The diagnostic value of anti-mullerian hormone in female infertility evaluation

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Abstract

The period of optimal fertility extends until the age of 30 yr and decreases gradually thereafter. The decrease in fertility is probably may be due to the decline of the number of primordial follicles after birth; by the age of 37-38 yr, the follicle ratio is significantly minimized as is the quality and quantity of oocytes. Anti-Mullerian hormone (AMH), or Mullerian Inhibiting Substance, is also known as one of the most important markers of ovarian reserve. The antral follicle count (AFC) is the number of antral follicles, generally in both ovaries taken together if not else specified. It can be determined by transvaginal ultrasonography. A low AFC is a major factor in the diagnosis of poor ovarian reserve, that is, low fertility characterized by low numbers of remaining oocytes in the ovaries, usually accompanied by high follicle-stimulating hormone (FSH) levels. Several studies show that an AFC test is more accurate than basal FSH testing for older women (< 44 years of age) in predicting IVF outcome. Antral follicle counts by ultrasound are one of the best ovarian reserve tests that we currently have available.

Keywords Fertility, Antral Follicle, Anti-Mullerian Hormone, Luteinizing Hormone, Follicle-Stimulating Hormone.

Introduction

Fertility is remarkably declines with age for females in both spontaneous conceptions and pregnancies following assisted reproductive methods (Templeton et al., 1996). The period of optimal fertility lasts until the age of about 30 yr and decreases gradually thereafter. The decrease in fertility is probably because of the decreasing number of primordial follicles after birth; by the age of 37-38 yr, the follicle pool is significantly decreased, (Faddy et al., 1992) as is the quality and quantity of oocytes (Novot et al., 1991). Anti-Mullerian hormone (AMH), or Mullerian Inhibiting Substance, is also known as one of the most important markers of ovarian reserve (Van Rooij et al., 2002). In the ovary, AMH inhibits initial primordial follicles recruitment and decreases the sensitivity of preantral and small antral follicles to follicle-stimulating hormone (FSH) (Durlinger et al., 2002), hence suggesting its role in intrafollicular and interfollicular coordination of follicle development (Salmon et al., 2004). It has been found that human antral follicles measuring < 6 mm express the greatest amount of AMH, and that levels decline as antral follicles increase in size. It has become clear that AMH also plays an important role in ovarian function, especially follicle development and follicle selection (Al-Kanaani et al., 2020). Furthermore, AMH is elevated in females with polycystic ovary syndrome (PCOS) compared with control, leading to the hypothesis that exaggerated AMH could be involved in follicular arrest seen in polycystic ovaries (Pigny et al., 2006). AMH is strongly correlated to antral follicle count (AFC), and both predictors have a linear relationship with age (Mustafa et al., 2019). Confirming the decline of ovarian reserve with age. Many researchers have found that basal AMH levels accurately reflect the total developing follicular cohort and ovarian response to gonadotrophin in ART cycles (Hazont et al.,
2004). As yet, AMH testing, like other predictive markers, doesn’t appear to predict accurately the probability of pregnancy after IVF treatment (Broer et al., 2008). Age, serum basal FSH levels, and serum basal estradiol (E2) levels are novel biomarkers for ovarian reserve (Bukulmez & Arici, 2004). AFC, serum inhibin B levels, and ovarian volume have also been studied as markers of ovarian reserve (Hendriks et al., 2005).

**Aim of the Study**

To investigate whether Anti-Mullerian hormone and antral follicle count can be useful in predicting the ovarian reserve and pregnancy rate in assisted reproductive technology cycles.

**Literature Review**

1. **Menstrual cycle**

Normal menstruation is defined as the periodic efflux of the sloughed endometrium and blood out of the uterine cavity into the vagina and ultimately outside of a woman’s body (Randal & Robinson, 2007). The period of the menstrual cycle is determined from the beginning of menstrual bleeding until the bleeding of the subsequent cycle. The idealized length of the normal human menstrual cycle is 28 days, the average amount of blood lost with each menstrual cycle is 30-80 ml (Randal & Robinson, 2007). The length of a female’s menstrual cycle is different; some have shorter periods and some longer periods. Eumenorrhea denotes normal, regular menstruation that lasts for a few days (usually 3 to 5 days), also periods from 2 to 7 days is considered normal (Strassman, 1996).

