Anti-Müllerian hormone: structure, properties and appliance

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ABSTRACT
Anti-Müllerian hormone (AMH) is a glycoprotein produced by the granulosa cells of preantral and small antral follicles. AMH concentrations reflect ovarian physiology with high precision, thus serving as a more sensitive marker of the ovarian reserve than the chronological age. This hormone plays a role in the pathogenesis of menstrual disorders and fertility in obesity and polycystic ovary syndrome. The evaluation of AMH may also be useful in the diagnosis or the monitoring therapy of granulosa cells ovarian tumors.
Key words: Anti-Müllerian hormone, fertility, gynecology

INTRODUCTION
Anti-Müllerian hormone (AMH), which was discovered in the fifties of the last century by Alfred Jost and his colleagues, is thought as an important factor involved in the differentiation of the internal sex organs of the embryo [1]. The expression of the AMH gene, also called MIS (Müllerian Inhibitor Substance) causes the loss of Müller ducts during the sex differentiation to the male [2]. In women, AMH is produced by granulosa cells in primordial preantral and small antral follicles, and participates in the regulation of folliculogenesis by inhibiting the recruitment process of the germinal vesicle by reducing the effect of FSH on the follicular growth of preantral and antral follicles.

The creation of commercially available diagnostic tests for measuring AMH concentration in serum in recent years has allowed for wide-scale research studies focused on the usefulness of this hormone as a clinical marker of the ovarian reserve and a predictive factor of a response to the ovarian stimulation with gonadotropins during the stimulation of ovulation.

The evaluation of AMH in women joining to the program “Treatment of infertility by in vitro fertilization” provided by the Ministry of Health has become one of the selection criteria for the procedure of the assisted reproduction. This work is an attempt to summarize the current knowledge about the role and use of the AMH hormone in gynecological practice.

THE STRUCTURE OF ANTI-MÜLLERIAN HORMONE AND ITS GENE
Anti-Müllerian hormone is a glycoprotein with a molecular weight of 140 kDa peptide belonging to a superfamily of growth factors TGF-β. AMH human gene located on the short arm of chromosome 19 comprises 275 base pairs, which are divided into five exons. AMH gene product is a 560 amino acid precursor proAMH. After the removal of the 24 amino acids consisting fragment a molecule is glycosylated and two identical subunits with a molecular weight of 70 kDa linked to each other by sulfide bridges are formed. ProAMH proteolysis leads to the formation of the N-terminal fragment called “pro-region” (115 kDa AMHN) and C-terminal domain ie. “mature region” (25 kDa AMHC). The C-terminal fragment is responsible for the biological
activity of the protein and receptor binding; but in order to be active it requires the N-terminal fragment unlikely to the other proteins in the TGF-β [3].

The action of the AMH particle is provided by two types of receptors: AMH-I and AMH-II, present in the mesenchymal cells. The anti-Müllerian hormone does not have the direct affinity to AMH-I, whereas it binds to the receptor type II, which allows for the connection of receptor I and the formation of a large complex consisting of the AMH protein dimer, two particles of AMH-RI and two AMH-RII. This combination induces tyrosine phosphorylation of AMH-RI and leads to further reactions during which the Smad protein complex (proteins involved in the signal transduction into the cell after the receptor type TGF-β activation) is transported to the nucleus where it regulates the transcription of certain genes [4, 5] (Fig. 1).

**AMH AND FOLLICULOGENESIS**

In a female fetus expression of AMH starts to be observed from the 36th week of pregnancy. In women, AMH is produced by granulosa cells in primordial preantral and small antral follicles. The production of Anti-Müllerian hormone in the follicle starts from the moment of its recruitment and lasts to the antral stage development. The greatest intensity of the hormone synthesis is observed in granulosa cells of preantral and small antral follicles (to 4 mm). In larger antral follicles (> 8 mm) AMH rate synthesis slowly decreases until it becomes undetectable [6].

