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HORMONE EXAMINATION IN MENOPAUSE

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ABSTRACT

The World Health Organization (WHO) and Stages of Reproductive Aging Workshop (STRAW) define menopause as a permanent endpoint of the menstrual cycle that occurs naturally or by induction of surgical procedure, chemotherapy, or radiation. Clinically, menopause involves females over 40 or 50 years old. Approximately, fifty million females in the world are experiencing menopause annually. The etiology of menopause is classified into physiological and non-physiological. Pathophysiology of menopause includes the decline of ovary function in menopause, response to a loss of ovarian feedback mechanism, the decline of hypothalamus and pituitary function. Endocrine changes in menopause lead to alteration of gonadotropin secretion cycle patterns, changes in steroid and peptide hormones through monophasic patterns to increase gonadotropin, a decrease of estrogen, progesterone, and inhibin. Clinical symptoms of menopause or estrogen deficiency syndrome are classified into short term clinical symptoms and long term clinical symptoms. Hormon alexaminations in menopause consist of Follicular Stimulating Hormone (FSH), Anti-Mullerian Hormone (AMH), Luteinizing Hormone (LH), Inhibin B and Estradiol. The importance of timing to examine the hormones should be considered carefully.

Key words: Menopause, follicular stimulating hormone, anti-mullerian hormone, luteinizing hormone, inhibin B, estradiol

INTRODUCTION

Menopause is a natural process occurring in the female body as a part of the normal aging process. The World Health Organization (WHO) and Stages of Reproductive Aging Workshop (STRAW) define menopause as a permanent end point of the menstrual cycle that occurs naturally or by induction of surgical procedure, chemotherapy, or radiation. Natural menopause occurs when it happens for 12 consecutive months without any menstrual period (amenorrhea), and does not relate with any physiologic process such as lactation or pathological condition.[1]

MENOPAUSE

Definition

Menopause comes from the Greek men (moon) and pausis (end of the process). Menopause is a permanent end point of the menstrual cycle, happening along with decreasing activity of ovary follicles. Clinically, menopause occurs in females over 40 or 50 years old.[1,2]

Epidemiology

It is expected 1.2 billion females all over the world will experience menopause in 2030. Approximately, fifty million females in the world are experiencing menopause annually.[3]According to Badan Pusat Statistik (BPS) Indonesia, in 2016 females experiencing menopause have reached 14 million (7.4%) from the total population. It is estimated that 60 million females will experience menopause in 2025. The average age estimation of menopause in Indonesia is 48 years old.

Etiology

The etiology of menopause is classified into physiological and non-physiological. Physiological menopause occurs naturally by decreasing ovarian hormone production. Non-physiological menopause is caused by permanent loss of ovarian function as follows, bilateral oophorectomy surgery, chemotherapy, radiotherapy or medication. Another condition is early menopause or Precock Climacterium. Early menopause happens before the age of 40 years as a part of Premature Ovarian Failure (POF). Most causes of early menopause are posted as neoplasm treatment or post autoimmune disease treatment.[4]

Menstrual Cycle Physiology

The first menstrual period experienced by a female is defined as menarche usually occurring at 12-13 years old. The duration of the menstrual cycle varies between 21-35 days. Two gonadotropins hormones physiologically playing a role in the

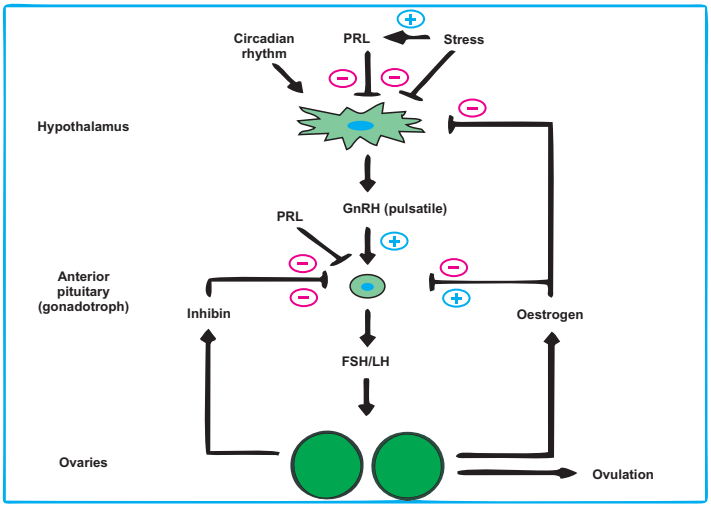

Figure 1. Hypothalamus-pituitary anterior-ovary axis[5]

