



# EVALUATING EFFECTS OF LOW SERUM AMH ON OOCYTE MATURATION, EMBRYO DEVELOPMENT AND CLINICAL OUTCOME IN ASSISTED REPRODUCTIVE TECHNIQUES

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## Abstract

In the current scenarios, female infertility is on a rise. There are many causes of female infertility, however, Low Serum AMH leading to Poor Ovarian Reserve is one of the leading cause of infertility in females with age 35 or less. AMH also known as Anti Müllerian Hormone is one of the key hormone for ovarian reserve markers. A lower level of AMH, often indicates poor ovarian reserve. A correlation between the low level of serum AMH, oocyte quality and quantity, embryo quality and clinical outcome is unclear yet. This study aims in finding out the correlation between the low level of serum AMH, oocyte quality and quantity, embryo quality and clinical outcome. A short retrospective cohort study is done and the patients are categorized in two groups - Group I having patients with low AMH (less than 1.1 ng/ml) and Group II having patients with normal AMH (more than 1.1 ng/ml). The oocyte quality and quantity, embryo quality and clinical outcome of the patients are assessed and statistical analysis is performed. The results indicate that there is a correlation observed between low serum AMH levels, oocyte quality and quantity, embryo quality and clinical outcome. With lower levels of AMH, there is a decrease in the quantity of oocytes as well as there is a decrease in the quality of oocyte and embryo, leading to an overall decrease in the clinical outcome.

**Keywords** - AMH, Oocyte quality, Oocyte quantity, Embryo quality and Clinical outcome.

## INTRODUCTION

Infertility is defined as the inability to conceive after having unprotected intercourse for a year. According to WHO 2022, infertility affects approximately in 6 couples. While infertility can arise due to various causes in both men and women, Assisted Reproductive Technologies (ART), including In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI), have emerged as effective solutions. IVF involves controlled ovarian stimulation, oocyte retrieval, fertilization, embryo culture, and embryo transfer to achieve successful pregnancy. AMH, produced by granulosa cells of small antral follicles, serves as a reliable marker of ovarian reserve and predicts the ovarian response to stimulation. AMH levels decline with age and low AMH is associated with diminished ovarian reserve (DOR), often leading to poor oocyte yield and compromised IVF outcomes (Cui et al., 2016). According to the Bologna criteria (ESHRE, 2010) and POSEIDON classification, patients with low AMH and poor ovarian response fall into distinct prognostic categories. (Ferraretti et al., 2011, Alviggi et al., 2016)

Oocyte quality, essential for successful fertilization and embryo development, is significantly influenced by AMH levels. Low AMH is correlated with poor nuclear and cytoplasmic maturation, leading to increased rates of abnormal oocytes. Furthermore, low AMH patients exhibit higher incidences of abnormal oocyte morphology, including variations in the perivitelline space (PVS), zona pellucida (ZP), polar body abnormalities, and cytoplasmic irregularities such as vacuoles, granulation, and SER aggregates. These abnormalities often result in reduced fertilization rates, impaired embryo quality, and lower implantation potential. Low AMH not only affects oocyte yield and embryo development but also contributes to suboptimal clinical outcomes, including lower



implantation rates, poor blastocyst formation, and increased miscarriage rates (Koysombat et al., 2023, Abu-Musa et al., 2020, Reig et al., 2023). Despite advancements in IVF protocols, achieving successful outcomes in low AMH patients remains a challenge, warranting further investigation into individualized ovarian stimulation and embryo transfer strategies.

The current study is aimed at finding a correlation between low AMH and its effects on oocyte quality and quantity, embryo fragmentation and clinical outcome.

## **METHODOLOGY -**

### **Study Design -**

This was a short retrospective cohort study done at Shachi Women's Hospital and DEV ART IVF Centre, Ahmedabad during the time period of December 2024 to March 2025. Patient selection - The patients when arrive for the first time at hospital, undergo transvaginal sonography to check for the AFC in both the ovaries. Once the sonography is done; the patient undergoes blood test to check for the level of Serum AMH. As soon as the report arrives, the patient is counseled for IVF stimulation.

### **Inclusion Criteria for Study -**

- a. Patients with primary infertility
- b. Patient with age  $\leq$  35 years of age
- c. Patient with AMH  $<$  1.1 ng/ml
- d. Patient with normal semen parameters

### **Exclusion Criteria for Study -**

- a. Patient with age more than 35 years
- b. Patient with abnormal semen parameters
- c. Patient with AMH  $>$  1.1 ng/ml
- d. Patient who have undergone any rejuvenation surgery
- e. Patient with previous obstetric history.

