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Ovarian activity regulation by anti-Müllerian hormone in early stages of human female life, an overview

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Abstract: The present study aimed at describing the anti-Müllerian hormone (AMH), with special focus on molecular background for ovarian activity, in particular the role AMH plays in sex determination and gonadogenesis process in early stages of prenatal life and folliculogenesis in postnatal life. It is a review of the literature currently indexed and abstracted in MEDLINE, SCOPUS and Google Scholars. The process of sex determination and gonad differentiation occurring during embryogenesis was discussed along with underlying molecular mechanisms. In the postnatal life the impact of AMH on the process of folliculogenesis was described. Clinical use of recent findings was shown as well. Genetic studies and molecular analyses have demonstrated that AMH is highly conservative, indicating its significance in reproductive process on the background of evolutionary processes.

Key words: AMH, sex determination, gonad differentitation, folliculogenesis, in vitro fertilization, androgens

Introduction

Anti-Müllerian hormone (hereinafter called AMH) was discovered by German medicine professor Peter Müller (1801–1858). He worked on embryology at the University in Bonn. He was able to observe pairs of structures characteristic of early fetal development and unknown substance acting on these structures. In 1940, Alfred Jost first described a substance of nuclear origin and influencing regression of Müllerian ducts. In 1986 Cate et al. identified and described the substance. Genes responsible for AMH expression have been isolated from human DNA.

Human gene coding AMH is located on the shortest arm of the 19 p.13.3 chromosome and it consists of 5 exons (Cohen-Haguenauer et al. 1987). The last codes the domain with C-terminus. AMH is a dimeric glycoprotein consisting of 560 amino acids, classified in the beta-TGF superfamily. An AMH molecule occurs in a precursor form. It contains two domains: a larger one, containing N-terminus region and a substantially smaller one, ended with a C-terminus. Primary form of the molecule possesses both domains bound by a non-covalent bond (AMHNC). After proteolytic enzymes cut the bond, the mature end with C-terminus and the proregion with N-terminus are bound with a disulfide bridge. It has not yet been determined, when proteolysis takes place-during hormone secretion or in the target tissue.

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Transformation of the primary, inactive form of AMH molecule facilitates attachment to the receptor and release of ligand, mature homodimer with C-terminus and proregion with N-terminus (di Clemente et al. 2010). Comparative model for AMH has been created based on human bone morphogenetic protein BMP-9, also included in beta-TGF superfamily. Perhaps the wrist site consisting of a prehelix loop and alpha-helix and form of concave fingers of the second monomer in AMH molecule constitutes the element that connects with the AMHRI receptor, while residues in the wrist site connect with AMHRII. All mutations in this region may lead to serious phenotypic and eventually sexual disturbances.

Anti-Müllerian hormone, also known as MIS (Anti-Müllerian Inhibiting Substance) is not species specific. Gene reading is equal for all species, and the transcription level mainly differs in terms of specific regulatory proteins connecting in the promoter region and due to the site and time of activation of signal cascades for the activation of AMH secretion. AMH coding gene as ortholog, is present in numerous mammal species, certain fish species, amphibians and reptiles, such as kangaroos, salamanders, alligators and sturgeons. An interesting discovery consisted of the fact of cloning AMH in fishes, which do not possess Müllerian ducts. It is suspected that the presence of AMH is associated solely with the process of gonad differentiation (Pfennig et al. 2015).

The present study aimed at describing the role of AMH, with special focus on molecular background for ovarian activity, in particular the role AMH plays in sex determination and gonadogenesis process in prenatal life and folliculogenesis in postnatal life.

Materials and methods

A literature search was performed of SCOPUS, PubMed, and Web of Science reviews for the years 2000-2017 under the following key phrases/words: <AMH in sex determination>, <AMH and gonad differentiation>, <AMH and folliculogenesis>, <AMH and in vitro fertilization (IVF)>, <AMH and follicle stimulating hormone (FSH)>, <AMH and androgens>. The following filters were used: abstract; full text; published in the last 10 years, women.

