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To determine the association of serum AMH levels with oocyte quality in infertile women who underwent controlled ovarian stimulation (COS) for IVF cycles

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***Abstract*---**There is well documented evidence showing a decline in female fertility with advanced age. This decline includes a significant reduction in the quantity as well as the quality of the oocyte available in the ovarian reserve. The present study was conducted to determine the association of serum AMH levels with oocyte quality in infertile women who underwent controlled ovarian stimulation (COS) for IVF cycles. The present retrospective study conducted in IVF Department of Nowrosjee Wadia Maternity Hospital for 10 months. Total 100 patients were included in the study. Serum AMH levels and number of antral follicle (on second day of menstrual cycle) on transvaginal sonography was done for all patients. Fertilization was checked followed by embryo quality. Statistical analysis was performed by ANOVA. Significance was attributed to events with a $p<0.05$. A Total 100 patients were studied. The patients were divided into five groups based on AMH levels; the groups had 0, 44, 20, 26 and 10 patients respectively. Age was found to be independent predictor of ovarian reserve. There was no significant difference between the groups in relation to BMI. Statistically significant difference was seen for antral follicle count when group 2, 3 and 5 were compared ($p>0.001$). Significant difference was observed in group 2 when compared with group 3,4 and 5 regarding the dose of gonadotropins($p<0.05$). No statistical significance was found regarding the duration of stimulus. There was significant difference in total number of oocytes retrieved and metaphase 2 oocytes between all the groups ($p<0.0001$). Age, antral follicle count, dose of HMG, number of days of stimulation, number of oocytes retrieved and number of metaphase 2 oocytes were thus found to be associated with AMH levels. Oocyte morphology was

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not related to fertilization rate. Significant reduction in cleavage rate was found in M2 oocytes with anomalies compared to those without anomalies. (p=0.03) A significantly reduced quality of embryos were observed when embryos developed from oocytes with one or more anomalies compared with those from normal oocytes. (p<0.001) The cleavage rate(p<0.05) and number of good quality embryos(p<0.001) was significantly higher in the group with extra cytoplasmic anomalies compared with cytoplasmic anomalies. Clinical pregnancy rate(33%) was significantly low in group 2 compared to 3,4 and 5. The present study concluded that AMH can be a reliable biomarker of oocyte quality as it allows not only quantification of the ovarian reserve , but also the prediction of an eventual ovarian response to the IVF stimulation. A prior measurement of AMH could enable a more appropriate formulation of gonadotropin doses.

***Keywords*---**AMH levels, oocyte quality, controlled ovarian, stimulation.

Introduction

To obtain higher success rates in Assisted Reproductive Techniques (ART), in vitro fertilization (IVF) programs must invest into improvement of various limiting factors including oocyte quality and endometrial receptivity. Indeed, only a small percentage of oocytes collected lead to the birth of a child. While techniques have evolved to diagnose low ovarian reserve[1,2], there have been few markers for the individual evaluation of oocyte quality. The polar body biopsy[3] or the analysis of cumulus oophorus[4] are both time consuming and cumbersome techniques rendering them difficult to be used routinely. The AMH, discovered in 1947 by Alfred Jost, is a homodimeric glycoprotein whose gene is located on the chromosome 19p13.3. and belongs to a TGF beta family growth factor[5,6]. Its function was initially noted as being exclusively responsible for female Mullerian duct regression[7]. Ovarian aging is characterized by a gradual decrease in both quantity and quality of the oocytes residing within the follicles. The availability of a test able to provide reliable information with respect to a woman's ovarian reserve within a given age category is of clinical importance. AMH is a known quantitative biomarker of the ovarian reserve.[8-12] Nevertheless, its ability to determine oocyte competence is a matter of debate.[13-16] The present study was conducted to determine the association of serum AMH levels with oocyte quality in infertile women who underwent controlled ovarian stimulation (COS) for IVF cycles.

Objectives

- Measure the serum AMH levels prior to COS
- Classify the study population according to the AMH levels
- Correlate the AMH levels to the oocyte number, dose of gonadotropin needed, number of days of stimulation, number of oocytes retrieved and oocyte quality.

