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Does Anti Mullerian Hormone value affect oocyte quality?

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ABSTRACT

AIM: To identify correlation between serum levels of Anti-Müllerian hormone (AMH) and oocyte quality among all infertile patients who underwent oocyte retrieval in department of Institute of Reproductive Medicine, Madras Medical Mission hospital.

METHODS: This is a retrospective observational study, including all subfertile women who underwent oocyte retrieval from 2019 to 2021 in the age group of 21 to 40 years. The study population will be divided into four groups based on AMH value. Group A (< 1.2 ng/ml), Group B (1.2 – 3.5 ng/ml), Group C (3.6 – 5 ng/ml) and Group D (>5 ng/ml). Controlled ovarian stimulation was done using antagonist protocol. Oocytes were retrieved after 35 to 36 hours and quality of oocytes were assessed based on six parameters and classified as good, average and poor.

RESULTS: 218 patients' records were analysed. The AMH values ranged from 0.13 to 9.3 ng/ml with a median of 2.6 ng/ml. Significant positive correlations were seen on comparing serum AMH levels with AFC, E_2 on the day of trigger, number of aspirated follicles, number of retrieved oocytes and number of mature oocytes. Poor quality oocytes and embryos were significantly higher in extremes of AMH values.

CONCLUSION: Serum AMH levels correspond to the number of antral follicles and is a reliable predictor of ovarian reserve and response to COH. Major variations of AMH levels will affect the number of follicles, total oocytes, mature oocytes collected and quality of oocytes and embryos. The ovarian response and success of IVF cycle depends, not only on the size of primordial follicle pool but also the quality of oocytes.

Keywords: Anti-Mullerian hormone, oocyte, embryo, pregnancy.

I. INTRODUCTION:

To obtain higher success rates in Assisted Reproductive Techniques, various factors like ovarian reserve, number of oocytes retrieved, oocyte quality and endometrial receptivity play an important role.The Anti-mullerian hormone, also known as Mullerian inhibiting substance, discovered in 1947 by Alfred Jost, is a peptide growth factor. It is a Dimeric glycoprotein, belonging to Transforming Growth Factor-beta family. It is produced by pre-antral and small antral follicles of the granulosa cells. The hormone production peaks after puberty and is characterized by a steady decline towards menopause when serum concentration becomes undetectable. Ovarian aging is characterized by a gradual decrease in both quantity and quality of the oocytes. [1]

AMH is a known quantitative biomarker of the ovarian reserve and correspond to the number of antral follicles and, therefore, provide a powerful means for predicting ovarian response to COH. Nevertheless, its ability to determine oocyte quality is a matter of debate. This retrospective study was undertaken to evaluate the impact of varied levels of serum AMH on oocyte quality and developmental competence.

II. MATERIALS AND METHODS

OBJECTIVE

This study aimed to identify a possible correlation between serum levels of anti-Müllerian hormone (AMH) and oocyte quality, embryo quality and implantation potential.

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STUDY DESIGN

This Retrospective observational study was conducted in the Department of Institute of Reproductive Medicine, Madras Medical Mission hospital. All subfertile women who underwent oocyte retrieval from January 2019 to December 2021 between the ages of 21 to 40 years were included in the study. Patients with endometriosis and women with known history of poor quality oocytes and oocyte donors were excluded from the study.

III. METHODOLOGY

AMH was measured using the enzyme amplified immunoassay (ELISA) between day 2 and day 5 of the menstrual cycle along with other predictors of ovarian reserve such as Serum FSH, Estradiol (E2) and Antral follicular count (AFC) measurements, within 3 to 6 months before starting ovarian stimulation. A transvaginal ultrasound scan was performed by an experienced ultra-sonographer to assess the AFC where the overall number of antral follicles sized between 2mm and 9 mm were counted in both ovaries.

Patients were subdivided into four groups based on AMH values as group A (<1.2 ng/ml), group B (1.2-3.5 ng/ml), group C (3.6-5 ng/ml) and group D (>5ng/ml).