Relevant manuscripts were identified and then performed secondary reviews of referenced articles, which previously had not been known or preceded the searched time period. A total of 89 publications were reviewed. The procedure of searching the existing literature is shown in Fig. 1.

The flow diagram depicts the screening process of retrieved articles, including the number and reason of exclusion.

Sex determination and gonadogenesis

AMH is synthesized in male embryos by Sertoli cells and in cooperation with testosterone produced by testes it maintains differentiation of male reproductive organs. AMH influences regression of Müllerian ducts, which constitutes the first stage of sex determination in the somatic aspect. The influence of testosterone results in Wolf ducts transformation into vas deferens (Birk et al. 2000). In the prenatal development of female embryos, AMH is found in trace amounts along with formation of ovarian reserve, which will constitute reproductive potential from puberty until the complete expiration of ovarian activity in women during menopause.

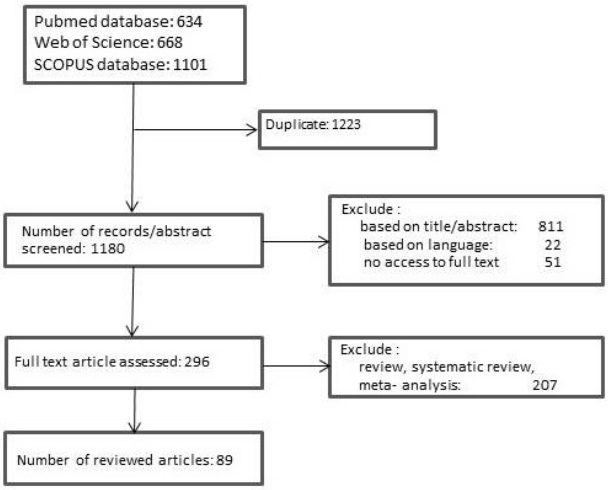

Figure 1. Flow diagram of literature review

Sexual determination of a developing embryo is strictly associated with the sex chromosome profile and depends on the presence of Y chromosome containing the TDF-Testis Determining Factor region (Nussey and Whitead 2001). Here, the SRY protein coding genes are present: The SRY factor initiates differentiation of Sertoli cells, already in a bipotential gonad of the embryo. These will constitute nearly half the volume of testes. These cells commence AMH secretion approximately 55–60 days after fertilization. This process is activated with approx. one week delay relative to Leydig cells that produce testosterone. The time and site for initiation of secretion of key hormones for the development of male embryo is significant due to the negative feedback of their influence in the implementation of male developmental pathway.

The process of female development appears to be more complex for humans than previously thought. Observations conducted on rodents suggest that the process of ovarian differentiation and transformation of Müllerian ducts into oviducts, uterus and upper portion of vagina is automatically activated in the absence of *Sry* gene. This has turned out not to be as obvious for humans, as the commencement of female developmental pathway, differentiation and stabilization of ovaries, seems to require the presence of several transcription factors (Tevosian 2013; Lin and Capel 2015; Biason-Lauber and Chaboissier 2015).

Primary urogenital system of a fetus is of mesodermal origin. During organogenesis, pairs of protrusions are formed from the epithelium that lines the body cavity, which form the precursor of the developing urogenital system. In the course of subsequent differentiation, they are transformed into Wolf ducts and an even meso-kidney. It consists of three segments: pronephros, mesonephros and metanephros.

Approximately 4 weeks after fertilization, strands of cells appear on both sides of mesonephros, known as genital ridges, which will become populated by primary sexual cells-gonocytes of endodermal origin. About 5 weeks after fer-

tilization, primary sex cords differentiate from the epithelium lining the body cavity, which constitute the scaffolding for gonocytes. This process initiates formation of morphologically undifferentiated gonads. Expression of *Sry* gene in a XY fetus promotes differentiation of testes with a network of ducts assuming the form of a horseshoe. Leydig cells are formed from the epithelium of sex ridges and are of mesenchymal origin. Eight weeks post fertilization, testosterone secretion becomes activated. Sertoli cells are differentiated from epithelial mesoderm, which commence AMH secretion at approx. one week time interval relative to Leidig cells. In the embryogenesis process, under the influence of maternal estrogens, pairs of germs of Müllerian ducts are formed from mesodermal epithelium.