A total of 30 patients were analyzed in two groups.

Group 1 consisted of 15 patient with low AMH while Group 2 consisted of other 15 patients with normal AMH.

### **Ovarian Stimulation protocol -**

All the patients were stimulated with HMG injection of dose 450 for 5 days starting from day 2 of menses. They were stimulated with the same dosage for 5 days after which transvaginal sonography was performed to check for the follicular growth. Once, the follicles reached a size of about 15 mm or more, antagonist injection was added. The same dosage was carried on for next 5 days. Again on 10<sup>th</sup> day of menses, transvaginal sonography was performed. If the follicles reached 20 mm or more in size, final trigger injection was given which was decided based on the levels of hormone Estradiol. Ovum pickup was performed 34 hours post trigger.

### **Oocyte retrieval and ICSI -**

Oocyte retrieval was performed 34 hours post trigger. The medias were aliquoted and kept in CO<sub>2</sub> incubator overnight. Oocyte retrieval was performed under short general anesthesia. A needle was inserted through transvaginal sonography and all the follicles were retrieved out through suction. The fluid was scanned under stereozoom microscope to pool out all the COC's. Once all the COCs were retrieved, they were transferred to 1 ml one step media and kept in 6% CO<sub>2</sub> incubator until denudation. Denudation was performed 2 hours post retrieval by diluting Hylase in HEPES media. Post denudation, ICSI was immediately performed. The injected oocytes were kept in cultural plate and were cultured in Trigas incubator. ICSI was performed immediately post denudation in all the MII oocytes. The injected oocytes were then kept in culture dish for further development. Fertilization check was performed the next day. In group I, the embryos were frozen on day 3 that is at the cleavage stage while in group II, the embryos were frozen on day 5 that is at blastocyst stage. Embryo transfer was performed

after a month. The embryos were thawed and transferred as soon as the endometrium was of 7 mm or more in size.

A confirmatory blood report;  $\beta$ hCG was performed 15 days after the embryo transfer.

The statistical data was analyzed using Prism Graphpad software. One way ANOVA was performed. The p value was <0.0001.

## RESULT

The mean number of oocytes retrieved in Group I were  $4.26 \pm 2.05$ , while that in group II were  $19.13 \pm 8.9$ . The p value was found to be significant meaning, the data was statistically significant. (Graph 1)

The mean number of MII oocytes retrieved in Group I were  $3.13 \pm 1.35$ , while that in group II were  $13.67 \pm 8.02$ . The p value was found to be significant meaning, the data was statistically significant. (Graph 2)

The mean number of oocytes fertilized in Group I were  $2.33 \pm 1.23$ , while that in Group II were  $10.87 \pm 6.85$ . The p value was found to be significant meaning, the data was statistically significant. (Graph 3)

The quality of oocyte in Group I, was observed as poor. The oocytes were abnormal with either extra - cytoplasmic, cytoplasmic or in some cases both (Fig 1). The major cytoplasmic abnormality observed is CLCG that is cytoplasmic Granulation. Whereas, in patients with Group II, the quality of oocytes was good. However, a very few number of oocytes have some slight cytoplasmic abnormality while some have only extracytoplasmic abnormalities (Fig 2).

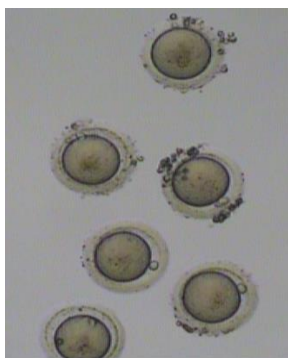


Fig 1 - Group I

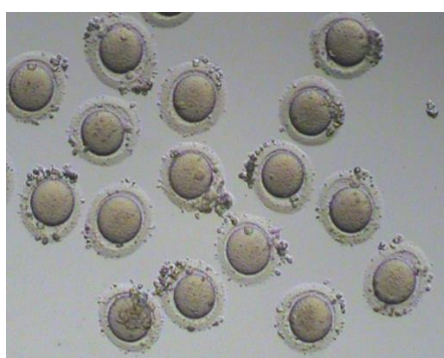


Fig 2 - Group II

The quality of embryo was average in Group I. On day 3, the embryos did reach 8 cell stage, but most of the embryos were fragmented leading to II and III grade embryos (Fig 3), while in Group II, the embryos were kept up to day 5 due to availability of more number of MII oocytes, thereby leading to more number of embryos. The majority of embryos were of good quality having good ICM and TE cells while few of the embryos were having compromised ICM and TE cells (Fig 4).