In the menstrual cycle, changes occur in the female reproductive system as well as other systems (which lead to breast tenderness or mood changes, for example). A woman’s first menstruation is termed menarche and occurs typically around age 12-13 (Anderson et al., 2003). The cessation of menstrual cycles at the end of a woman’s reproductive period is termed menopause. Menopause before the age of 40 is considered premature and the age of menopause is largely a result of genetics; however, illnesses, certain surgeries, or medical treatments may cause menopause to occur earlier (Greenberg et al., 2007).

1.1. **Follicular phase**

It is also called the proliferative phase because a hormone causes the lining of the uterus to proliferate, within this time (Losos Jonathan et al., 2002). The low levels of serum inhibin A, estrogen, and progesterone allow for increases in gonadotropin-releasing hormone (GnRH) pulse frequency. This increase in GnRH pulsatility begins in the last luteal phase with the decline in serum progesterone, estrogen, and inhibin A that occurs if a pregnancy has not developed and the corpus luteum involutes. GnRH pulses increase from 3 per day to 14 per day. This increase in GnRH pulses frequency results in an increase in FSH production from the anterior pituitary (Randal & Robinson, 2007). In general, FSH in the presence of E2 induces the formation of LH receptors on the granulosa cells. If the LH level is too high, theca cells produce a large amount of androgen that causes follicular atresia (Campbell et al., 2000). This follicular atresia is important because it normally allows only one follicle to grow large enough each month to ovulate, therefore usually preventing more than one child from developing with each pregnancy (Guyton & Hall, 2001).

1.2. **Ovulation**

Ovulation is the process of releasing an egg. When the egg has nearly matured, E2 levels in the body have increased enough to trigger an abrupt release of LH from the anterior pituitary gland. In the average cycle, this LH surge starts around cycle day 12 and may last 48 hours (Weschler & Toni, 2002). These opposite responses of LH to E2 may be stopped by two different estrogen receptors in the hypothalamus, which are: estrogen receptor alpha, which is responsible for the negative feedback E2-LH loop, and estrogen receptor beta, which is responsible for the positive E2-LH relationship (Hu et al., 2008).

1.3. **Luteal phase**

It is also called the secretory phase. After ovulation, the pituitary hormones FSH and LH lead the remaining parts of the dominant follicle to move into the corpus luteum, which produces progesterone (P). The hormones produced by the corpus luteum also work on suppressing the production of the FSH and LH that the corpus luteum requires to maintain itself. Consequently, the level of FSH and LH fall quickly over time, and the corpus luteum subsequently atrophies (Losos Jonathan et al., 2002).

Falling levels of (P) trigger menstruation and the beginning of the next cycle. From the time of ovulation until (P) withdrawal has caused menstruation to begin, the process typically lasts two weeks, with 14 days considered normal (Weschler & Toni, 2002). In the middle to late secretory phase, evidence of the increased secretory capacity of the endometrial glands becomes more apparent. Vascularization of the endometrium increases, the glycogen content increases, the endometrial glands become engorged with secretions, they become tortuous and achieve maximal secretory activity at approximately day 20 (Randal & Robinson et al., 2007).

2. **Regulation of the menstrual cycle**

The menstrual cycle is regulated at both endocrine and paracrine levels, the early stages of follicle development in the human are independent of gonadotropins. Further progression to the antral and pre-ovulatory stages appear to be completely dependent on the presence of gonadotropins (William & Ledger, 2007). The neuroendocrine system that controls the human menstrual cycle is the pulsatile nature of gonadotropins secretion, which is a direct result of episodic release GnRH from the neuronal terminals at the arcuate nucleus median eminence region and delivery to the gonadotroph through the portal vessels (Yen et al., 2009). These trophic hormones, LH and FSH stimulate the ovary to synthesize and secrete the sex steroids estrogens and progesterins. The ovaries also produce peptides called inhibins and activins. Together, these ovarian steroids and peptides exert both negative and positive feedback
on both the hypothalamus and the anterior pituitary. Because the cyclic secretion of estrogens and progestins primarily controls endometrial maturation, menstruation reflects these cyclic changes in hormone secretion (Ervin & Jones, 2009).