Functional development of a gonad takes place about 7 weeks after fertilization, which is exhibited via secretion of trace amounts of estradiol by as yet phenotypically undifferentiated gonads. It should be emphasized that at the same time, testis commence testosterone production. Forty-five days since the beginning of human embryogenesis, ovary is identifiable only because the gonad has not yet transformed into early testes. In the third trimester of gestation, in the case of absence of SRY protein female developmental pathway becomes activated and gonads differentiate into ovaries, regression of Wolf ducts is activated and Müllerian ducts begin development towards formation of oviduct, uterus and upper portion of vagina (Munsterberg and Lovell-Badge 1991). Approximately 5–6 weeks after fertilization the primary sex ridges, and then sex cords with primary sexual cells initiate formation of ovary, which will constitute the source of AMH secretion. The Wolf ducts degenerate. Cords undergo fragmentation and regression giving the onset of the ovarian medulla, along with vascularized stroma. At the same time, cortical cords are created from the proliferating gonad epithelium. They will give the beginning of a line of granular cells, which, by forming a single layer around oogonia will form primordial follicle with theca of follicle. This takes place around 10 weeks after fertilization. First primordial follicles are formed in fetal ovaries approximately 15–16, while ready Graafian follicle in 23–24 week post fertilization (Reynaud et al. 2004). Due to proliferation of granulosa cells, granular and thecal layer are formed, which constitute important sources for secretion of steroid hormones and AMH.

The moment of AMH expression during embryogenesis is of key significance. The time shift associated with differentiation of male and female fetus may be associated with the fact of AMH influence on regression of Müllerian ducts. AMH receptors located on cellular membranes of Müllerian ducts of a female fetus are subject to regression. In this manner, even trace amounts of AMH produced by granular cells of the forming follicles cannot have a destructive impact on Müllerian ducts, which are then transformed into oviducts, uterus and the upper portion of vagina. AMH production is detectable in 24 week of gestation in granular cells of preantral follicles (Kuiri-Hanninen et al. 2011). In both sexes, initiation of AMH transcription is subject to a strict control in terms of place and time. It is of key importance for normal development and formation of the most important biological function of organisms-reproduction.

Mechanism of molecular regulation

Determination of the direction and sexual determination during embryogenesis

take place already in first weeks after fertilization. In the initial stage of development, i.e. approx. 6 weeks, bipolar, phenotypically undifferentiated gonads are formed. The genetic program of a fetus is determined by a set of sex chromosomes: XY, XX imposes the sex determination direction, provided the developmental process has normal course. This consists of numerous genetic and signaling connections with synergistic or antagonistic relationship, regulating the correct developmental pathway.

Chromosome Y determines the developmental processes of a male fetus by recording the *Sry* gene, coding the TDF (Testis Determining Factor). Formation of programmed testes requires activation of such factors as NR5A1, SF1 incorporating them in the expression of *Sox9* gene which is of key significance for the subsequent reactions. The activated signaling cascade acts as positive or negative interaction of factors or reactions. As a result of activation of developmental program for male fetus by *Sry* gene, the possibility for activation of reactions for female developmental pathway is automatically terminated (Barrionuevo 2005; Vidal et al. 2001; Chaboissier et al. 2004).

Absence of *Sry* gene results in signaling activation characteristic of initiation of the development of ovaries and repressing effect for activation of nuclear signaling network. In Vertebrata, sexual determination of gonads is associated with determination of lines of germ cells: spermatozoa or oocytes (de Falco and Cambel, 2009).

System of supporting cells, Sertoli and granular cells, is activated, which, at later stages of development, in relationship with the hypothalamic-pituitary-gonadal axis will play the key regulatory and endocrine role. Both types of supporting cells will be responsible for secretion of AMH, an important regulator during implementation of developmental programs (different for both sexes) and the functional performance of the reproductive system.

Research has confirmed that the primary sex determination associated with chromosome Y, activating programmed cascade of molecular interactions is more conservative than previously thought.

