Association Between AMH, Oocyte Number and Availability of Embryos for Cryopreservation in IVF

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Abstract. Aim: To investigate possible associations between serum anti-Müllerian hormone (AMH) levels, the number of oocytes collected and the availability and suitability of produced embryos for cryopreservation in in-vitro fertilization (IVF) cycles. Patients and Methods: Sixty women in their first IVF/intracytoplasmic sperm injection (ICSI) cycle were studied. The short stimulation protocol was used for controlled ovarian hyperstimulation. AMH levels were measured during the menstrual cycle preceding treatment. Results: A strong, positive correlation between AMH and the number of collected oocytes was found. The patients with available and suitable embryos for cryopreservation had significantly higher levels of AMH. Conclusion: AMH appears to be a valuable marker mainly for ovarian reserve and response to IVF treatment. AMH levels are strongly associated with the number of retrieved oocytes and the availability of supernumerary embryos suitable for cryopreservation.

Ovarian reserve is determined by the size of the ovarian follicle pool and the quality of the produced oocytes. It declines as female age increases, resulting in a decrease in a woman’s reproductive ability with time (1).

Assessing ovarian reserve is a useful but challenging process in the field of in vitro fertilization (IVF). Various hormonal tests have been used, such as basal (day 3) follicle stimulating hormone (FSH) levels, the ratio between FSH and luteinizing hormone (LH) levels, estradiol (E₂) and inhibin B levels. The most commonly used is basal FSH, the higher it is, the lower that ovarian reserve is expected to be. Ultrasonography is also used to assess the quantitative aspect of ovarian reserve. Ovarian volume has also been used to estimate ovarian reserve (2). Measurement of the number of antral follicles has also been shown to be predictive of ovarian response to external stimulation (2). Of course, additional transvaginal ultrasounds during the early follicular phase are required to monitor the ovarian response. A serum marker assessing ovarian reserve would be easier to measure, and anti-Müllerian hormone (AMH) appears to be a candidate.

AMH is expressed in granulosa cells from pre-antral and small antral follicles and continues to be expressed in the growing follicles until they have reached the size and differentiation state at which they are to be selected for dominance (3). This occurs at the antral stage when the follicle is 4-6 mm (3). AMH decreases with age as a sign of follicular reserve exhaustion (3). Serum AMH levels have been measured at frequent time-points during the menstrual cycle, with the results suggesting complete absence of fluctuation (3). This absence of variation may be consistent with the continuous non-cyclic growth of small follicles throughout the cycle (3). Due to this stability, AMH was used as a marker for ovarian response to controlled ovarian stimulation independently of the day of the cycle in which the blood sample was obtained (3).

Most IVF programs employ embryo cryopreservation to enhance pregnancies from a single ovarian stimulation (4). There are two main cryopreservation methods, vitrification and slow freezing. Vitrification was the method used in this study. The objective of this study was to investigate possible correlations between serum AMH levels, the number of oocytes collected in IVF cycles and also the availability and suitability of produced embryos for cryopreservation.

Patients and Methods

Patient characteristics and IVF procedure. Samples from a total of sixty women aged between 23 and 35 years scheduled to undergo their first IVF/intracytoplasmic sperm injection (ICSI) treatment were used in this study. Inclusion criteria were written consent for treatment, normal gynecological ultrasound and cervical smears. Exclusion criteria were infectious diseases, severe psychiatric
illnesses, or being a carrier of severe genetic diseases. Most couples presented due to a male cause of infertility. Other common causes (apart from unexplained infertility) were ovulation problems, including polycystic ovary syndrome, and tubal blockage/removal.

The short stimulation protocol was used for controlled ovarian hyperstimulation (COH). Pituitary desensitization was achieved with the gonadotropin releasing hormone (GnRH) agonist triptorelin (0.1 mg/day). Follicle growth was stimulated using recombinant FSH doses ranging from 150 to 300 IU/day. Follicle growth was monitored by E2 levels and transvaginal ultrasound. Ovulation was induced with human chorionic gonadotropin (10,000 IU). Oocyte retrieval was performed 36 hours later. After ICSI, fertilization was assessed and the best morphologically graded embryos were chosen for embryo transfer. The remaining embryos were considered for cryopreservation. Embryos suitable for cryopreservation had to have good morphology and normal cleavage rate. Cryopreservation was made according to the vitrification technique with Medicult Vitrification Kit (Origio A/S, Måløv, Denmark) and McGill Cryoleaf (Origio A/S, Måløv, Denmark) as vitrification device.

Enzyme-linked immunosorbent analysis (ELISA) of AMH. Soluble AMH levels in blood serum samples were measured by standard ELISA methods using an AMH detection kit in accordance with the manufacturer’s instructions (USCN Life Sciences, Houston, TX, USA). The limit of quantification was 13.2 pg/ml, the intra-assay precision was ≤10% and the inter-assay precision ≤12%. Blood was taken during the menstrual cycle preceding treatment.