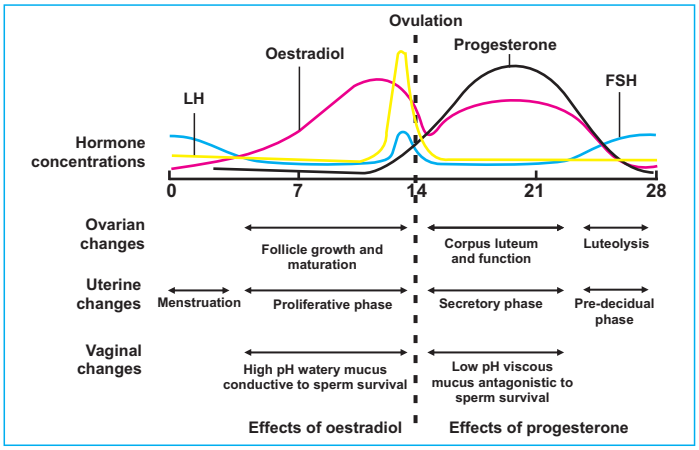

Figure 2. Menstrual cycle[5]

menstrual cycle are Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Follicle stimulating hormone and LH are produced by the anterior pituitary, their secretion controlled by Gonadotropin-Releasing Hormone (GnRH) secreted by the hypothalamus (Figure 1). A total 30-40 follicles grow as a response to FSH stimulation, but only one mature ovum is released.[5]

A normal menstrual cycle is divided into the ovarian cycle and the uterus cycle. The ovarian cycle consists of three phases:

1. Follicular or pre-ovulation phase

The production of estradiol, progesterone, and inhibin A is low in the early follicular phase. Those hormones cause a negative feedback response of pulsatile GnRH. Pulsatile GnRH increases and causes high stimulation of FSH and LH secretion by the anterior pituitary. Increased FSH secretion results in stimulation of ovarian follicle growth. Luteinizing hormone surge stimulates ovulation in 36 hours.[3,5]

Ovarian follicle (tertiary follicle or Graafian follicle) becomes mature through folliculogenesis during the follicular phase. It may contain mature ovum which is ready to be released. The low level of Inhibin A and progesterone can cause the regression of corpus luteum so that FSH and LH levels are increased. It can cause the formation of the new follicle and starts next menstrual cycle.[3,5]

2. Ovulation phase

The luteinizing hormone surge stimulates ovulation. Ovulation phase varies but generally occurs for 28 days. Variation of the ovulation cycle reflects the ovum production rate (follicular phase).[5] Gonadotropin-releasing hormone surge precedes the LH surge, indicating that the main effect of estrogen in the hypothalamus is controlling GnRH secretion.[3,5] This condition encourages further gonadotropin secretion, an increase of GnRH, FSH, and LH, ending in ovulation (Figure 2).[5]

Follicular cells of the ovary become corpus luteum and secrete progesterone and inhibin A through luteinization after ovulation. Progesterone and inhibin A suppress FSH and inhibit the

development of other ovarian follicles. If there is no fertilization or embryo implantation, the corpus luteum will degenerate. [3,5]

1. Luteal phase

The luteal phase is the final phase of the ovarian cycle and related with uterus secretion phase. During the luteal phase, FSH and LH cause the remaining dominant follicles to turn into corpus luteum. Corpus luteum secretes estradiol which provides negative feedback on LH, FSH, and progesterone.[5]