All the patients were stimulated using a GnRH antagonist protocol. In this regimen, recombinant FSH or highly purified HMG was started on day 2 of the cycle and GnRH antagonist was administered after 5–6 days of stimulation depending on the presence of a 12–13 mm follicle in the ultrasound scan. Trigger was given using HCG alone or in selected patients with dual trigger or agonist trigger using Triptorelin. Oocyte retrieval was done 35 to 36 hours after trigger. After a 2 to 4 hours incubation, oocytes were denuded from their cumulus complex using the enzyme hyaluronidase and glass pipettes. Following this denudation, maturation and morphological features of the oocytes were investigated immediately before ICSI using 6 parameters and oocytes quality was graded as good, average and poor.[2] (Table 1.)

Table 1: Parameters determining oocyte quality:

PARAMETER	GOOD QUALITY	AVERAGE QUALITY	POOR QUALITY
Oocyte morphology	Normal oocyte coloration , round shape	Less dark general oocyte coloration and less ovoid shape	dark general oocyte coloration and/or ovoid shape
Oocyte size	> 130 µm and <150 µm	Size did not deviate from normal by more than 10 µm	Size below 120µm or greater 160µm
Ooplasm	Absence of granularity and inclusions	Slightly granular and/or demonstrated only few inclusions	Very granular and/or very vacuolated and/or demonstrated several inclusions
PVS	Normal size PVS with no granules.	Moderately enlarged PVS and/or small PVS and/or a less granular PVS	Abnormally large PVS, an absent PVS or a very granular PVS.
Zona pellucida	Normal zona (> 12 µm and <18 µm)	Did not deviate from normal by more than 2 µm	Very thin or thick (<10µm or >20µm)
Polar body	Round shape and clear borders	Fair but not excellent	Flat and/or multiple PBs, granular and/or

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			either abnormally small or large PBs

The assessment included 2337 oocytes obtained from 218 patients undergoing ICSI cycles. Analysed anomalies included dark central granulation of the cytoplasm, refractile bodies, vacuoles, aggregation of smooth endoplasmic reticulum (sER) and perivitelline space granularity and zona elasticity.

Fertilization rate, which was assessed 16 to 18 h after ICSI, was characterized by the presence of two pronuclei. Early embryo development was assessed by the number of blastomeres and the percentage of fragmentation, 42– 44 h after ICSI and the embryos were categorized as grade A, B and C according to Gardner Embryo Grading System.

Clinical pregnancy was defined by the presence of a gestational sac with heartbeat viewed on ultrasound examination 4-6 weeks after embryo transfer.

We have analysed the correlation between serum levels of AMH with (i) maternal age, (ii) maternal BMI, (iii) Antral follicular count (iv) FSH dose for ovarian stimulation (v) number of oocytes aspirated (vi) number of retrieved MII mature oocytes, (vii) oocyte quality, (viii) fertilization rate, (ix) number of obtained embryos, (x) embryo quality and (xi) clinical pregnancy rate.

Data were analysed using SPSS software (version 17). The statistical methods used were Chi square test, ANOVA test and post HOC test for multiple comparisons. Significance was defined as $p< 0.05$.

IV. RESULTS

218 patients' records were analysed. Among this study population 50 patients had AMH of < 1.2 pg/ml, 83 patients had AMH of 1.2 to 3.5 pg/ml, 46 patients had AMH between 3.6 to 5 ng/ml and 39 patients had AMH of >5 ng/ml.

The mean (±SD) value of day 3 AMH for all 218 patients was 3.21 (±2.4) ng/ml. The range, however, was from 0.13 to 9.3 ng/ml with a median of 2.6 ng/ml

We found that majority of participants who had irregular menstruation and PCOS had higher AMH levels ($p<0.05$) and there was no statistical significance between AMH and type and cause of subfertility. (Table 2)

HISTORY		AMH (ng/ml)				P value
		<1.2	1.2-3.5	3.6-5	>5	
Menstrual history	Regular	40 (28.6%)	61 (43.6%)	26 (18.6%)	13 (9.3%)	<0.001
	Irregular	10 (12.8%)	22 (28.2%)	20 (25.6%)	26 (33.3%)	
Type of infertility	Primary	38 (26.6%)	52 (36.4%)	28 (19.6%)	25 (17.5%)	0.360
	Secondary	12 (16%)	31 (41.3%)	18 (24%)	14 (18.7%)	
Cause of subfertility	Male	14 (20%)	32 (45.7%)	18 (25.7%)	6 (8.6%)	0.052
	Female	22 (30.6%)	27 (37.5%)	10 (13.9%)	13 (18.1%)	
	Combined	11 (37.9%)	10 (34.5%)	5 (17.2%)	3 (10.3%)	
	Un explained	3 (6.4%)	14 (29.8%)	13 (27.7%)	17 (36.2%)	
PCOS	Present	6 (6.3%)	21 (21.9%)	30 (31.3%)	39 (40.6%)	<0.001
	Absent	44 (36.1%)	62 (50.8%)	16 (13.1%)	0	

