# Evaluating the Role of Serum Anti-Mullerian Hormone (AMH) Levels in Predicting Embryo Quality and Pregnancy Outcome Among Women Who Underwent In Vitro Fertilization (IVF)

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**Objective:** The objective of this study was to evaluate the role of serum Anti-Mullerian hormone (AMH) concentration in predicting embryo quality and pregnancy outcome among women who underwent in vitro fertilization (IVF) cycles.
**Design**: Retrospective cohort study
**Setting**: Center for Advanced Reproductive Medicine and Infertility (CARMI), St. Luke's Medical Center, Global City
**Patients**: There were 94 women who underwent an IVF procedure at this facility from January to October 2012.
**Methodology**: Serum AMH levels of these women were determined prior to initiation of ovulation induction. Embryo quality and pregnancy outcome were determined for each patient.
**Results**: Higher percentages of patients with high AMH levels were observed to have excellent quality embryos transferred (67%) and positive pregnancy outcome (46%), particularly among women aged 36 years old and above. However, no statistical significance between AMH levels and embryo quality or AMH levels and pregnancy outcome was found.
**Conclusion**: Serum AMH has no predictive role on embryo quality and pregnancy outcome.

**Key words**: Anti-Mullerian hormone, embryo quality, oocyte quality, pregnancy outcome

## Introduction

Traditionally, there have been a number of parameters, commonly referred to as ovarian reserve markers, early follicular phase follicle stimulating hormone (FSH) and antral follicle count (AFC) in particular, which have been comprehensively utilized as predictors of ovarian responses to gonadotropin stimulation during in vitro fertilization treatment.

More recently, serum AMH has been extensively investigated for employment in the assessment of egg quantity. AMH is a dimeric glycoprotein that belongs to the transforming growth factor beta family synthesized from birth to menopause exclusively by the functional granulosa cells surrounding the preantral and small antral follicles. These follicles have already undergone recruitment from the primordial follicle pool, but have not been selected for dominance. AMH is believed to deter initial primordial follicle recruitment and progression by decreasing the sensitivity of prenatal and small antral follicles to FSH stimulation. This denotes that AMH may have a function during intrafollicular and interfollicular coordination of follicle development. Pellatt and his colleagues were able to demonstrate in their study that involved isolated granulosa cells and their exposure to AMH that certain factors that promote follicle evolution of granulosa cells from follicles of the size from which the dominant follicle would be selected are in effect inhibited by AMH.[1] Human antral follicles measuring <6mm manufacture the greatest amount of AMH, and those levels precipitously decline as follicles increase in size. As AMH levels decrease with follicle growth, the inhibitory action of AMH is removed, and the follicles become responsive to FSH and selection for dominance can then proceed. It is for this reason that serum AMH has been widely accepted as a marker of the total antral follicle pool, and consequently, a measure of ovarian aging.

**Table 1.** Mean age, AMH levels and outcomes.

| Total Sample | n = 94 |
|---|---|
| Age (mean ± s.d.) | 36 ± 5 |
| AMH Level (in ng/mL) | 2.1 ± 2.2 |
| Low (<0.3 ng/mL) | 12 (13%) |
| Medium (0.3-1.0 ng/mL) | 30 (32%) |
| High (>1.0 ng/mL) | 52 (55%) |
| Number of embryos (mean ± sd) | 3 ± 1 |
| 1 or 2 embryos | 36 (38%) |
| 3 or more embryos | 58 (62%) |
| Highest quality of embryo transferred | |
| Excellent | 55 (59%) |
| Average | 36 (38%) |
| Poor | 3 (3%) |
| Pregnancy Outcome | |
| Positive | 37 (39%) |
| Negative | 57 (61%) |

Table 2A shows the relationship of AMH levels to the number of embryos transferred, embryo quality and pregnancy outcome. There was a statistically significant association between AMH concentration and the number of embryos transferred. Specifically, 67% of those patients with low AMH values had only 1 or 2 embryos transferred, while 73% of subjects with high AMH had 3 or more embryos transferred.

Examining further, the statistical significance between AMH levels and number of embryos transferred was found only among subjects aged 36 years old and above (Table 2B). In particular, 80% of the women from this age group with high AMH concentrations had 3 or more embryos transferred. Similarly, those with low AMH concentrations had only 1 or 2 embryos transferred (67%). There was not enough evidence to show the said statistical relationship among the younger subject group (Table 2C). Likewise, no statistical significance was found between AMH level and outcomes in either younger or older subject groups. The significant association between AMH level and number of embryos transferred established earlier with the total population was a mere reflection of the significant association present solely within the older age group.