Gonad differentiation

The process of formation and stabilization of initially bipotential gonads in humans commences on the 32 day post fertilization. Numerous transcription factors are activated during embryogenesis, which activate the process of development and differentiation. Several genes were distinguished in research conducted on mice, such as: *Lh9*, *Gata4*, *Emx2*, *Wt1*, *Chx2*, *Nr5a* and *Six1/4*, influencing formation of bipotential gonads in embryos of both sexes (Svingen and Koopman 2013). WT1 (+KTS) and LH9 bind and activate the *Sf1* gene promoter. Similarly, *Cbx2* will activate *Sf1* expression. *Emx 2* is responsible for normal proliferation at the early stage of gonad development, further contributing to the signal reaction of the developmental pathway of supporting cell line, which will commence secretion of AMH at a different time and site of gonadogenesis. This is of particular importance, as they are differentiated at an earlier stage than germ cells. First, Sertoli cells and then granular cells are formed. *SOX9* and *SF1* initiate expression of *AMH* (*MIS*) gene. AMH, by binding with AMH-RII receptor commences signal via activation of AMHRI. AMH is regulated by SF1 and other transcription factors such as WT1(+KTS) and GATA4. Activation

of signaling elements takes place on the basis of positive and negative regulation correlation. AMH results in regression of Müllerian ducts formed during embryogenesis and development of XY fetus. *Pdgf* and *Dhh*, genes are also expressed in Sertoli cells, which subsequently contribute to the process of Leydig cells formation, where the steroidogenesis process further includes SF1. Testosterone secretion initiates directional organization of testes and development of internal genital organs (Tanaka and Nishinakamura 2014). Following formation of testes and involution of Müllerian ducts, AMH will remain at a stable, relatively low level. In female fetus, approx. 10 weeks post fertilization and regression of AMH receptors in cellular membranes of Müllerian ducts, the neutral gonad differentiates into ovary. This process is automatically activated in the case of absence of SRY and signal cascade associated with the protein, leading to testes differentiation. *Wnt4* and *Rspo1* and the negatively correlated beta-catenin effector substance activate the program for female developmental pathway. Expression of the *Fox* gene is activated in the postnatal period and it becomes a part of maintaining female developmental pathway. Analogous function is played in male fetus by the *Dmrt1* gene.

Folliculogenesis and anti-Müllerian hormone

Thirty two weeks post fertilization, the process of development of female gonad is accomplished. From this moment, ovary in correlation with the hypothalamic-pituitary axis assumes its key endocrine function. Pituitary gland of female fetus produces gonadotropic hormones, FSH and LH, which act through receptors located on membranes of thecal cells of the theca and granular cells of ovarian follicles (Juuula et al. 2012).

Cells of the developed follicular apparatus exhibit high dynamism, participating in cyclical transformations under the impact of hormones and successfully entering the pathway of apoptosis. This process commenced in embryonic life will cease only during menopause. In 18 week of gestation, ovaries contain 7 million follicles (te Velde and Pearson 2002). Gonads of female fetus contain as many as 1–2 million of them. Before a girl attains sexual maturity, they will amount to 400 thousand, and after menopause approx. 1000 follicles will remain in the ovaries. Value of AMH concentration is reduced during embryonic life, as well as after the birth (Lasala et al. 2011; Lukas-Croisie et al. 2003; Rey et al. 2006). Numerous transcription factors participate in the process of primary follicle formation, whose expression and signal reactions have not been fully researched (Fowler et al. 2009). FIG-alpha is one of the factors with real impact on the process of primary follicle formation that has been discovered (Soyal et al. 2000). The transition from primordial to primary stage of a follicle is regulated by key transcription factors: NOBOX, SOHLH1 and SOHLH2. GDF9 (Laitinen et al. 1998; Dong et al.1996) and BMP15 (Di Pasquale et al. 2004) factors regulate the development of preantral and antral follicle. At this developmental stage, attained in 24 week of gestation, the follicle commences AMH secretion. AMH regulates follicle recruitment and transition to the subsequent developmental stage via inhibition of FSH receptors, which may lead to selection of one, that will attain the stage of Graafian follicle and will become released during ovulation (Durlinger et al.1999,