Table 2: AMH and basic parameters:

We found that maternal age had an inverse correlation with AMH and was statistically significant. Requirement of FSH dose and LH dose for controlled ovarian hyperstimulation (COH) decreased with increasing AMH levels. Significant positive correlations were seen between serum AMH levels and AFC, E_2 on the day of trigger, number of aspirated follicles, number of retrieved

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oocytes and number of mature oocytes where all these were statistically significant when one-way ANOVA was applied ($p<0.05$) . (Table 3) (Fig.1,2,3)

Mean ± SD	AMH (ng/ml)				P value
	<1.2 (n=50)	1.2-3.5 (n=83)	3.6-5 (n=46)	>5 (n=39)	
Age	34.9±4.8	32.6±3.6	30.1±4.6	31.7±4.2	<0.001
BMI	27.11±3.9	28.4±4.9	28.3±4.9	28.9±3.8	0.251
AFC	7.6±4.3	12.2±5.5	17.8±6.4	22.03±6.6	<0.001
FSH dose (IU)	4316.7±785.9	3994.5±714.8	3473.8±819.2	3677.3±727.4	<0.001
LH dose (IU)	3283±1111.6	2860.6±1117.9	2525.5±1193.3	2449.03±1415.3	0.003
E_2 (pg/ml) on day of trigger	1675.8±1191.4	2921.4±1902.8	4304.2±2117.2	6652.2±2620.3	<0.001
Agonist trigger	0	1 (16.7%)	4 (66.7%)	1 (16.7%)	<0.001
Dual trigger	21 (16.9%)	42 (33.9%)	29 (22.6%)	34 (26.6%)	
Hcg trigger	29 (34.1%)	40 (47.1%)	12 (14.1%)	4 (4.7%)	
No. of follicles	6.06±3.6	9.94±5.03	14.2±7	18.46±5.03	<0.001
No. of oocytes	5.48±3.6	9.51±4.8	13.57±7.02	16.67±5.4	<0.001
No. of metaphase II oocytes	4.56±3.2	7.43±4.2	10.7±4.4	13.7±4.5	<0.001

Table 3: Comparison of demographic data and controlled ovarian stimulation (COH) details with respect to anti-Müllerian hormone (AMH) group affiliation.