Material and Methods

The present retrospective study conducted in_IVF Department of Nowrosjee Wadia Maternity Hospital for 10 months. Total 100 patients were included in the study. The primary end point of the study was the oocyte quality. Fertilization, embryo quality and clinical pregnancy rate were evaluated as secondary outcomes. Patients of age between 21-39 years, patients with first or second ICSI cycle, Tubal factor infertility and unexplained infertility were included in the study. Patients with three or more than three failed ICSI cycles,_Severe male factor infertility requiring sperm retrieval procedures, Women with genetic abnormalities, Previous ovarian surgery, history of endometriosis, Previous history of irradiation therapy, Previous history of gonadotoxic chemotherapy, Polycystic ovaries were excluded from the study.

Methodology

Serum AMH levels and number of antral follicle (on second day of menstrual cycle) on transvaginal sonography was done for all patients as a part of basic infertility workup. Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using the AMH/MIS ELISA kit (Immunotech-Beckman). Patients were classified into five categories based on the reference range of the AMH levels used in our hospital.

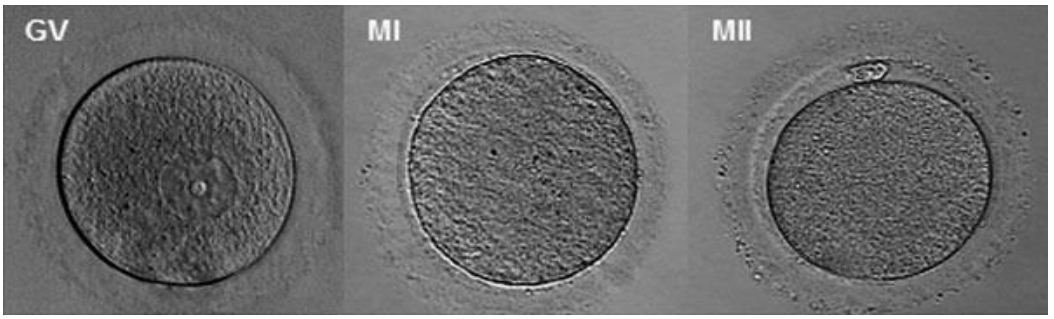
Ovarian fertility potential	AMH (ng/ml)
Very low/ undetectable fertility	0.0-0.3
Low fertility	0.3-2.2
Satisfactory fertility	2.2-4.0
Optimal fertility	4.0-6.8
High level	>6.8

The patients were down regulated using combined oral contraceptive pills for 21 days. COS was done using the antagonist protocol starting from the second day of the subsequent menses. The drugs used for stimulation were intramuscular injections of highly purified human menopausal gonadotropin (Injection Menotas, Intas Pharmaceuticals Ltd) starting with dose of 300 IU and increasing up to 450 IU as per the patient response. Serial transvaginal ultrasound was performed to monitor follicle growth. The antagonist used was subcutaneous injections of cetrorelix in a dose of 0.25mg (InjCetrolix, Intas Pharmaceuticals Ltd.), using the flexible protocol i.e. when the leading follicle >14mm. The drug used for triggering final maturation of follicles was subcutaneous injection of leuprolide acetate (InjLupride, Intas Pharmaceuticals Ltd) in a dose of 2mg (Agonist trigger). Trigger was given once the leading follicles were in the range of 18mm while others in ranges of 14-15mm. Oocyte retrieval was performed 34-36hours after the trigger by standard transvaginal method. The follicular fluid was scanned for oocytes. The retrieved oocytes were maintained in culture medium (Global® for fertilisation, LifeGlobal, Connecticut,USA) supplemented with 10% protein supplement (LGPS,LifeGlobal, Connecticut, USA) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, Connecticut, USA) for two to three hours before the removal of cumulus cells. The surrounding cumulus cells were removed after

exposure to a HEPES-buffered medium containing hyaluronidase (80IU/mL, LifeGlobal, Connecticut,USA). The remaining cumulus cells were gently removed with a denuding pipette.[7] The oocytes were examined for their morphology under Olympus Inverted Microscope.

