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TITLE: Clinical Applications of Anti-Müllerian Hormone Measurement

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Hello, my name is Aaron Geno. I am a Clinical Chemistry Fellow at Dartmouth-Hitchcock Medical Center in Lebanon, New Hampshire. Welcome to this Pearl of Laboratory Medicine on “Clinical Applications of Anti-Müllerian Hormone Measurement.”

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To begin, what is anti- Müllerian hormone?

Anti-Müllerian hormone, or AMH, is a protein hormone from the transforming growth factor beta, or TGF-ß, family. TGF-β family proteins regulate tissue growth and differentiation. AMH is a homodimeric protein complex of approximately 140 kDa in weight composed of two identical subunits linked by disulfide bonds. Anti-Müllerian hormone is also known by the names, “Müllerian inhibiting factor, Müllerian-inhibiting hormone, and Müllerian-inhibiting substance”

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So, why is it called anti-Müllerian hormone? AMH gets its name from its relationship to Müllerian structures. Müllerian structures are, as their name suggests, structures derived from the Müllerian ducts in the course of development. In males, the Müllerian ducts atrophy. In females, the ducts persist and go on to form the uterus, fallopian tube, and vagina.

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You may have guessed, then, that anti-Müllerian hormone inhibits the formation of the Müllerian structures in men. During male uterine development, sertoli cells in the testis produce high amounts of AMH. The resulting elevated concentration of AMH suppresses the production of Müllerian structures (namely, the vagina, uterus, and fallopian tubes). At the onset of puberty, as testosterone begins to climb towards adulthood concentrations, AMH declines in parallel, though it remains at concentrations greater, on average, than those generally observed in females.

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In females the absence of AMH during development allows the development of the vagina, uterus, and fallopian tube structures. After puberty, AMH plays a central role in maintaining the ovarian reserve. Primordial follicles grow in response to follicle-stimulating hormone, or FSH. As follicles develop into preantral and antral follicles, AMH is produced in follicular granulosa cells, reaching peak production in the small antral follicles. AMH inhibits additional follicular recruitment and inhibits FSH-mediated maturation of additional small antral follicles until selection of the dominant follicle occurs. AMH has also been proposed to titrate the estrogen released by granulosa cells through desensitizing granulosa cells to FSH.

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In the clinical laboratory, anti-Müllerian hormone is measured by immunoassays. Originally, this was performed via manual, two-site, or “sandwich” format ELISA. However, as clinical utility has been demonstrated and interest has increased, automated two-site immunoassays are also common. As a quick reminder, a two-site immunoassay is one in which an antibody captures the target of interest, and a second antibody binds to the target in a second site. Depending upon the format, a streptavidin conjugate may be required to bind to a biotin tag on the second antibody, as shown in

this figure, or a label may be directly conjugated to the second antibody. Any biotin-containing immunoassay may be subject to biotin interference. In either case, the complex is ultimately stimulated, in the case of electrochemiluminescence or fluorescent tags, or incubated with a substrate in the case of an enzyme conjugate, and the resulting signal is in direct proportion to the concentration of the target contained within the sample.

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The first commercial assays for anti-Müllerian hormone were produced by Immunotech in 1999 and Diagnostic Systems Laboratories in 2003. Both companies were acquired by Beckman-Coulter by 2005, and in 2011, Beckman Coulter introduced the Gen II AMH assay. However, it was observed in stability studies that the apparent concentration of AMH *increased* over time – unusual for a directly proportional assay. Subsequent studies revealed that complement component C1q from patient serum bound the antibodies in the assay and activated complement upon them via the classical pathway, resulting in steric hindrance that prevented AMH recognition. As specimens aged, complement degraded, and interference lessened, resulting in increased apparent AMH. The discovery of this issue created confusion and necessitated multiple updates to the assay, including adding a pre-dilution step to neutralize complement, and it likely delayed more widespread implementation of AMH testing. Current automated assays are not impacted by complement interference.

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Why would one want to measure anti-Müllerian hormone? We'll devote the remainder of this presentation to this question. This list highlights a number of these applications; due to the time restriction of this format, we'll only touch on a few of them today. It's important to note that while clinical utility has been demonstrated for many of these applications, commercial AMH assays may not be FDA approved for all applications.

For example, the Roche and Beckman assays are approved only for assessment of ovarian reserve, while AnshLabs' assay is FDA approved only for assessing menopausal status.

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We'll begin with a bad use of anti-Müllerian hormone testing. A single AMH measurement should not be utilized for an assessment of general fertility or for determinations of where a woman may be on her "biological clock." AMH has not been demonstrated to correlate with the likelihood of becoming pregnant! In one study, women with low serum AMH achieved pregnancy at similar rates to women with normal to high AMH. Another study found that women with serum AMH in the lowest 20% of participants exhibited similar fecundability—that is, the probability of becoming pregnant within one menstrual cycle—to women with AMH in the central 60% of participants. Notably, in this study, women with the highest 20% of AMH concentrations had reduced likelihood of becoming pregnant, which may be related to polycystic ovarian syndrome, as we'll see in the next few slides. Finally, in a study of 750 women aged 30-44, AMH did not predict fertility as defined by ability to achieve pregnancy within 12 menstrual cycles.