Visser et al. 2006). AMH secretion by preantral and small antral follicles occurs independently of the influence of FSH. Follicles in excess of 8 mm are FSH-dependent. The increasing level of FSH at the follicular stage influences increase of estrogen concentration, which, acting via negative feedback influence the decrease of AMH concentration in the follicle. It has been demonstrated that the level of AMH correlates with the concentration of estrogens in small antral follicles. This statement is confirmed by observation of polymorphisms occurring in AMH and AMHRII receptor. Damaged genes influenced the clear decrease of the level of E2. Action of AMH is based on its affinity to AMHRII receptor, which attaches functionally to AMHRI. The formed complex triggers tyrosine phosphorylation reaction, leading to commencing reaction via SMAD protein activation. They are involved in transfer and transduction of signal in cellular nucleus, influencing the transcription of target genes by attaching to specified elements of the promoter. The initialized signal cascade is accompanied by elements with activator and effector functions (Shi and Massague 2003). SMAD are associated with transferring signal to the nucleus and are activated solely by ligands of the large beta-TGF family (Massague et al. 1996). AMHRII receptor possesses affinity to one ligand type, contrary to AMHRI, which attaches to several types of ligands. Type I receptor is common for selected factors belonging to the beta-TGF superfamily, including human bone morphotic factor BMP, apart from AMH ligand it may attach e.g. ALK, corresponding to BMP. AMHRI could probably result in a defective or lethal morphogenesis of bones. AMH type II receptor was discovered and cloned by two independent research teams: Baarends et al. (1994) and di Clemente et al. (1994). Genomic sequence recording AMHRII is located on chromosome 12 (13q12) and it consists of 11 exons. 3 first constitute the record of extracellular domain, 4 exon is a transmembrane domain while 7 remaining ones-intracellular domain. The sequence is 8.7 kb long. AMHRII are present in Müllerian ducts and gonads of both sexes (Baarends et al., 1994; di Clemente et al. 1994). AMH type II receptors have also been found in the endometrium (Renaud et al. 2005) and in mammary and prostatic glands (Hoshiya et al. 2003). AMH type I receptor in combination with AMHRII participates in signal transfer.

The fact of a different interaction between sexual cells and AMH secretion in Sertoli and granular cells during gonadogenesis leads to interesting observations. They have been confirmed by the study conducted by Behringer et al. (1990) on transgenic mice. It has been demonstrated that Sertoli cells can survive independently, even if reproductive cells have been damaged or destroyed, while granulocytes in the case of female line have a different behavior.

High AMH levels appear to be toxic for oocytes undergo premature meiosis. Concentration of the hormone in the follicle within specified time interval may be slightly increased and toxic for germ cells. It is suspected that this mechanism constitutes a certain type of regulation, optimizing time and site of subsequent developmental processes.

AMH concentration reflects secretion of only those follicles that are vascularized. Despite the auto-and paracrine secretion, AMH value in blood serum reflects well the pool of ovarian follicles (La Marca, Sunkara 2014).The hormone influences aromatase inhibition in gran-

ular cells, which converts androgens to estrogens via blockage of cytochrome expression (Grossman et al. 2008).

Interesting variability is expressed by AMH depending on the season of the year, which is associated with exposure to sunlight and expression of D3dihydroxyvitamin. 25OH-D influences negative regulation of AMH via AMHRII receptor mechanism and SMAD 1/5/8 protein mediation (La Marca, Sunkara 2014, Su et al. 2014; Gassner et al. 2014). As an effect of D3 vitamin, expression of the receptor gene is reduced, SMAD phosphorylation is reduced and its location in the nucleus is altered. Reproductive potential is increased due to repression properties of AMH and reduction the sensitivity of AMHRII receptor and SMAD proteins by 25OH-D. This is exhibited by a more efficient differentiation of granular cells in the follicle and enables their major portion to develop properly. This increases the chance for the selection of the best dominant follicle during ovulation. Reproductive potential increases significantly, as 25OH-D concentration correlates negatively with the level of AMH, balancing inhibition in developmental and control processes. Research on the seasonal variability of AMH concentration was conducted by Dennis et al. (Dennis et al. 2012), which translates into better understanding and directing reproductive processes and thus the reproductive success.