Oocyte grading

Oocytes were divided according to the stage of maturation (GV/M1/M2)



Morphological evaluation

- Normal
- Cytoplasmic anomalies
- Extra cytoplasmic anomalies

Subgroup analyses were performed according to five AMH ranges

- group 1- very low (<0.3 ng/ml)
- group 2- low (0.3-2.2 ng/ml)
- group 3- satisfactory (2.2-4 ng/ml)
- group 4-optimal (4-6.8 ng/ml)
- group 5-high (>6.8 ng/ml)

Observed variables included

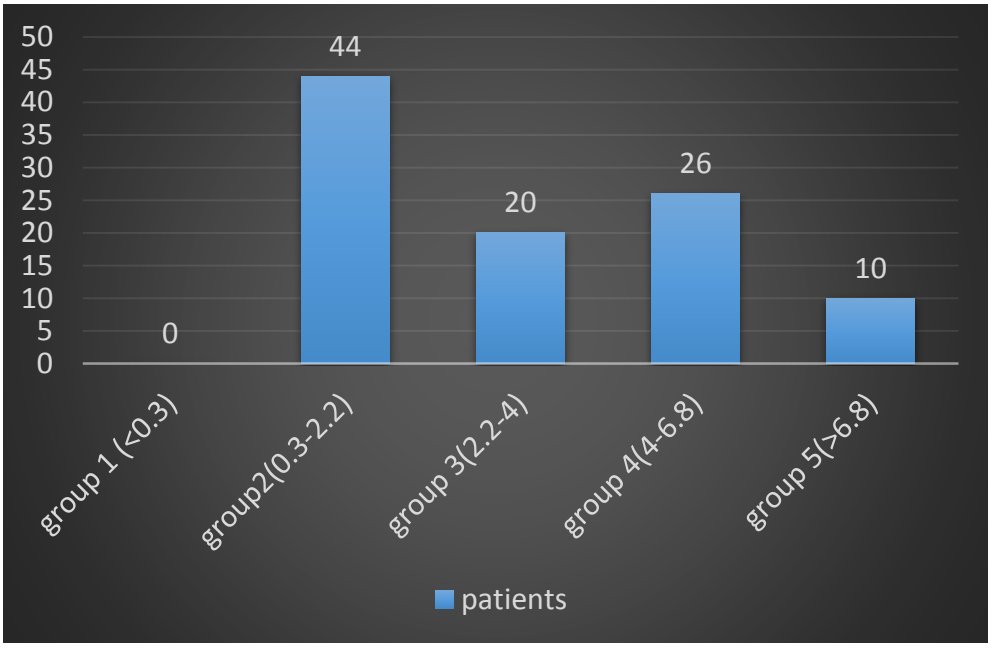
- Patient's age
- Antral follicle count
- BMI
- Dose and number of days of stimulation
- No. of oocytes retrieved
- No. of M1,M2, GV
- Fertilization was checked followed by embryo quality but was not considered as the sole purpose of our study.

Statistical analysis was performed by ANOVA. Significance was attributed to events with a $p<0.05$.

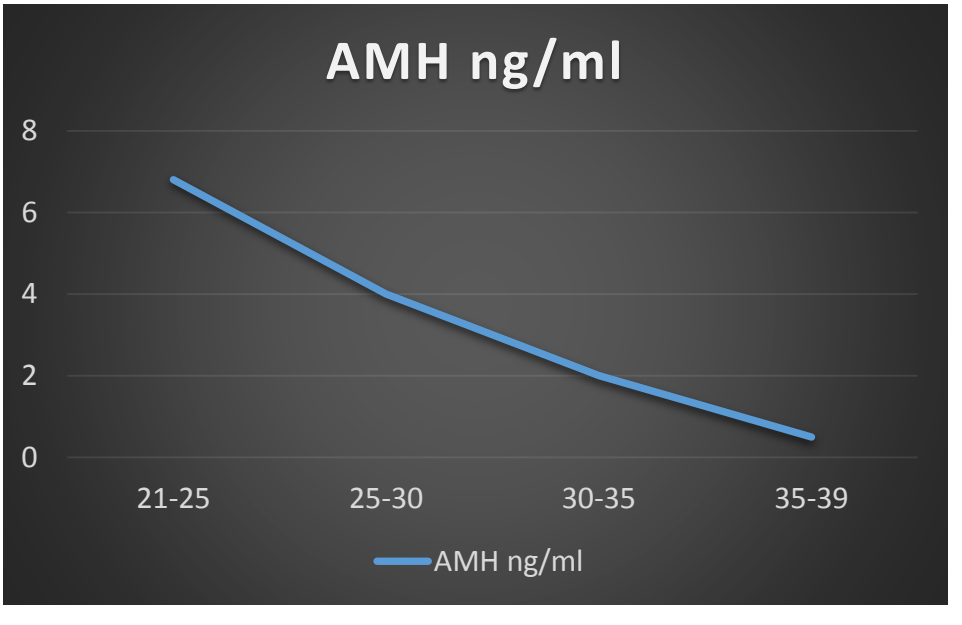
Results

A Total 100 patients were studied. The patients were divided into five groups based on AMH levels; the groups had 0, 44, 20, 26 and 10 patients respectively.