Figure 1: Correlation between AMH and Antral follicular count

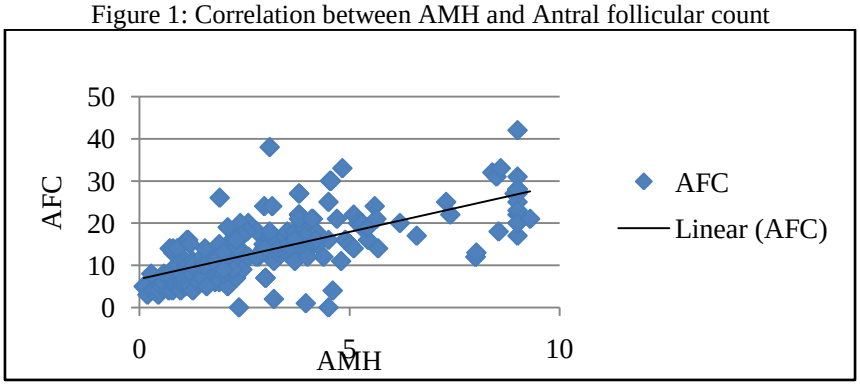

Figure 2:Correlation between AMH and number of oocytes retrieved

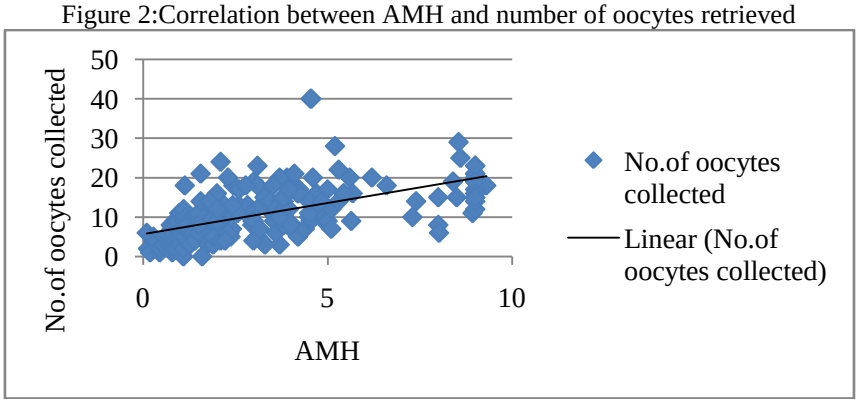

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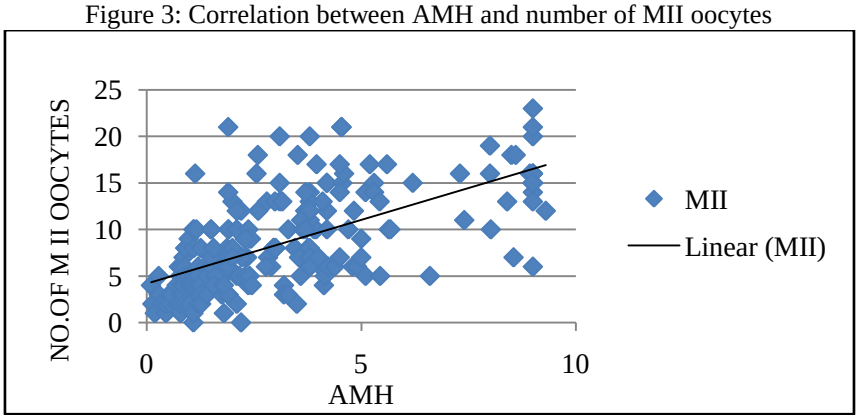
Figure 3: Correlation between AMH and number of MII oocytes



Focusing on oocyte and embryo quality there was a significantly higher number of good quality oocytes and embryos in the normal range AMH (Group B) while the number of poor quality oocytes and embryos were higher in Group D with higher AMH values (p< 0.01). (Table 4, Fig4, Fig5)

Table 4: Comparison of AMH and quality of oocytes and embryos

		AMH (ng/ml)				P value
		<1.2	1.2-3.5	3.6-5	>5	
Oocyte quality	**Good**	0.38±0.85	3.92±2.79	2.79±2.96	2.54±3.73	**<0.001**
	Average	2.76±2.01	3.9±2.52	6.2±3.36	7.08±3.76	**<0.001**
	Poor	1.2±1.34	0.77±1.1	1.7±1.68	2.92±2.45	**<0.001**
Embryo quality	**A (Good)**	0.84±0.98	5.66±2.97	4.93±3.25	4.10±3.23	**<0.001**
	B (Average)	2.44±1.92	3.45±2.09	4.65±2.58	5.64±3	**<0.001**
	C (Poor)	0.89±1.1	0.25±0.56	0.78±1.04	2.03±1.56	**<0.001**