3.1. Oogenesis

Oogenesis is the sequence of events by which primitive germ cells called oogonia are transferred to mature oocytes. Ovarian follicles are the main functional units of the mammalian ovary, includes two functional pools, resting and growing follicles. The resting follicle pool represents the “ovarian reserve” whereas follicles will be ready for maturation throughout life (McGee & Hsueh, 2000).

3.2. Folliculogenesis

Folliculogenesis is the maturation of ovarian follicles, the progression of several small primordial follicles into large preovulatory follicles that enter the menstrual cycle. So oocyte maturation passes in two periods, prenatal and postnatal periods (Fortune, 2000).

3.2.1. Pre-natal period

In humans, folliculogenesis begins before the 13th-week post-conception. At about 6-8 weeks of gestation in the interior part of the ovary. The first sign of ovarian differentiation starts during the rapid mitotic multiplication of germ cells (Speroff et al., 1999). These mitotic divisions are completed by the middle fetal life and oogonia enter their first meiotic division. These cells are now called "primary oocytes" (Boron & Boulpeap, 2005). Starting from about 26,000 oogonia at 6 weeks of conception. The germ cell number increases with gestational age until it peaks at about 6-7 million at 20 weeks of gestation. Germ cell number then decreases to about 2 million at term pregnancy (Bendsen et al., 2006), at which time more than half are still not engaged in primordial follicles. The meiotic division of the primary oocyte which begins at 11-12 weeks is arrested in the diplotene stage of prophase 1 (Speroff et al., 1999). The meiotic division will produce haploid germ cells from diploid germ cells, so it is called reduction division. The completion of the first meiotic division does not occur until adolescence because of oocyte maturation inhibitor (OMI) which is secreted by cells surrounding the oocyte (Scott & Hodgen, 1990).

3.2.2. Post natal maturation of the oocyte.

The growing follicles that are recruited from a pool of primordial follicles undergo multiple changes in size, morphology, and physiology, which results in the transition from primordial follicle to Graffian follicle stage (Boron & Boulpeap, 2005). In the late luteal phase of menstrual cycle, the recruitment of three to eleven small antral follicles (2–5 mm) occurs (Gougeon, 1979). A single follicle is then selected from this cohort in the early follicular phase to continue growth and ovulation happen at midcycle. The primary oocytes are progressively depleted until no more than a few thousand follicles remain in both ovaries at menopause (Richardson et al., 1987). Of these only about 400 follicles become secondary oocytes and are expelled at ovulation (Scott & Hodgen, 1990), the remainder become atretic and degenerate. It was documented that in the last 10-15 years before menopause, there is an acceleration of follicular loss which is related to the rise in FSH level and brings the female to menopause stage (Fowler et al., 2003).

4. Role of hormones in the ART programs

4.1. Role of follicle-stimulating hormone (FSH)

Follicle-stimulating hormone is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH regulates the development, growth, puberty maturation, and reproductive processes of the body. FSH and Luteinizing hormone (LH) act synergistically in reproduction (Fowler et al., 2003). It is well documented that FSH plays an essential role in the recruitment, selection, and dominance processes during the whole follicular phase (Messinis & Templeton, 1990). FSH stimulates transcription of several genes within the granulosa cells, leading to the synthesis of proteins like, aromatase, inhibin, and the LH receptor, whose expression reflects follicle differentiation.

4.2. Role of luteinizing hormone (LH)

Luteinizing hormone is a hormone produced by the anterior pituitary gland. In females, a high rise of LH called the LH surge that triggers ovulation and development of the corpus luteum. In males, LH had also been called interstitial cell-stimulating hormone (ICSH), it stimulates the production of Leydig cell of testosterone. It acts in parallel with FSH. LH acts directly on the ca cells where LH receptors are constitutively present and ensure the atomic production of androgen during the whole follicular phase (Yong et al., 1992). High LH levels are usually related to the increased incidence of infertility or miscarriage, while low day 3 LH values are predictive of reduced response to ovarian stimulation (Kubilay & Isik, 1999).