In addition, though not statistically significant with a P value of 0.088, 75% of women aged 36 years old and above with high AMH values produced embryos of excellent quality. This association was not observed with AMH value and pregnancy outcome (Table 2C).

Age was further evaluated in terms of its relationship with AMH levels and outcome parameters among the subjects in this study (Table 3). A statistically significant relationship was found between age and AMH levels. Majority of the subjects aged 35 years old and below (78%) had high AMH levels and none had low AMH. All twelve women classified with low AMH levels belonged to the 36 year old and above age group. A smaller portion of these women (38%), compared to the younger group, had high AMH levels. Otherwise, no statistically significant relationship was found between age and overall outcomes.

## Discussion

In the present study, serum AMH level was found to be significantly associated with the number of embryos transferred for women 36 year old and above. There is a standard protocol that dictates the number of embryos to be transferred based on the age of the patient. Generally,

**Table 2A.** Number of embryos transferred, highest quality of embryo and pregnancy outcomes by AMH level.

| | | AMH Level | | | |
|---|---|---|---|---|---|
| Total Sample | Total n = 94 | Low n = 12 | Medium n = 30 | High n = 52 | P-value |
| No. of embryos transferred | | | | | 0.020 |
| 1 or 2 embryos | 36 (38%) | 8 (67%) | 14 (47%) | 14 (27%) | |
| 3 or more embryos | 58 (62%) | 4 (33%) | 16 (53%) | 38 (73%) | |
| Highest quality of embryo transferred | | | | | 0.108 |
| Excellent | 55 (59%) | 3 (25%) | 17 (57%) | 35 (67%) | |
| Average | 36 (38%) | 8 (67%) | 12 (40%) | 16 (31%) | |
| Poor | 3 (3%) | 1 (8%) | 1 (3%) | 1 (2%) | |
| Pregnancy outcome | | | | | 0.287 |
| Positive | 37 (39%) | 3 (25%) | 10 (33%) | 24 (46%) | |
| Negative | 57 (61%) | 9 (75%) | 20 (67%) | 28 (54%) | |

**Table 2B**. Number of embryos transferred, highest quality of embryo and pregnancy outcomes by AMH level. (Among patients aged 36 years old and above)

| | | AMH Level | | | |
|---|---|---|---|---|---|
| Total Sample | Total<br>n = 53 | Low<br>n = 12 | Medium<br>n = 21 | High<br>n = 20 | P-value |
| No. of embryos transferred | | | | | 0.031 |
| 1 or 2 embryos | 21 (40%) | 8 (67%) | 9 (43%) | 4 (20%) | |
| 3 or more embryos | 32 (60%) | 4 (33%) | 12 (57%) | 16 (80%) | |
| Highest quality of embryo transferred | | | | | 0.088 |
| Excellent | 31 (58%) | 3 (25%) | 13 (62%) | 15 (75%) | |
| Average | 19 (36%) | 8 (67%) | 7 (33%) | 4 (20%) | |
| Poor | 3 (6%) | 1 (8%) | 1 (5%) | 1 (5%) | |
| Pregnancy outcome | | | | | 0.242 |
| Positive | 19 (36%) | 3 (25%) | 6 (29%) | 10 (50%) | |
| Negative | 34 (64%) | 9 (75%) | 15 (71%) | 10 (50%) | |

**Table 2C**. Number of embryos transferred, highest quality of embryo and pregnancy outcomes by AMH level. Among patients aged 35 years old and below.

| | | AMH Level | | | |
|---|---|---|---|---|---|
| Total Sample | Total<br>n = 41 | Low<br>n = 0 | Medium<br>n = 9 | High<br>n = 32 | P-value |
| No. of embryos transferred | | | | | 0.181 |
| 1 or 2 embryos | 15 (37%) | 0 (0%) | 5 (56%) | 10 (31%) | |
| 3 or more embryos | 26 (63%) | 0 (0%) | 4 (44%) | 22 (69%) | |
| Highest quality of embryo transferred | | | | | 0.331 |
| Excellent | 24 (59%) | 0 (0%) | 4 (44%) | 20 (63%) | |
| Average | 17 (41%) | 0 (0%) | 5 (56%) | 12 (38%) | |
| Poor | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| Pregnancy outcome | | | | | 0.970 |
| Positive | 18 (44%) | 0 (0%) | 4 (44%) | 14 (44%) | |
| Negative | 23 (56%) | 0 (0%) | 5 (56%) | 18 (56%) | |