Figure 4:AMH and oocyte quality

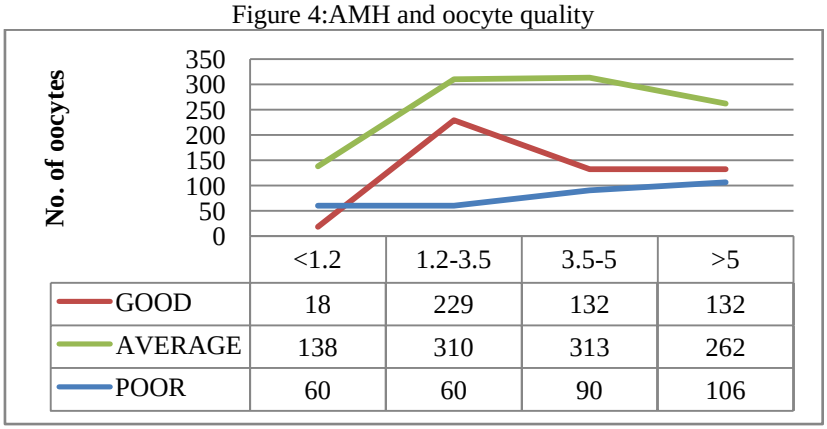


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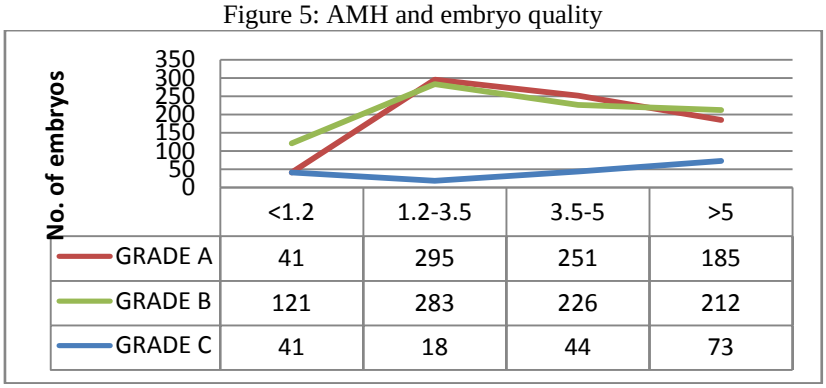
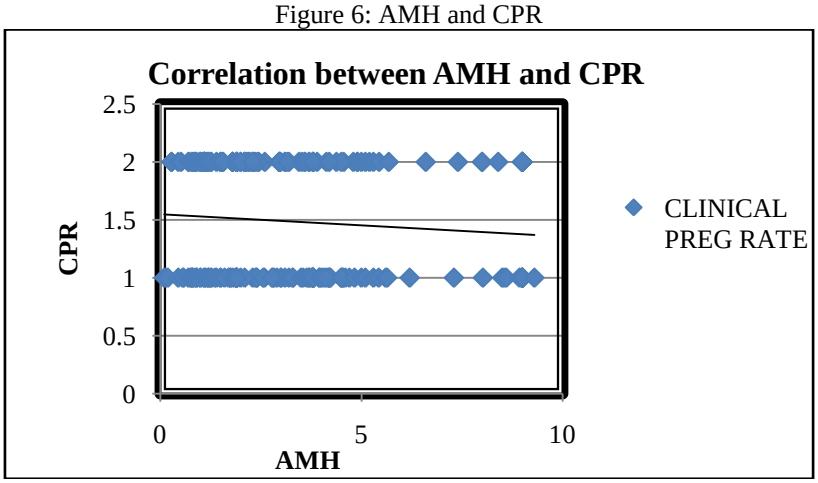
Figure 5: AMH and embryo quality



	<1.2	1.2-3.5	3.5-5	>5
GRADE A	41	295	251	185
GRADE B	121	283	226	212
GRADE C	41	18	44	73

The clinical pregnancy rate (CPR) had no significant correlation with AMH values (p - 0.343) (Fig 6), but the number of pregnancies were more in the normal AMH group (37.9%) and miscarriage rate was more in group A (26.7%) and group D (40%).

Figure 6: AMH and CPR



V. DISCUSSION

AMH is the best hormonal marker of ovarian reserve [3-6]. Its stable value in the menstrual cycle allows a more objective evaluation independent of cycle day [7,8]. There is a linear decrease in AMH over age after reaching a maximum in the mid-twenties, indicating that AMH exactly mirrors the decrease in the follicular pool with time, and thus, it is one of the preferable ovarian markers.AMH and AFC are predictive of ovarian response in ART cycles and indirectly predict ART success rates and therefore can predict a woman's total fertility potential. Women having low age-specific AMH and AFC are at a high risk of premature and rapid loss of fecundity, early premature ovarian insufficiency and early menopause [9,10].

In our study 66% of women more than 35 years of age presented with low AMH values (<1.2 ng/ml) and we found a positive correlation between AMH and AFC which was reliable indicator of ovarian response to stimulation.