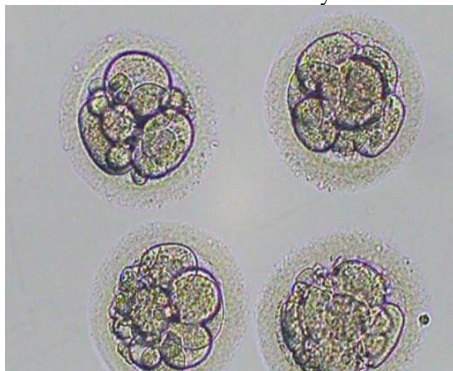


Fig 3 - Day 3 Embryo

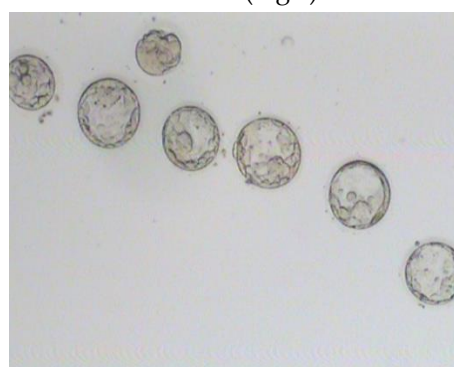
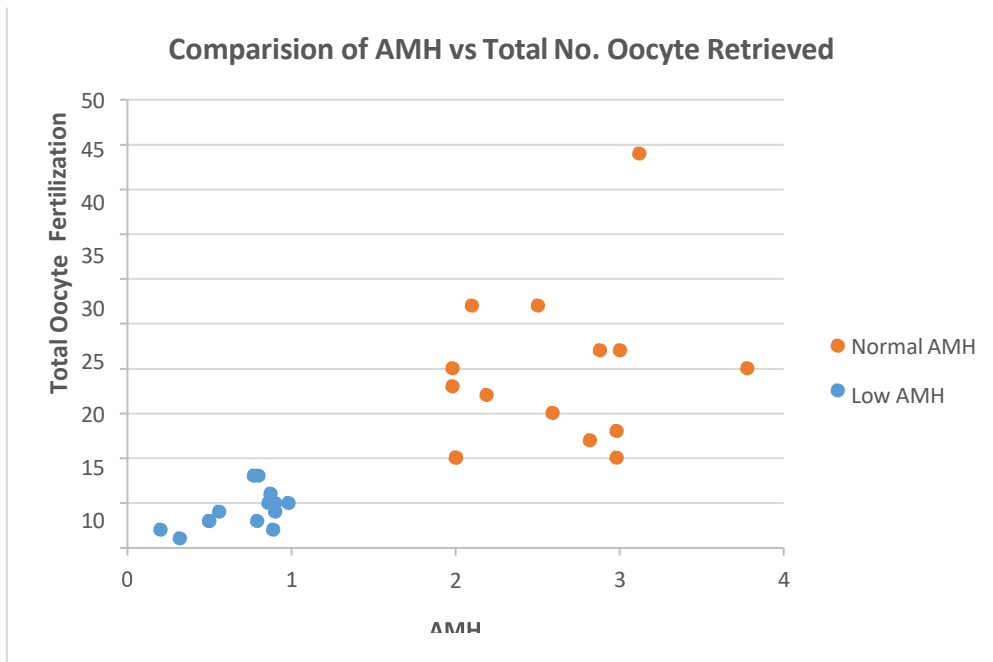