AMH regulates ovarian follicle recruitment for subsequent stages of folliculogenesis via reduction of their sensitivity to FSH (Durlinger et al. 2002; Visser et al. 2006). This process requires strong molecular basis in the form of a range of transcription factors, which will implement the process by means of positive or negative regulation. The fact that AMH is highly conservative in evolutionary terms allows to assume that the above presented developmental process with its contribution appears to be most advantageous in biological terms.

Recent studies on women with polycystic ovaries (PCOS) who have higher level of androgens have shown that early follicular growth may be promoted by androgens (Fig. 2). During the pre-antral (gonadotropin-independent) follicle growth, FSH stimulates follicle growth via theca cell-derived androgens. Selected androgens may

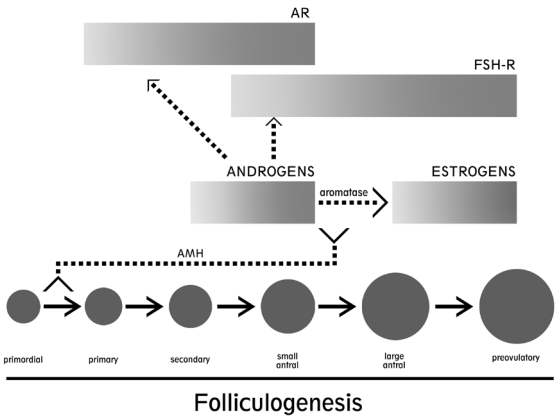

Figure 2. Working model showing how selective androgens appear capable of improving early stages of folliculogenesis. See text for explanation. Modified after Lebbe and Woodruff (2013).

exert effects via androgen receptors (AR) through transcriptional regulation but also via enhanced FSH receptor expression modulating follicle stimulating hormone (FSH) activity in granulosa cells. The AMH is produced by preantral and small antral follicles. AMH through the inhibition of FSH receptors regulates premature recruitment of ovarian follicles. FSHR expression / activation occurs under androgen control. AMH inhibits the conversion of androgens to estrogens by blocking the expression of aromatase induced by FSH (Lebbe and Woodruff 2013).

The relationship between anti-Mullerian Hormone and FSH and their effect on the regulation of steroid hormones is shown in Fig. 3. Positive or negatively conjugated loops maintain balance during folliculogenesis limiting excessive recruitment of ovarian follicles. The maintenance of the proper concentration of androgens and estrogens in the individual stages of ovarian follicle development is responsible for the regulation of the menstrual cycle and ovulation. The effect of androgens on the expression of AMH has not been fully understood yet (Dewailly et al. 2016).

Experimental studies

AMH, as a member of a large family of molecular growth factors beta-TGF, constitutes an important component in the control over the ovarian follicle pool, occurrence at trace amounts and for a short period during prenatal development, determs correct course of a range of interactions implementing genetically programmed sex determination pathway, while in the reproductive period it constitutes a significant regulator of the ovarian balance (Yigong S, Massagué J. 2003).

AMH molecular model, due to highly conservative C-terminus of its molecule, constitutes the object of scientific research and investigations. A series of studies utilizing animal models were conducted, which have proven that the evolutionary process adopted and fixed main regulatory mechanisms in the process of developmental pathway realization. In certain species, they have undergone some modifications, yet a common trend has been observed in the field of function of AMH in the majority of Vertebrata, including humans.

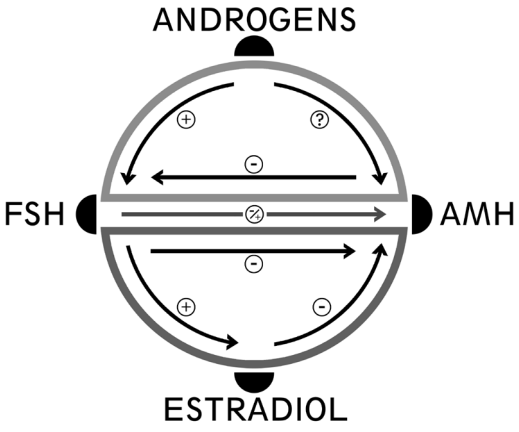

Figure 3. Relationship between anti-Müllerian hormone and FSH and their effect on the regulation of steroid hormones during early stages of folliculogenesis. See text for explanation. Modified after Dewailly et al. (2016).

