7] In our study, the patients who had a poor ovarian response according to AMH or follicular phase FSH underwent the short flare protocol which could be a reason for nonsignificant difference between the two in our study.

Literature reviews have shown AMH-tailored protocols as a better predictor in terms of cycle cancellation rates due to poor response or risk of ovarian hyperstimulation;[6,10] however, results of our study showed the similar incidence of cycle cancellation and excess response leading to OHSS in both groups and thus showing that conventional protocol design has the equal predictive accuracy to AMH-tailored protocol. The number of mature oocytes and the total number of fertilized oocytes were similar in each group disfavoring the previous studies which showed an increased number of mature oocytes in AMH personalized protocol.[16,17]

Hence, the conventional predictive model was equally good as AMH-tailored protocol in deciding the protocol and gonadotropin dose in ART patients when other variables such as age and BMI were matched and thus suggesting that the true utilization of these ovarian reserve tests as predictive models depends on the availability of tests and calibration of the laboratory performing the tests. Limitations of FSH testing such

as high intracycle variation and timing of test makes it cumbersome, but AMH testing is also limited by suboptimal standardization of laboratory values due to the availability of various assay kit and thus the difficulty in interpretation. Hence, the usefulness of each predictive model for individualizing ART protocol and dose based on various ovarian reserve tests depends on the individual ART clinics and consideration of limitations of each test is justified.

The main limitation of our study was the small sample size. We also failed to look into the cost-effectiveness of each protocol over the other which would help further in decision-making.

Conclusion

The outcome analysis of the present study showed similar effectiveness in terms of clinical pregnancy rate, number of mature oocytes, cycles cancelled, and incidence of OHSS when personalized treatment regimens of AMH-tailored protocol were compared to the conventional protocol for ART. Hence, we suggest that before incorporating the AMH-tailored protocol in routine IVF practice further prospective and randomized studies which look into clinical outcome and cost-effectiveness should be undertaken to confirm the findings.

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Conflicts of interest

There are no conflicts of interest.

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