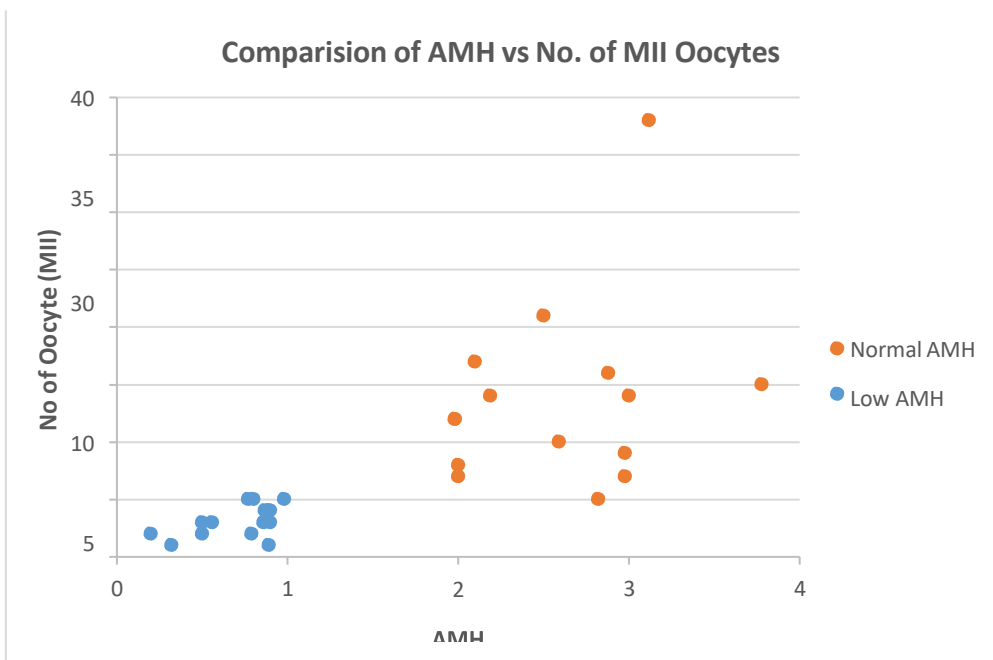
Fig 4 - Day 5 Embryo



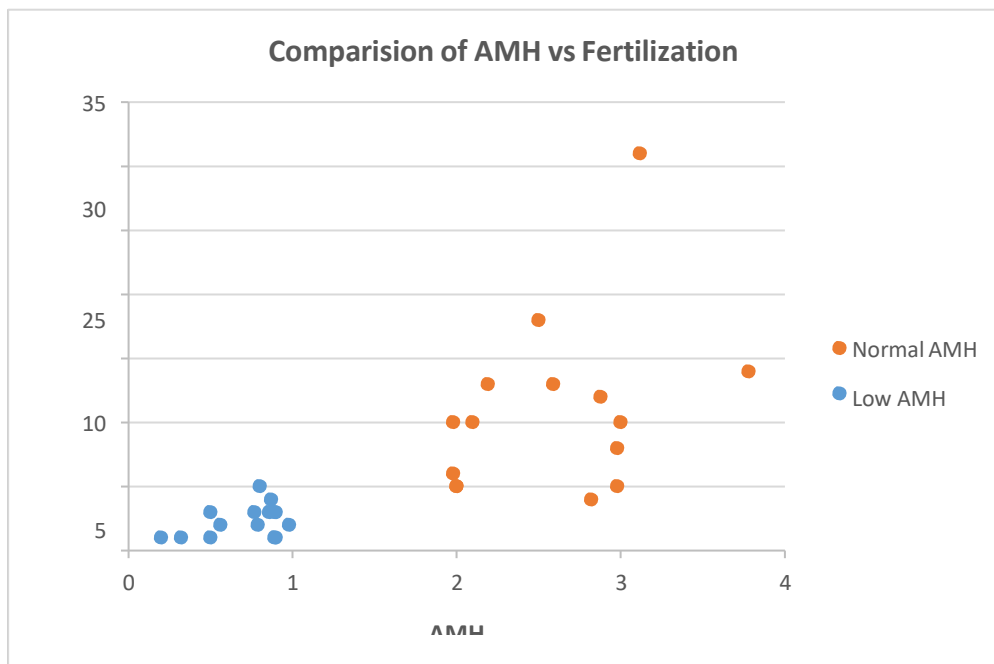
The clinical outcomes in terms of achieving pregnancy, was analyzed in both the groups which was 40% in Group I, while Group II outcome was higher at 66%.



Graph 1 – Comparison of AMH vs Total no of oocytes retrieved.



Graph 2 – Comparison of AMH vs No of MII oocytes.



Graph 3 - Comparison of AMH vs No of Oocytes fertilized

## DISCUSSION

This study investigated the correlation between low serum Anti-Müllerian Hormone (AMH) levels and various reproductive outcomes, including oocyte quantity and quality, embryo quality, and clinical outcome. A statistically significant correlation ( $p < 0.0001$ ) was found, indicating that low AMH levels negatively impact all these factors. Specifically, the clinical outcome for the low AMH group was significantly lower (40%) compared to the normal AMH group (66%). While the exact reasons for poor oocyte quality with low AMH are debated (granulosa cell secretion vs. direct AMH effect), the study suggests that low AMH affects not only oocyte quantity but also quality, potentially leading to decreased fertilization and embryo quality due to cytoplasmic and extracytoplasmic abnormalities, even though the precise cause remains unclear. The study concluded that low AMH negatively affects embryo quality, aligning with other research showing a positive correlation between AMH and euploid embryo numbers (Borges et al., 2017, Iliodromiti et al., 2014, Setti et al., 2023, Gat et al., 2017).

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