4.3. Inhibin and activin

Two closely related protein complexes that have almost directly opposite biological effects. Activin enhances FSH biosynthesis and secretion and participates in the regulation of the menstrual cycle. Many other functions have been found to be exerted by activin, including roles in cell proliferation, differentiation, apoptosis (Chen et al., 2006). metabolism, homeostasis, immune response, wound repair, and endocrine function. Conversely, inhibin down-regulates the synthesis of FSH and the inhibition of FSH secretion (Sulyok et al., 2004).

5. Antral follicle

An antral follicle is an ovarian follicle during a certain latter stage of folliculogenesis. Antral follicles are small follicles (about 2-8 mm in diameter) that we can see - and measure and count - with ultrasound. Antral follicles are also referred to as resting follicles. The early stages of follicular development, including the early antral follicle are independent of the FSH
and LH. The low responsiveness of antral follicles to gonadotropamines results presumably from the low number of gonadotropine receptors on follicle cells at this stage of development (Visser et al., 2005).

6. Antral follicle count

The antral follicle count (AFC) is the number of antral follicles. It can be determined by transvaginal ultrasonography. A low AFC is a major factor in the diagnosis of poor ovarian reserve, that is, low fertility characterized by low numbers of remaining oocytes in the ovaries, usually accompanied by high follicle-stimulating hormone (FSH) levels. Several studies show that an AFC test is more accurate than basal FSH testing for older females (< 44 years of age) in predicting IVF outcome (Klinkert & Ellen et al, 2005). Antral follicle counts by ultrasound are one of the best ovarian reserve tests that we currently have available. Antral follicle counts are a good predictor of the number of mature follicles that we will be able to stimulate in the woman's ovaries when we give injectable FSH medications that are used for in vitro fertilization. The number of eggs retrieved correlates with IVF success rates.

7. Ovarian reserve

The term "ovarian reserve" refers to a woman's current supply of eggs and is closely associated with reproductive potential. In general, the greater the number of remaining eggs, the better the chance for conception. Conversely, low ovarian reserve greatly diminishes a patient's chances for conception (Zorn, 1995). Diminished ovarian reserve is a natural physiologic occurrence that is noted in most women during their mid to late thirteenth's, and occasionally earlier (Scott & Hofman, 1995).

8. Anti-müllerian hormone (AMH)

Anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting substance, which is synthesized in the granulosa cells of preantral and small antral follicles, is a member of the transforming growth factor-β (TGFβ) superfamily. AMH includes more than 35 structurally related peptides including activins, inhibins, bone morphogenetic proteins (BMPs), and growth differentiation factors (Itman et al., 2006). AMH is a homodimeric, disulfide-linked glycoprotein that has a molecular weight of 140kDa. The gene for AMH is AMH, which is located on the short arm of chromosome 19, band 19p 13.3 in humans, while the gene AMHR2 codes for its receptor on chromosome 12 (Visser et al., 2006). AMH is strongly expressed in the sertoli cells from testicular differentiation up to puberty and to a much lesser degree in the GCs also. It is responsible for the ipsilateral regression of the müllerian duct by eight weeks. AMH acts on its own specific type II receptor, AMHR2 to signal through a BMP-like pathway, by recruiting one of the types I receptors; activin receptor-likekinase (ALK) 2 (Clarke et al., 2001), 3 (Jamin et al., 2002) or 6 (Gouedard et al., 2000). The downstream signaling of the AMH receptor involves cytoplasmic effectors which are known as receptor-related SMAD proteins (RSmads 1, 5, and 8) and a common SMAD4 protein (Josso & Di Clemente, 2003). Once AMH binds to AMHR2, the type I receptor becomes recruited, thus forming a receptor complex. This results in the activation of the type I receptor, which causes the phosphorylation of R-Smads. These proteins bind to the common SMAD4 protein, resulting in the translocation of this complex into the nucleus and it's binding directly to the DNA to regulate gene expression or interacting with other DNA-binding proteins (Massagne & Wotton, 2006). The type II receptor, AMHRII, has been identified and is specifically expressed in the gonads and mesenchymal cells adjacent to the Müllerian ducts (Baarends et al, 1994). The type II receptor imparts ligand-binding specificity and the type I receptor mediates downstream signaling when activated by the type II receptor (Ingraham et al., 2000).