Overweight and obesity are increasingly common health problems associated with increased risks of morbidity, lower quality of life and metabolic and reproductive health concerns. The potential effects of obesity on reproductive health may be very complex, given that different hormones, like insulin, leptin, and adiponectin, may affect follicle growth, corpus luteum function, early embryo development, trophoblast function and endometrial receptivity. So, BMI may influence fertility in terms of follicular development or endometrium receptivity [11, 12] but its effect on ovarian reserve is yet to be proven. Albu ét al reported that AMH is positively correlated with BMI, especially in infertile patients younger than 35 years of normal weight and with normal ovarian reserve [13]. Sahmay and Halawaty ét al. reported no relationship between these two markers [14]. In our study we found no correlation between AMH and BMI.

Ali Abara ét al in their cohort study of 187 patients suggested that AMH is a strong

predictor for menstrual disturbance due to PCOS and that the risk of menstrual disturbance was increased with the degree of elevation of AMH.[15] Our study also showed that women with higher AMH levels had increased rates of menstrual disturbance and an increased number of features of PCOS. This would indicate that PCOS affects up to a fifth of women of reproductive age and is the commonest cause of anovulatory subfertility. Also women with PCO have LH-predominant gonadotropin secretion leading to increased rates of menstrual disturbance.

Serum AMH is strongly correlated with the size of the primordial follicle pool since it is secreted by the developing follicles and there is a good correlation between AMH and AFC. During COH these antral follicles will respond to the gonadotropins and grow to form the dominant follicle pool. Hence AMH and AFC are useful in predicting low and high response to controlled ovarian stimulation. The initial assessment of AMH helps in deciding the initial dose of gonadotropins in COH. AMH also helps in predicting risk of premature ovarian failure [16,17]. In our study we found that the women with lower AMH needed higher doses of FSH and as AMH increased FSH dose requirement reduced.

In a retrospective, observational study by Kozlowski IF ét al, which included the data of about 1500 patients, a positive correlation was found between serum AMH levels and total number of oocytes and mature oocytes retrieved from stimulated cycles [18]. We found a positive correlation between AMH and AFC, number of total oocytes and MII oocytes retrieved as well. This proves that the number of antral follicles available at the time of stimulation is very important in determining the final number of oocytes retrieved.

Both extreme forms of ovarian response (hypo and hyper-response) may be associated with diminished oocyte quality [3,19], we had hypothesized that extreme levels of AMH might have a negative effect on oocyte and embryo development. Our results demonstrated that the number of poor quality oocytes were higher with AMH value of >5 ng/ml and poor quality embryos were high in both low (<1.2ng/ml) and high (>5ng/ml) AMH values.This is because, in PCOS patients, chronic inflammation and reactive oxidative species can result in alteration of normal ovarian follicular dynamics thus impairing oocyte maturation and quality.

Morales ét al, in their retrospective study concluded that Anti-Müllerian hormone levels correlated with embryo quality on Day 5, but had no correlation with embryo quality on Day 3 or pregnancy rate.[20].In our study embryo quality had a positive correlation with the AMH value, with poor quality embryos being more in the extremes of AMH values especially when AMH >5 ng/dl. This could provide us with a very vital information that low or high AMH can cause a decrease in oocyte quality producing poor quality embryos.

Tal ét aland Umarsingh ét al, have described AMH as a weak predictor for clinical pregnancy and we found no differences between pregnancy rates in our results. [21, 22]

VI. CONCLUSION

Serum AMH levels correspond to the number of antral follicles andis a reliable predictor of ovarian reserve and response to COH. Major variations of AMH levels will affect the number of follicles, total oocytes, mature oocytes collected and quality of oocytes and embryos.This helps us to anticipate the patient's response to stimulation and also poor quality oocytes and outcome in patients with AMH <1.2 ng/ml and > 5 ng/ml, thus emphasizing the need for proper patient counselling regarding chances for cancellation of cycles, hyper-response, prognosis and IVF outcome before starting a stimulation cycle.

We need further studies to prove the effect of AMH on the embryo quality and clinical pregnancy rate.

LIMITATIONS: Need a larger sample size. Quality of embryos can be affected by various other factors like male factor, which was not excluded in my study.

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Ethical approval: Taken

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