Graph 1. distribution according to AMH levels

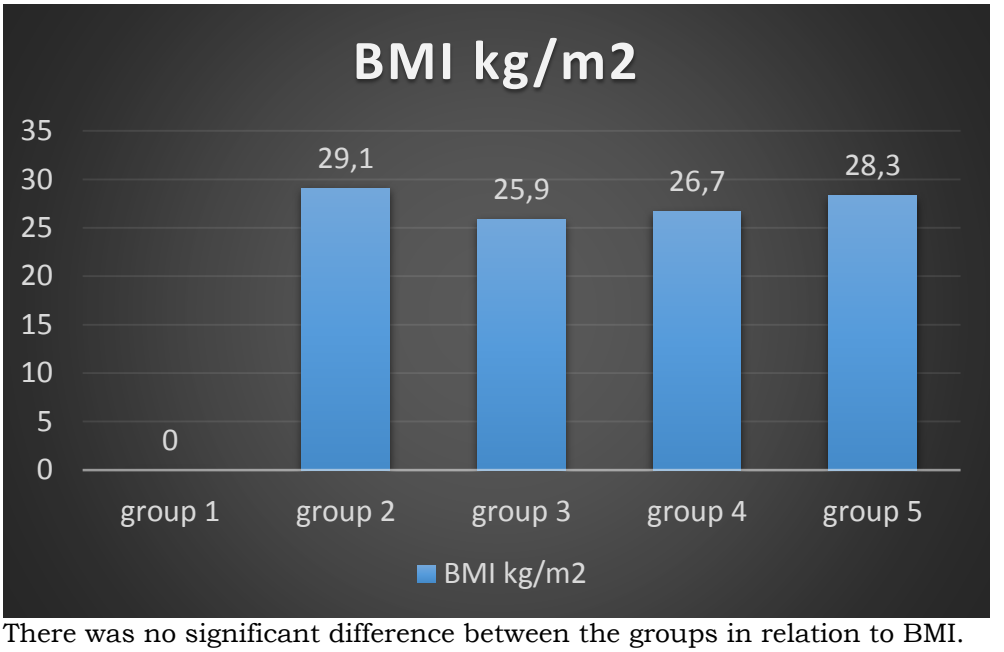


Graph 2. AMH in relation to age groups



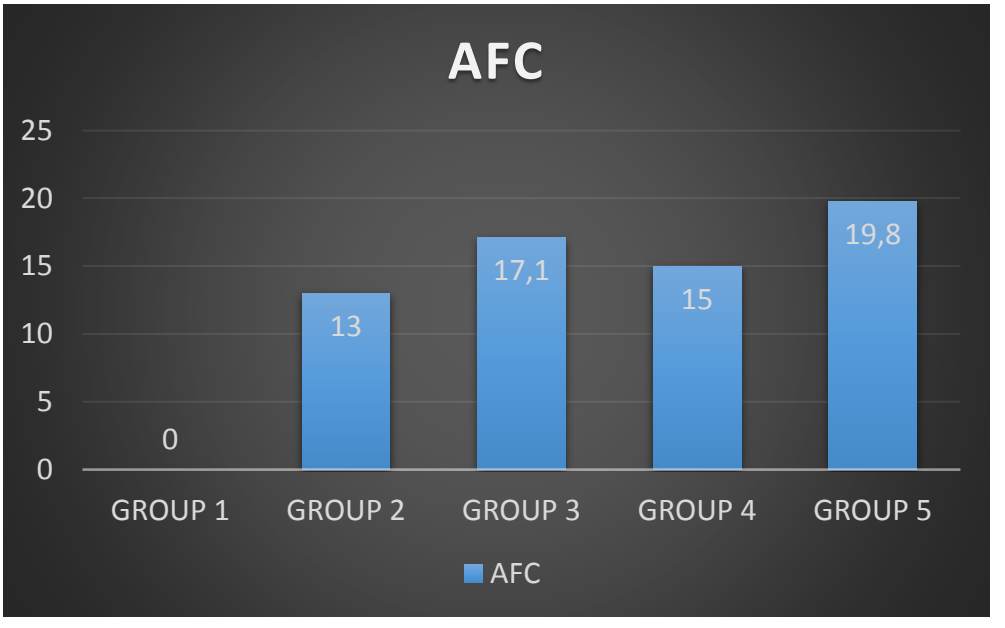
Age was found to be independent predictor of ovarian reserve.