8.1. Anti-Müllerian hormone (AMH) levels in women

In the female, AMH levels are almost undetectable at birth. After an initially slight rise in a few weeks after birth, AMH levels increase, peaking during late puberty and then show a noticeable decline throughout reproductive life as the follicular reserve becomes depleted (Guibourdenche et al., 2003), and finally becoming undetectable after menopause. Further evidence that circulating AMH appears to be solely of ovarian origin comes from a study in which AMH was undetectable 3–5 days following bilateral oophorectomy (La Marca et al., 2005).

8.2. Source and pattern of serum AMH levels

Ovarian hyperstimulation by exogenous FSH has allowed for the examination of the contribution of different follicle stages on AMH serum levels because it forces many small antral follicles to transform into large dominant follicles. Hyperstimulation then results in a remarkable decline in peripheral AMH levels, with a progressive reduction in the number of small antral follicles along with an increase in the number of large, dominant follicles (Catteau-Jonard et al, 2007). Under such hyperstimulation, the serum level of AMH correlates with the number of smaller (<12 mm diameter) but not larger (≥12 mm) follicles. These data indicate that AMH is preferentially secreted into the serum by small antral follicles (Fanchin et al., 2003).

8.3. The role of AMH in ovarian Folliculogenesis

AMH can first be detected in the human fetal ovary at 36 weeks of gestation in the columnar granulosa cells of maturing, primary follicles. AMH expression persists in these growing follicles and is at the maximal expression in granulosa cells of preantral and small antral follicles (up to 6 mm in diameter). At the larger antral follicle stage (>8 mm), AMH expression diminishes and ultimately becomes undetectable once FSH-dependent follicular growth has been initiated (Rajpert- De Meyts et al., 2005). No AMH expression is detected in atretic follicles. This pattern of expression strongly indicates that AMH has an important role in regulating the number of follicles that grow from the primordial pool. Moreover, AMH might regulate the selection of the dominant
follicle from the FSH-sensitive follicle cohort (Visser & Themmen, 2005).

8.4. Relation of AMH and F.S.H.

Normal results do not necessarily mean that the egg quantity is good. There are a significant number of women with normal FSH values that have a reduced egg supply. The lower egg supply is not being reflected in their FSH value. This is why doing antral follicle counts and AMH levels can be useful. However, the FSH level is not as reliable as the AMH level for 3 reasons (Visser & Themmen, 2005).

1. The FSH level varies according to the cycle dates.
2. It depends upon the estradiol level (a high estradiol level will artificially suppress a high abnormal FSH level into the normal range).
3. It varies from cycle to cycle, so is not always reliable or dependable.

8.5 Age-specific FSH, AMH Levels

Follicle-Stimulating Hormone (FSH) is usually the most vastly used measurement to assess a women's ovarian function. High FSH is indicative of low ovarian reserve, and significantly lower pregnancy chances with IVF. Similarly, abnormally low AMH and/or AFCs also denote a relatively poor prognosis, in addition to high FSH levels. It is important to remember that FSH levels increase and AMH levels as well as AFCs decline as women age (Visser & Themmen, 2005).

9. Assisted reproductive techniques.

The generally accepted clinical definition of infertility is the inability to conceive after a year of unprotected intercourse during the fertile phase of the menstrual cycles in women under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002). Most studies of infertility also include couples under 35 years, or after 6 months in women 35 years or older (Evers, 2002).

9.1. Artificial insemination

The term artificial insemination (AI) covers a range of techniques for insemination including intravaginal, intracervical, intrauterine, intra-fallopian, intra-follicular, and intraperitoneal (Shields, 1950).

A-Intrauterine insemination (IUI)

IUI had been used to reduce the effect of factors that may impede the progress of spermatozoa such as vaginal acidity and cervical mucus hostility and to benefit from the deposition of a bolus of concentrated, motile, morphologically normal sperms as close as possible to the oocytes.