Statistical analysis. Statistical tests were performed using SPSS software version 20.0 (SPSS Inc, Chicago, IL, USA). Correlation between variables was evaluated with Spearman rank R test. Differences in mean values were analyzed using the Kolmogorov-Smirnov and the Mann-Whitney U-test. All p-values were two-sided and 5% was chosen to denote significance. Values are presented as mean±standard deviation.

Results

Oocyte, vitrification and AMH levels. A total of sixty individuals were included in this study. In five patients the IVF cycle had to be canceled either due to ovarian hyperstimulation syndrome or lack of proper response to treatment. The number of oocytes collected ranged between two and 27. The mean number of oocytes was approximately eight per patient. AMH levels ranged from 0.2 to 10.3 ng/ml. The number of vitrified embryos ranged only from 0 (not available, not suitable for vitrification) to 1 (at least one available and suitable for vitrification) per patient. In 11 completed IVF cycles, there were no embryos available and suitable for cryopreservation.

There was a strong correlation between AMH levels and oocyte numbers (Spearman R=0.86, p<0.05) (Figure 1). There was also a strong correlation between AMH and antral follicles count (Spearman R=0.88, p<0.05). A strong negative correlation was found between AMH and basal FSH (Spearman R=−0.84, p<0.05) as well as between antral follicle count and basal FSH (Spearman R=−0.83, p<0.05).

As expected, patients with vitrified embryos had significantly higher number of collected oocytes (11.86±5.72) than patients not having available or suitable embryos for vitrification (3.56±1.67) (Mann-Whitney U=8.00, Z=−4.59, p<0.001). AMH levels of patients with vitrified embryos (3.79±2.81, range: 1.2-10.3 ng/ml) were also statistically significant higher than those of patients without vitrified embryos (0.64±0.48, range: 0.2-1.7 ng/ml) (Mann-Whitney U=15.00, Z=−4.44, p<0.001).

Discussion

Ovarian aging is a continuous process that is characterized by a gradual decrease in both quality and quantity of a woman’s oocytes. Assessing ovarian reserve is an extremely useful but challenging process in the field of assisted reproduction.

The purpose of this study was to evaluate the correlation between the serum levels of AMH with the number of oocytes collected during IVF cycles and also the availability and suitability of produced embryos for cryopreservation.

We observed that in the case of AMH versus the number of oocytes there was high correlation between the two variables. Larger numbers of oocytes were collected when the patient had high levels of AMH. Strong correlation between AMH levels and the number of oocytes has been mentioned in the literature before (3, 5-18). Other studies have shown the opposite, however. For example, a study by Takahashi et al. (19) showed that there was no significant correlation between AMH levels and oocyte number.

Serum AMH has an important advantage as a marker. It decreases with age as a sign of follicular reserve exhaustion (3) and its level exhibits no intracycle fluctuation (3). Ultrasonography is also used to assess ovarian reserve by
ovarian volume and measurement of the number of antral follicles seems to be predictive of ovarian response (2). A combination of two methods could give additional information and a better picture of ovarian reserve (2).

The present study showed that high levels of AMH are related with the availability of embryos suitable for cryopreservation. A previous study by Majumder et al. (7) revealed a correlation between serum AMH and the number of embryos available for freezing. They performed a prospective observational study involving 162 women (<40 years old) undergoing their first IVF cycle. They found that serum AMH and antral follicle count were significantly associated with the number of high quality embryos available for transfer and the number of embryos frozen (7).

Some studies showed a correlation between serum AMH and the number of embryos produced (5, 6, 8) whereas others did not (9, 16). On the other hand, the relationship between serum AMH and embryo quality seems controversial. There are studies to support it (6, 10, 11, 20) while others disagree (8, 15, 17-19).

It is now routine practice for IVF programs to use embryo cryopreservation to enhance pregnancy yields from a single ovarian stimulation cycle, to reduce the risk of ovarian hyperstimulation syndrome and to minimize high order gestations by limiting of the number of transferred embryos (4). Transferring frozen embryos is also less expensive and less invasive than repeating an ovarian stimulation required for a fresh embryo transfer and there is no risk of ovarian hyperstimulation (4). Although pregnancy rates with cryopreserved embryo cycles may be lower than those achieved from fresh cycles, the cumulative effect of pregnancies achieved with thawed cycles to those attained with fresh cycles must also be considered (4). Moreover, there is no apparent negative effect on perinatal outcome or health of children born as a result of these procedures (4). For these reasons, we believe that the relationship between an easily measured marker like AMH with the suitability and availability of embryos for cryopreservation appears to be clinically useful.

In conclusion, AMH appears to be a valuable marker mainly for ovarian reserve and response to IVF treatment. It can be combined with ultrasound measurements for a better overall assessment. Serum AMH levels are strongly related to the availability of supernumerary embryos suitable for cryopreservation. However, the relationship between AMH and the availability and suitability of produced embryos for cryopreservation needs further confirmation in larger clinical studies.

References


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