AMH inhibits the gene and protein level expression of cytochrome P450 in aromatase granular cells [7]. By reducing the effect of FSH on the preantral and antral follicles growth the Anti-Müllerian hormone participates in the regulation of folliculogenesis, inhibiting the recruitment of germinal vesicles.

Information about the AMH effect on folliculogenesis was provided by the research on mice lacking the gene AMH (type null). The ovaries of four-months old mice type null were twice large and contained a lower number of germinal vesicles and a three times higher number of small growing vesicles than the wild type mice [5]. This was due to the increased recruitment of primordial follicles and their transformation to preantral and antral follicles.

Mice type null with the larger number of follicles also showed a lower level of FSH which allowed for the hypo-

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**Figure 1.** The mechanism of AMH action via receptor Proteolysis of AMH pro leads to a conformational change in the C-terminal domain that allows binding AMRH II. AMH-RII induces binding type I receptor, which is phosphorylated by a kinase receptor type II. The activated AMH-RI in turn phosphorylates further Smads proteins. Phosphorylated Smads specific receptors translocate to the nucleus, where they regulate gene expression.
thesis that in the state of AMH absence follicles are more sensitive to FSH influence. In *in vitro* studies on mice, the AMH addition to follicles culture inhibited FSH — the dependent follicular growth resulting in a smaller diameter of follicles [8]. In *in vivo* studies on mice type null the greater growth of follicles was observed comparing wild type both at low and at high exogenous FSH administration. The AMH inhibitory effect on FSH follicular sensitivity may take part in the follicle selection process [5].

Current theories suggest that AMH acts as a co-regulator of steroidogenesis in granulosa cells. As it has been shown the concentration of AMH correlates with estradiol levels in the fluids of small antral follicle.

The confirmation of this thesis are studies which showed the relations between AMH gene polymorphism or AMH receptor type II and the level of estradiol in the follicular phase, which points the AMH role in the FSH-dependents steroidogenesis in the human ovary [9].

The AMH hormone is detected in the serum, although its role relates mainly to the auto and paracrynic action on the follicular development. Antral follicles seem to be a major source of AMH in serum because of the larger number of granulosa cells. The presence of AMH in the blood stream has been the subject of many studies focused on the possibility of the use of hormone evaluation in the diagnosis of endocrine and oncological disorders.

**AMH AS A MARKER OF FEMALE FERTILITY**

During the life of a woman AMH concentration in serum is low after the birth and prepubertal time. At puberty the concentration of AMH in blood increases reaching the highest values at the age about 20–25 years. After this time the hormone concentration decreases to undetectable levels after menopause [10].

AMH seems to be a good marker in the assessment of ovarian reserve. In women AMH concentration decreases with age before there are any signs of upcoming menopause like an increase in the concentration of FSH or a decrease in the number of antral follicles. During the menopause time the hormone becomes undetectable, which is associated with the depletion in the follicular reserve. Thus the reduced concentration of AMH in serum may indicate the physiological or pathological aging of the ovary [11].

During one menstrual cycle hormone levels are not significantly affected [11, 18], although Wunder et al. showed lower concentrations of the hormone in the early luteal phase [12]. The described variation in the cycle is not significant enough to recommend the measurement of AMH on specific days of the menstrual cycle.

Further described factors influencing the differences in the concentrations of AMH are: ethnicity, smoking, vitamin D levels and obesity. It was found that Afroamerican and Span-
also been demonstrated in adolescent girls (12-18 years) with PCOS [19], as well as in 4-7 years old daughters of women suffering from this disease. The AMH concentration in women with PCOS declines with age, but it clearly occurs after the 40-th decade of life.

THE USE OF AMH IN ASSISTED REPRODUCTION TECHNIQUES

The level of AMH slowly drops down after gonadotropin treatment during a controlled ovarian stimulation [20]. The decrease of the hormone during stimulation could be an effect of a direct or indirect negative influence of FSH on AMH ovarian secretion. The exogenous FSH treatment gives estradiol elevation, which could be the reason of AMH decrease because estradiol influences negatively the regulation of mRNA for AMH and AMHII in the ovary [21].