**Table 3**. AMH levels and pregnancy outcomes by age group.

| | | Age Group | | |
|---|---|---|---|---|
| Total Sample | Total<br>n = 94 | 35 yo & below<br>n = 41 | 36 yo and above<br>n = 53 | P-value |
| AMH Level | | | | 0.000 |
| Low | 12 (13%) | 0 (0%) | 12 (23%) | |
| Medium | 30 (32%) | 9 (22%) | 21 (40%) | |
| High | 52 (55%) | 32 (78%) | 20 (38%) | |
| No. of embryos transferred | | | | 0.764 |
| 1 or 2 embryos | 36 (38%) | 15 (37%) | 21 (40%) | |
| 3 or more embryos | 58 (62%) | 26 (63%) | 32 (60%) | |
| Highest quality of embryo transferred | | | | 0.285 |
| Excellent | 55 (59%) | 24 (59%) | 31 (58%) | |
| Average | 36 (38%) | 17 (41%) | 19 (36%) | |
| Poor | 3 (3%) | 0 (0%) | 3 (6%) | |
| Pregnancy outcome | | | | 0.428 |
| Positive | 37 (39%) | 18 (44%) | 19 (36%) | |
| Negative | 57 (61%) | 23 (56%) | 34 (64%) | |

2 or 3 embryos are transferred to patients less than 33 years of age to minimize the risk of multiple pregnancies, and more than 5 embryos, if available, in patients older than 39 years old to enhance the establishment of pregnancy in older women.[15] Intracytoplasmic sperm injection (ICSI) has become widely used as a therapeutic tool in overcoming fertilization failure, specifically those factors that determine various degrees and types of male infertility. Therefore, fertilization in the IVF cycles studied in this population could be largely dependent on the quality of the oocytes retrieved. The number of embryos transferred was not included as a measure of outcome for this study. However, since there was a significant association elucidated between the number of embryos transferred and the AMH level of women belonging to the age group 36 years old and above, it seemed prudent to explore this association further. The number of embryos transferred for the older women with high AMH levels among the subjects aged 36 years old and above could be viewed as an indirect reflection of the quality of the oocytes recovered. It could be surmised that women with high AMH levels from this age group could be associated with high quality oocytes, and that such oocytes could be retrieved and undergo successful fertilization with ICSI, resulting in viable embryos for eventual transfer. Determining AMH level for women 36 years old and above could be more foretelling of the outcome of the IVF treatment, not only in terms of ovarian responsiveness and quantity of eggs retrieved, but more so the quality of the oocytes that will undergo fertilization with ICSI. However, further investigation with a larger sample size would be necessary to uncover a statistically significant association.

Embryo quality has long been recognized as mainly age-dependent. In this study, it was noted that 75% of the women aged 36 years old and above with high AMH levels were associated to have excellent embryo quality. Though not statistically significant, a 90% confidence interval would provide an entirely different implication. Regardless, these data would still be useful in terms of interpreting AMH levels of women belonging to this age group and prognosticating IVF outcome, considering that these women are immediately stereotyped as having low AMH values and poor oocyte quality. Further studies would be required to elucidate the potential value of AMH level on predicting oocyte and embryo quality for women aged 36 years old and above.

Pregnancy outcome is multifactorial. The results of this study are consistent with earlier published works that AMH level has no effect on pregnancy outcome.[16,17] The implantation potential of the embryo transferred, mostly dependent on the quality of the embryo produced, lack significant association with AMH level. Customarily, embryos from women of advanced age are considered to demonstrate significant impairment in cleaving status and ultimately weaken the implantation ability of such embryos. Prospective cohort studies involving this particular group of women that aim to establish a greater impact of the role of serum AMH determination on embryo quantity and quality would be necessary.

## Conclusion

Serum AMH is not predictive of embryo quality and pregnancy outcome in IVF cycles. Age of the woman is still the most reliable predictor of oocyte quality that will ultimately affect the cleavage capability of the resulting embryo and its implantation potential.

## Recommendation

Serum AMH determination may have a role in predicting oocyte and embryo quality for women aged 36 years old and above who will undergo IVF treatment. Additional studies involving these women would be needed.

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