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Polycystic ovarian syndrome, or PCOS, is a condition characterized by hyperandrogenism, ovulatory dysfunction, polycystic ovarian morphology, insulin resistance, and diabetes. PCOS is largely a diagnosis of exclusion. Once alternative explanations are eliminated, the 2003 Rotterdam criteria for PCOS define PCOS as having at least two of the following three symptoms: hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. The Rotterdam Criteria are currently the most widely-utilized diagnostic criteria and those endorsed by the Endocrine Society.

Estimates for prevalence vary, but the United States Centers for Disease Control and

Prevention estimates that between six and twelve percent of women of reproductive age are affected by PCOS, making it the most common cause of infertility.

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As we discussed on the previous slide, polycystic ovarian morphology is one of the parameters assessed by the 2003 Rotterdam criteria in the evaluation for PCOS. This is currently assessed by counting of antral follicles by ultrasound. The Rotterdam criteria established an antral follicle count of 12 or greater to be indicative of polycystic ovaries. However, this is problematic for diagnosis, as ultrasound technology has advanced since 2003, and higher image resolutions have led to more accurate follicle counts that are higher than what was achievable before. A 2013 study found that the Rotterdam threshold of 12 was no longer appropriate – while the sensitivity remained high, specificity was low, as shown in the table. Other proposed thresholds were evaluated, up to 26 follicles per ovary, which the authors deemed the best balance of sensitivity and specificity. Given a shifting goalpost for follicle number per ovary and the inherent subjectivity of manual counting from an image, an objective measure, such as a biomarker surrogate, is a desirable goal. Anti-Müllerian hormone has been found to correlate well with antral follicle count, and AMH is being evaluated as a possible substitute for this metric; nonetheless, AMH has not yet been incorporated into consensus clinical guidelines.

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Anti-Müllerian hormone has several advantages as a surrogate biomarker in PCOS. It correlates well with antral follicle count, and it has been observed to be substantially elevated in individuals with PCOS, in whom its concentration correlates with the severity of PCOS symptoms. Additionally, its concentrations do not change throughout a woman's menstrual cycle. However, it is not without limitations.

AMH concentrations have been reported to change with a number of other variables, including weight, age, smoking status, and oral contraceptive use. Some studies have also proposed that it can vary with ethnicity. There are also technical challenges, such as the lack of a standardized reference material, an essential need if AMH is to be incorporated into diagnostic criteria. In addition, inappropriate specimen handling can dramatically alter the AMH recovery of a specimen. None of these challenges is viewed as insurmountable, however, as the in vitro fertilization field is successfully incorporating these additional factors into patient management.

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We'll close our look at clinical use of AMH in fertility with a more established use. In recent years, AMH has found clinical utility in the prediction of ovarian response to stimulation prior to oocyte harvest for in vitro fertilization. At the lower extreme, it can identify women who will respond poorly to normal courses of ovarian stimulation and who will require additional treatment to maximally stimulate follicle recruitment. At the upper extreme, it can identify women who will respond overly well to normal stimulation and thus be at high risk for developing ovarian hyperstimulation syndrome.

Ovarian hyperstimulation syndrome occurs when ovaries become enlarged due to the recruitment and sustained development of a large number of antral follicles, leading to overly aggressive vascularization, which leads to escape of follicular fluid and perifollicular blood containing large amounts of VEGF into the peritoneal cavity. This leads ultimately to fluid shift from the intravascular space into the third compartment--known as "third spacing"—and can be life-threatening, causing strokes, ischemia in extremities, and other thromboembolic events. Identification of women at risk for hyperstimulation and altering their treatment accordingly can reduce much of the risk associated with in vitro fertilization.

A sample protocol for oocyte harvest, from the citation provided, stratifies women based on their AMH concentrations. A normal AMH concentration receives the normal course

of stimulation, using gonadotropin-releasing hormone agonists. A low AMH concentration predicts a poor response to the normal protocol, and a modified protocol known as a “flare” protocol is employed, using several microdoses of a gonadotropin-releasing hormone agonist to prevent premature LH surges that can work against a collection that is already likely to be suboptimal. A high AMH concentration, on the other hand, predicts a patient who is likely to over-respond to the normal stimulation protocol. In this situation, long-acting gonadotropin releasing hormone antagonists can be given to titrate the response to the agonists and reduce the likelihood of ovarian hyperstimulation syndrome.

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In addition to fertility-related uses, AMH also has utility in the evaluation of disorders of sexual development. Disorders of sexual development are situations in which genitalia are discordant with relation to the chromosomes or gonads. Because *in utero* AMH is produced solely in the sertoli cells of the testis, a male concentration of AMH indicates the presence of testicular tissue. This can help to clarify etiology for the disorder when placed in the context of other findings such as karyotype, ultrasounds, and concentrations of other relevant hormones such as 17-hydroxyprogesterone. In addition, identification of testicular tissue via AMH or other means can inform the decision of whether to pursue gonadectomy, as the presence of Y chromosomes in a dysgenetic gonad carries a high risk of malignancy.

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Finally, AMH is also useful as a tumor marker. As we learned earlier, anti-Müllerian hormone is secreted by granulosa cells. Granulosa cell tumors account for 2% of ovarian cancers and characteristically secrete anti-Müllerian hormone. Historically, inhibin B was the tumor marker used in following patients with granulosa cell tumors; however, recent studies have found that AMH was also significantly elevated in patients

with primary or recurrent tumors and also correlated with tumor size. Combining inhibin B and AMH measurement improved the detection of recurrent disease.

Slide 16: References

[no spoken content]

Slide 17: Disclosures

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Slide 18: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Clinical Applications of Anti-Müllerian Hormone Measurement.**"