1. Indications for intrauterine insemination

There are several indications for IUI using the husband semen; these are ejaculatory failure is the classical indication, since the male partner is unable to ejaculate into the vagina, while cervical mucus hostility is a logical indication for IUI, as it bypasses the mucus in the cervical canal. The most known indications for IUI are the less severe forms of male-factor infertility and idiopathic or unexplained infertility. Other indications, for which conclusive evidence of effectiveness is lacking, are immunological causes of infertility and endometriosis (Richard et al., 2010).

2. Sperm preparation

The sperm separation technique should be quick, easy, and cost-effective. Types include swim-up procedure, migration-sedimentation, density gradient centrifugation, glass wool filtration, transmembrane migration (Ralf et al, 2003).

3. Complication

The complication which causes couples the most concern, but which is almost certainly very rare, is the possibility that the participant might be inseminated with the wrong semen sample. Painful uterine contractions that may occur during insemination can usually be minimized by inseminating slowly. Intrauterine infection and anaphylaxis rarely may also occur, especially if neat semen is used – which it should never be. The two most serious complications of ovulation induction (OI) are multiple pregnancy and ovarian hyperstimulation. There is increased awareness of the dangers of multiple pregnancies, including twins (Richard et al., 2010).

B- Intra cervical insemination (ICI)

This is the easiest way to inseminate, involves the deposit of raw fresh or frozen semen (which has been thawed) by injecting it high into the cervix with a needle-less syringe. This process closely replicates how fresh semen is directly deposited on to the neck of the cervix by the penis during vaginal intercourse (Demirol & Gurgan, 2007).

C- Intra uterine tube peritoneal insemination (IUTPI)

The improvement of this method of insemination lies in the 10ml of properly prepared sperm and clamping of the cervix, which is best achieved with the specially designed Double Nut Bivalve (DNB) speculum.

The volume of 10 ml of inseminate is sufficient enough to fill the uterine cavity, pass through the interstitial part of the tubes and the ampulla, finally reaching the peritoneal cavity and the pouch of douglas where it would be mixed with the peritoneal and follicular fluid. In this way (IUTPI) is achieved (Dankert et al, 2006).
D- Intra – tubal insemination (ITI)

This is the least commonly-performed type of artificial insemination, because it is more invasive than other types of artificial insemination and is associated with much higher costs. ITI works by placing the partner's sperm directly into the fallopian tubes. Here, the sperm has a better chance of fertilizing the eggs and producing a pregnancy (Vermeylen et al., 2006; Mustafa et al., 2020; Mustafa & AL-Samarraie, 2020).

Conclusion

It is well known that follicle stimulation hormone FSH plays an essential role in the recruitment, selection, and dominance processes during the whole follicular phase. FSH stimulates transcription of several genes within the granulosa cells, leading to the synthesis of proteins such as aromatase, inhibin, and the LH receptor, whose expression reflects follicle differentiation. FSH threshold is required to induce follicular growth. Increasing FSH supply in the early stage of the cycle is a key factor for the follicular recruitment process. The number of eggs retrieved correlates with IVF success rates. Studies have validated the use of serum AMH levels as a marker for the quantitative aspect of ovarian reserve. Because of the lack of cycle variations in serum levels of AMH, this marker has been suggested to be used as part of the standard diagnostic procedures to assess ovarian dysfunctions, such as premature ovarian failure, some studies have shown AMH to be a better marker than basal FSH for women with proven (prior) fertility in measuring the age-related decline in ovarian reserve. It appears that a decrease in AMH levels is an essential part of the reproductive response to treatment. AMH is strongly correlated to antral follicle count (AFC), and both predictors have a linear relationship with age, confirming the decline of ovarian reserve with age. Many researchers have shown that basal AMH levels accurately reflect the total developing follicular cohort and ovarian response to gonadotrophin in ART cycles. As yet, AMH testing, like other predictive markers, doesn’t appear to predict accurately the probability of pregnancy after IVF treatment. No AMH expression is detected in atretic follicles. This pattern of expression strongly indicates that AMH has an important role in regulating the number of follicles that grow from the primordial pool. Moreover, AMH might regulate the selection of the dominant follicle from the FSH-sensitive follicle cohort.

Authors’ contributions

All authors have contributed significantly to the conception and design of the study, the interpretation of data, and the drafting and revision of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors hereby declare no conflict of interest.

Consent for publication

The authors declare that the work has consent for publication.

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