Results of many studies pointed out the presence of strong positive correlation between the AMH level in serum and the number of obtained egg cells [20]. It seems to be that the pretreatment level of AMH is a better prognostic marker than the patient age, the level of FSA on the 3rd day of the cycle, the level of estradiol or inhibit B. A number of antral follicles (AFC-antral follicle count) in the USG image were also strongly positively correlated with AMH concentration. Low levels of anti-Müllerian hormone and AFC are markers of a weak prognosis for stimulation in in vitro fertilization (IVF) also they are considered to be equivalent parameters to the prediction of the treatment success. The risk of the IVF cycle cancellation could be 13 times higher in patients with undetectable AMH level than in those women with AMH level more than 2.0 ng/ml [22]. Low values of AMH do not exclude the possibility of getting pregnant, although the success rate is much lower. In the study of Kedema et al. [23], conducted in 769 IVF/ICSI cycles the rate of achieved pregnancies was the same and equal to 4.4% in the group of patients with low AMH level of (0.2–1.0 ng/mL) as well as extremely low AMH level (< 0.2 ng/mL). In the group of women older than 42 years with AMH < 0.2 ng/mL the pregnancy was not achieved. However, in the analysis presented by Lukaszuk et al. [24], conducted on 101 women with AMH < 0.4 ng/mL treated with 188 cycles of in vitro fertilization he achieved 14 live births, which is being projected to 7.45% effectiveness of IVF procedures. Thus low concentrations of AMH require different stimulation protocols, in particular short and ultrashort with high initial dosages of gonadotropins [22].

The evaluation of anti-Müllerian hormone is an important predictor factor of the risk of ovarian hyperstimulation syndrome (OHSS, ovarian hyperstimulation syndrome). AMH is a better prediction marker for an excessive ovarian response due to the stimulation rather than the gonadotropins level, age or BMI and is more precise than the number of follicles in the USG image or the estradiol level after HCG administration. The value 3.5 ng/mL is considered as the cut-off point, which indicates a high risk of ovarian hyperstimulation syndrome. During the cycles of in vitro fertilization it is recommended to use low initial doses of gonadotropins and protocols with the GnRH antagonist [20].

In the prospective study, Nelson et al. showed that the higher rate of live births is associated with higher concentrations of AMH. However, the authors suggest that such a good correlation may be related to the greater number of oocytes [25]. Other randomized studies conducted by Brodin et al. [26] on 842 women confirmed the strong positive correlation between the serum AMH levels and the amount of received oocytes, the rate of achieved pregnancies, as well as a cumulative indicator of live births. It seems that AMH is a good marker for quantitative rather than qualitative techniques of assisted reproduction. The individual selection of the type of stimulation protocol and doses of gonadotropins in IVF programs on the basis of the concentration of an anti-Müllerian hormone allows for a safer and more effective use of the procedures of in vitro fertilization.

AMH AS CANCER MARKER

The expression of AMH by granulosa cells of small antral and primordial follicles in the ovary was used as an idea to try to use AMH as a marker of cancer derived from granular cells (GTCs, granulosa cell tumors). These tumors comprise about 3 to 5% of all ovarian tumors. It was noted that AMH concentration was elevated in 76–93% of women with tumor cells derived from the granular cells [27]. Furthermore, higher concentrations of AMH were observed up to 16 months before tumor relapse. Therefore, the determination of the hormone value is a useful marker in detecting recurrences of GTCs, in particular by using new generation ultrasensitive assays.

Recent studies indicated that a large number of primary epithelial ovarian tumors originate from the scrap of the fallopian tubes or the components of the secondary Muller system. Based on the fact that AMH is responsible for the regression of the Mullerian ducts there has been an attempt to use this hormone in the treatment of epithelial ovarian cancers. The inhibition of the growth in lines of epithelial tumor cells after the incubation with the AMH was achieved in in vitro studies [28].