The human gene coding AMH has the length of 2.8 kb (Cate et al. 1989) and it is mapped in the 19p.13.3 position. It has been cloned for numerous mammal as well as fish, salamander, reptile and bird species (Halm et al. 2007; Pala et al. 2008). The expression pattern of genes coding AMH is similar in Vertebrata, yet the expression method and certain modifications in the genomic sequence reading stem from species particularities.

It has been determined that the ortholog group is characterized, similarly to humans, regression of Müllerian ducts during the embryogenesis of the male sex. This includes a marsupial-tammar wallaby (Pask et al. 2004), American alligator (Western et al. 1999) or chicken (Carré-Eusèbe et al. 1996; Neeperet al. 1996). This observation has not been confirmed in representatives of such species as *Caudata amphibia, Pleurodeleswaltl,* the Iberian ribbed newt, representatives of salamanders. Male individuals retain Müllerian ducts (Al-Asaad et al. 2013). The discovery of AMH orthologs among teleost fishes came as a surprise, for which AMHRII genes could be cloned and full regression of Müllerian ducts was observed.

In fishes, AMH plays the key role for proliferation of reproductive cells and gonad maturation. This allows to conclude that in the course of evolution, AMH was responsible for an increasing range of molecular reactions. This is confirmed by the fact that in higher vertebrates, the scope of AMH effect has covered regulation of the follicular system in the ovaries of adult females (Visser and Themmen 2005) or control of Leydig cells in males (Racine et al. 1998). Its previous role was limited solely to the function of differentiation of bipolar gonads. Regulation of human embryogenesis enables confirmation of the fact of high sexual dimorphism in prenatal development due to the effect of AMH. Time shift caused by varying AMH coding gene expression is of high significance. In Sertoli cells it is activated on 8 week post fertilization, whereas in female fetus this process takes place on 24 week in granular cells of the formed ovarian follicles. AMH actively participates in the process of folliculogenesis, which is the expression of high evolutionary specialization (Durlingr et al. 2002).

The carboxylic portion of the AMH glycoprotein molecule remains strongly conservative in evolutionary terms, which is confirmed by the comparison of amino acid sequence between: mouse and human and mouse and cattle in terms of the percentage contribution of homologues: 95% and 94 % of homology. The minor differences between the species stem from the respective 5 and 6 changes in the group, which comprises approx. 106 amino acids.The presence of cysteine has also been considered, as it is highly valuable as predictor of evolutionary conservationism (Cate et al., 1986).

The observed pattern of gene expression: *amh*, *sox9a*, *sox9b* and *cyp19a1*a and regulatory reaction in the zebrafish comply with that observed for mammals, thus confirming the conservatism of the expression pattern. However, different species may exhibit various regulatory mechanisms and interactions between genes, as in the case of e.g. chicken or alligator embryos, where amh expression precedes sox9 expression (Smith et al., 1999, Western et al.,1999, Oreal et al., 2002).

AMH constitutes a negative regulator of the *CYP19a1* gene in mammal ovarian follicles. The product of the gene expression is aromatase, an enzyme controlling

conversion of androgens to estrogens. This female steroid hormone affects reproductive success. Expression of the *Sox9b* gene-regulating AMH expression, occurs directly in the ooplasm of oocytes, which appears to favor directional regulation of the AMH in the correct place and time. (Rodriguez-Mari et al. 2005)

The research team of Rodriguez-Mari (2005), who carried out molecular testing on the zebrafish obtained results that have confirmed the similarity concerning AMH expression in human granular cells of ovarian follicles, from the fetal live, through reproductive period until the menopause (Lasala et al., 2004). Identical AMH expression pattern was determined in the granulosa cells in both tested cases, with differences of histological nature being determined. The result suggests that genes coding AMH sequence originate from a common ancestor to fishes and humans, thus confirming the role fulfilled by the AMH in reproductive process.