Graph 3. Groups according to BMI



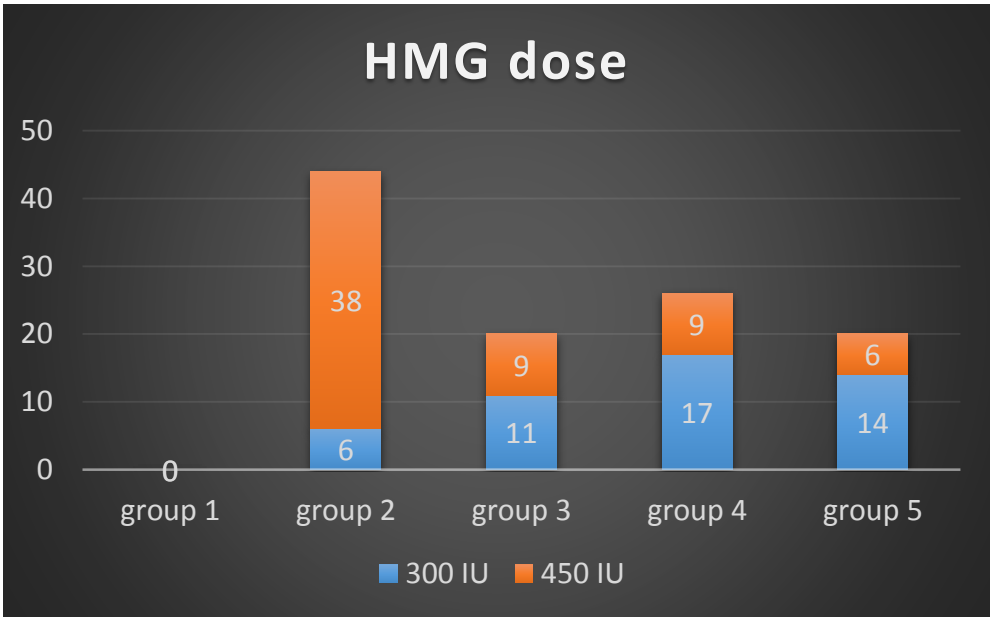
There was no significant difference between the groups in relation to BMI.

Graph 4. Antral follicle count in different groups



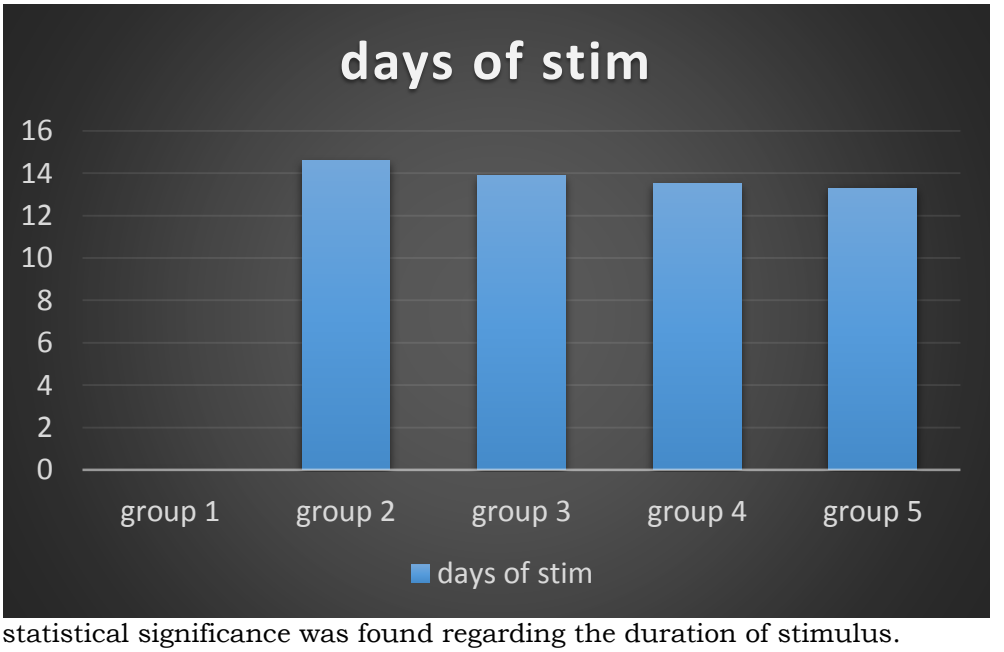
Statistically significant difference was seen for antral follicle count when group 2, 3 and 5 were compared ($p>0.001$).