Chemotherapy is particularly toxic for the ovaries by promoting follicles injury. It has been shown that after chemotherapy AMH concentration is significantly lower than in healthy women at a similar age, which confirms the idea that the hormone is a sensitive indicator of ovarian reserve. Depending on the drugs and the doses used in a therapy, the AMH levels after treatment may vary. Hormone levels
were significantly lower or almost undetectable after the therapy with alkylating agents in comparison to other types of chemotherapy. The elevation of AMH after chemotherapy depends on the type of the treatment protocol [29].

AMH was found as a marker in classifying patients for fertility preservation procedures before the anticancer therapy. Therefore, it is so important to direct women to infertility treatment facilities as soon as possible to select the appropriate treatment options like: cryopreservation of the obtained embryos, oocytes or ovarian tissue freezing for the afterward transplant.

**AMH MEASUREMENT**

The first reports about the determination of the level of anti-Müllerian hormone emerged in the 90’s. The I-st generation commercially available tests were produced by Diagnostics Systems Ltd. (DSL) and Immunotech Ltd. (OIT) and contained different types of antibodies and standards, which was the reason for differences in AMH obtained results [30]. The subsequent analysis showed a similarity in AMH levels, which could be an effect of kits improvement. The consolidation of the two companies by The Beckman Coulter resulted in the introduction of one test — AMH Gen II Assay which contained antibodies from the DSL kit and standards from IOT kit. The AMH results obtained with using the II-nd generation test were about 22–40% higher than the I-st generation test results; however they corresponded to each other [31]. Further studies discredit the serum sample stability during storage time, dilution and even AMH measurement [32]. Rustamov et al., noticed that the level of AMH obtained with using AMH Gen II kit was by about 20% lower than the results obtained by DSL kit. The decrease in the quality by II-nd generation tests (also these which have been produced since the year 2012 AMH Gen II kit with IVD certificate) was demonstrated during the analysis conducted in 8323 samples. Levels of AMH obtained by II-nd generation test Gen II IVD were by about 40% lower than the previous generation of Gen II kit and by about 70% lower than OIT kit [33]. In response to reports regarding the correctness of the test, from June 2013 Beckmann Coulter introduced a new protocol for AMH evaluation with the recommendation of serum preincubation with a suitable buffer (ESHRE, London 2013) before measurement. The dilution of the sample was to prevent errors resulting from the effect of binding of complement proteins. An additional action to improve the stability of the sample was an absolute recommendation to freeze the serum within 2 hours after its collection. The newest generation tests like Ultrasensitive (2012) and PicoAMH (2013) produced by AnsLabs show a higher sensitivity and detection threshold than AMH Gen II IVD (0.023 vs. 0.001 vs. 0.08 ng/mL) and they could be more useful in the recognition of low levels of anti-Müllerian hormone [34].

Data published so far have pointed out the necessity of a cautious interpretation of the results obtained using the AMH Gen II kit. That is why till now it seems to be rather impossible to compare the results, cut-off points or clinically important values obtained with the test produced by different manufacturers.

The introduction of the first automatic AMH test in September 2014 by Elecsys (ECLIA method) Roche could be a next step in the creation of a test appropriate in clinical practice. The producer assigned individual reference ranges for women at different age and defined the cut-off value for AMH in patients with PCOS [35]. The determination of the international AMH standard could be an important step towards the creation of a test completely useful in clinical practice.

**SUMMARY**

Anti-Mülllerian hormone reflects the ovarian function highly precisely. AMH is a more sensitive marker of ovarian reserve than the chronological age; that is why it seems to be very helpful in planning reproduction in women using both natural and assisted reproductive technologies. This hormone is also one of the elements of the pathogenesis of menstrual disorders and fertility in obese women in cases with and without PCOS coexistence. The evaluation of AMH may also be very useful in diagnosing or monitoring the treatment of ovarian cancer derived from granulosa cells.

**REFERENCES**