Clinical studies

Determination of the AMH level in humans is increasingly commonly applied in highly developed countries. In clinical practice, determination of AMH serum concentration is applied primarily for the procedures of assisted reproduction performed in larger clinical centers.

AMH constitutes a stable marker reflecting normal prenatal sexual development (Josso et al. 2012; Rey and Grinspon 2011). The diagnostic value of AMH further includes postnatal developmental disorders, in particular of boys at prebupertal age (Grinspon and Rey 2010; Rey et al. 2013; Rohayem et al. 2015).

AMH is widely used for determination of ovarian reserve in women at reproductive age (van Rooij et al. 2002; van Rooij et al. 2005) and for prediction of time to menopause (Tehrani et al. 2013). It has been observed that AMH concentration is subject to minor fluctuations during menstruation cycle, which is not of clinical importance (van Disseldorp et al. 2010; La Marca et al. 2013.) The hormone maintains its high diagnostic value.

Determination of AMH serum concentration is used for the basic examination of patients with suspected granular cells cancer (Gustafson et al. 1992; Long et al. 2000). AMH is used for standard IVF procedures, where it constitutes the main diagnostic element for determining causes for infertility (Alvaro Mercadal et al. 2015). Depending on the hormone level determined, and the use of other diagnostic tools, the cause is determined with high likelihood, which greatly facilitates treatment.

Diagnostic methods

The lack of uniform standards for laboratory determinations constitutes a disputable issue. Results obtained differ markedly, and depend on the applied method and laboratory tools. Clinical centers establish own norms used to compare the obtained results, yet different sensitivity of applied systems disables comparison of results in the global aspect.

The Beckman-Coulter Gen-II system utilizes the developed DSL antibodies (Li et al. 2012; Han et al. 2014), however, a precise determination according to the rhAMHImmunotech curve requires repetition of the procedure to eliminate additional reaction with the complement. Results of AMH determinations turned out to be approx. 22–40% higher in comparison to the results with the use of previously used, I generation system (Wallace et al. 2011).Widely used

is the Ultrasensitive picoAMH ELISA system by Anshlabs (2013), as well as a version of this system-analysis with dried blood (Gassner and Jung 2014) by the same company (Roberts et al. 2016), as well as Beckman Coulter Access AMH (Pearson et al. 2016). Laboratory diagnosticians value the Roche Elecsys product, which was introduced to the marked in 2014. It is a modern, fully automatic diagnostic system, which utilizes electrochemiluminescence effect (Gassner et al. 2014).

The process of AMH value determination has been abbreviated to 18 minutes.

In June 2013, during the ESHRE Congress (European Society of Reproduction and Embryology), the Backman Coulter company applied for an improvement of the diagnostic value of AMH by means of proper preparation of samples with buffers or freezing after their collection and prior to determination. Establishing international standards for AMH constitutes the current challenge in the clinical practice.

Conclusions

Application of AMH in clinical practice appears to have a wide perspective of development. The AMH diagnostic value enables an insight into developmental processes, both during prenatal life as well as after the birth. It is of particular significance in woman reproductive period, since AMH is the most precise marker reflecting biological processes taking place in the ovaries.

The conservative mechanism of sex determination and differentiation during embryogenesis is regulated by activation of expression of genes associated with chromosome Y, which activates biochemical cascades, including AMH gene expression. This hormone, indispensable for the process of sexual formation and regulation of male and female embryo, acts according to different signal patterns. In those processes, cascades of biochemical reactions are activated, including transcription factors, cofactors, signal proteins and genes themselves. Positively or negatively coupled interaction loops in biological processes realize genetic pattern for differentiation, development and maintaining of the reproductive potential in the adult life.

This conservative regulatory mechanism, with the important role of AMH, is observed in the majority of Vertebrata.

A more extensive use of AMH diagnostic value in the future may contribute to better understanding of processes associated with dysfunction of the reproductive system and fertility disorders.

Authors' contributions

Both authors contributed equally to this manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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