Graph 5. Human gonadotropins in different groups



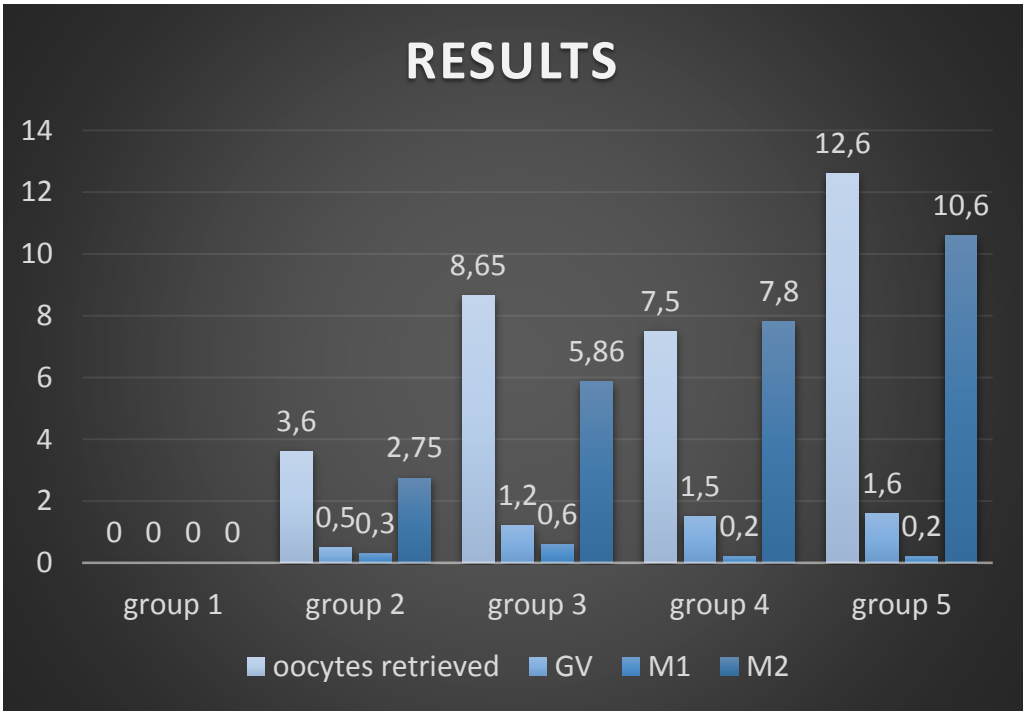
Significant difference was observed in group 2 when compared with group 3,4 and 5 regarding the dose of gonadotropins.($p<0.05$).

Graph 6. Days of stimulus



No statistical significance was found regarding the duration of stimulus.

Graph 7. Oocytes retrieved



There was signifigant difference in total number of oocytes retrieved and metaphase 2 oocytes between all the groups ($p<0.0001$).
Age, antral follicle count, dose of HMG, number of days of stimulation, number of oocytes retrieved and number of metaphase 2 oocytes were thus found to be associated with AMH levels.

Table 1
Oocyte quality

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
TOTAL M2 OOCYTES	0	121	117	150	106
NO ANOMALIES	-	95	110	132	98
CYTOPLASMIC ANOMALIES	-	9	1	6	2
EXTRA CYTOPLASMIC ANOMALIES	-	17	6	12	6
FERTILISED	-	99	92	113	88

CLEAVED	-	87	64	88	56

Oocyte morphology was not related to fertilization rate. Significant reduction in cleavage rate was found in M2 oocytes with anomalies compared to those without anomalies. (p=0.03). A significantly reduced quality of embryos were observed when embryos developed from oocytes with one or more anomalies compared with those from normal oocytes. (p<0.001). The cleavage rate(p<0.05) and number of good quality embryos(p<0.001) was significantly higher in the group with extra cytoplasmic anomalies compared with cytoplasmic anomalies. Clinical pregnancy rate(33%) was significantly low in group 2 compared to 3,4 and 5.

Discussion

Our study suggests that AMH could be a reliable biomarker of oocyte quality. Interestingly, we found that the mean implantation rate was lower for patients younger than 35 years with AMH<1 ng/ml. In addition, for patients with serum AMH <0.46 ng/ml (<25th percentile), the study showed that they sustain six times more chances of having a cycle cancellation, twice less likely to perform an embryo transfer, to obtain frozen embryos and to obtain an ongoing pregnancy independent of their age, total exogenous gonadotropins dosage and the number of eggs retrieved after a first IVF, compared to our reference population (25–75th percentile). AMH has demonstrated its effectiveness in many areas. It is the best hormonal marker of ovarian reserve.[8-11,16,17] Moreover, its stable value in the menstrual cycle allows a more objective evaluation independent of cycle day.[11-13,15,18] AMH is a useful tool to predict low response to controlled ovarian stimulation and conversely the risk of excessive response.[17,19] A prior measurement of AMH could enable a more appropriate formulation of gonadotropins doses.[13-15] A prior measurement of AMH could enable a more appropriate formulation of gonadotropins doses.[13-15] Other applications included diagnosing patients with polycystic ovaries syndrome with AMH higher than 4–5 ng/ml[20] and conversely low or normal AMH could be found in specific causes of primary ovarian insufficiency patients.[21-22] Nevertheless, its ability to determine the oocyte competence is a matter of debate.[2]

The dogma that AMH doesn't predict pregnancy rates has often been adopted. Smeenk et al. (2007)[23] showed that basal AMH is not related to embryo quality nor to the probability of achieving a pregnancy. Nelson et al. (2007)[24] explained the close relationship between AMH and the pregnancy rate in IVF through the oocyte yield. Recent studies concluded that AMH could be a reliable biomarker of oocyte quality.[25,26] Hazout et al. (2004)[27] demonstrated an association between AMH level on day 3 and the number of mature oocytes, the number of embryos and clinical pregnancy rates in 109 IVF patients. Blazar et al. (2011)[28], showed in a prospective study including 190 IVF cycles that AMH is a useful predictor of cycle outcome and strongly predicts an increased number of oocytes and ongoing pregnancy (P<0.0001). In 2011, Irez et al.[29], demonstrated that AMH levels may predict the oocyte quality. Wang et al. (2010)[30] found that for women between 34 and 41 years of age, AMH concentrations was associated withgreater chances of pregnancy (P<0.01). To the best of our knowledge, this is the largest retrospective

analysis investigating the relationship between serum AMH and the rate of ongoing pregnancy after first IVF cycles.

Our findings imposes a greater duty to cautiously counsel our infertility patients prior to initiating sIVF cycle and more specifically in patients with AMH<0.47 ng/ml of the poorer prognosis compared to our reference population. Accordingly, physicians would be able to provide a better counseling for their patients. AMH level could be a useful tool to adjust the number of embryo transferred for patients younger than 35 years. Therefore, elective double embryo transfer (eDET) should be evaluated for patients in this category of age with anticipated poor prognosis in a future randomized control trial. AMH plays a major role in assisted reproductive technology. It allows not only the quantification of the ovarian reserve, but also the prediction of an eventual ovarian response to the IVF stimulation. Our results suggest that AMH is a reliable biomarker of oocyte quality. We emphasize the fact that each fertility center should develop his own normogram of AMH stratified by age in order to be able to deliver a better patient counselling. Further, we believe in a score based on clinical (patient age) biological (AMH) and sonographic (automated 3D AFC) features. These three elements are the cornerstone that could enable a more individualised patient's management based on oocyte quality.

Conclusion

- AMH can be a reliable biomarker of oocyte quality as it allows not only quantification of the ovarian reserve , but also the prediction of an eventual ovarian response to the IVF stimulation.
- AMH is useful predictor of quality of ovarian response to stimulation, independently from patient age.
- AMH is useful tool to predict low response to controlled ovarian stimulation as well as excessive response.
- A prior measurement of AMH could enable a more appropriate formulation of gonadotropin doses.
- We emphasize the fact that each fertility center should develop their own normogram of AMH stratified by age for better patient counselling.

Wider implications of the findings

- Our study can help us individualize the dose of gonadotropins used for ovarian stimulation according to AMH levels of the patients in order to optimize the oocyte retrieval rate and subsequently pregnancy rates.
- Optimization of the oocyte retrieval would also help to minimize the incidence of ovarian hyperstimulation syndrome.

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