PATHOPHYSIOLOGY OF MENOPAUSE

The decline of ovary function in menopause

The maximum amount of primordial follicles occurs during fetal life and continues to decrease with age. The number of follicles is decreased at 38 years old. The loss of follicles results in a decrease of estrogen and inhibin A. However, androgen secretion (especially testosterone) continues and does not significantly change the number of follicles. Anatomical changes in ovary due to aging process selectively occur in females with menopause such as vascular sclerosis, decreasing amount of primordial follicle, and decreasing activity of steroid hormone synthesize. The low level of estrogen can cause disturbances of its physiological function.[3,6]

Response to loss of ovarian feedback mechanism

The decline of ovary function in menopause causes decreasing of feedback mechanism to hypothalamus and pituitary. Thus, it can induce the increase of hypothalamic GnRH secretion followed by the increase of anterior pituitary FSH secretion 10-20 times folds, and LH 5-6 folds. Primordial follicle maturation failure and unformed corpus luteum cause decreasing production of progesterone.[1]

Furthermore, the decrease of ovary function induces more frequent pulsatile GnRH in basal medial hypothalamus and causes longer half-life elimination of LH and FSH in the menopause period.[6]

The decline of hypothalamus and pituitary function

The decline of hypothalamus function leads to decreasing pulsatile GnRH about 22% at the age of 50-75 years and 30-40% during post-menopause. A decreasing response of LH/FSH toward GnRH in post-menopause is higher in the older age. Estrogen negative feedback to the hypothalamus in post-menopause in the older is still the same as in younger age.[6]

MENSTRUAL CYCLE AND ENDOCRINE CHANGES IN MENOPAUSE

Endocrine changes in menopause lead to alteration of gonadotropin secretion cycle patterns, change of steroid and peptide hormones through monophasic patterns to increase gonadotropin, a decrease of estrogen, progesterone, and inhibin. The most essential hormone in menopause is estradiol. Estradiol has many target organs including brain, breast, bone, cardiovascular system, liver, uterus, and vagina.[1,7]

The Stages of Reproductive Aging Workshop (STRAW) working group in 2001 define a system that classifies females reproductive age based on menopause (Table 1).[8]

Pre-menopause

Pre-menopause is a phase from menarch to the

The 2011 Stages of Reproductive Aging Workshop + 10 staging system for reproductive aging in women

Menarche — FMP (0)

Stage	-5	-4	-3b	-3a	-2	-1	+1a	+1b	+1c	+2
Terminology	REPRODUCTIVE				MENOPAUSAL TRANSITION			POSTMENOPAUSE		
	Early	Peak	Late		Early	Late	Early			Late
					Perimenopause					
Duration	Variable				Variable	1–3 years	2 years (1+1)	3–6 years		Remaining lifespan
PRINCIPAL CRITERIA										
Menstrual cycle	Variable to regular	Regular	Regular	Subtle changes in flow/ length	Variable length Persistent ≥7-day difference in consecutive cycles	Interval of amenorrhea of ≥60 days				
SUPPORTIVE CRITERIA										
Endocrine										
FSH			Low	Variable*	↑ Variable*	↑ >25 IU/L**	↑ Variable	Stabilizes		
AMH			Low	Low	Low	Low	Low	Very low		
Inhibin B			Low	Low	Low	Low	Very low	Very low		
Antral follicle count										
DESCRIPTIVE CHARACTERISTICS										
Symptoms						Vasomotor symptoms *Likely*	Vasomotor symptoms *Most likely*			*Increasing* symptoms of urogenital atrophy

*Blood draw on cycle days 2–5 ↑ = elevated
**Approximate expected level based on assays using current international pituitary standard

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Table 1. The Stages of Reproductive Aging Workshop (STRAW) + 10 staging system for females reproductive age[8]

beginning of peri-menopause. Pre-menopause is identical with late productive years, it occurs at early 40 years and starts with their regular menstrual cycle (prolonged, with a lesser or greater amount of bleeding), even accompanies with pain. Vasomotor symptoms or pre-menstrual syndrome (hot flushes, mood swings, sleep disturbances) may appear at this phase. Ovulation may still occur in pre-menopause but with shortened follicular phase (from 14 days into 10 days).[9,10]

Peri-menopause (menopause transition)

Peri-menopause or menopause transition is a time period that surrounds the final years of a female's reproductive life. It begins at a mean of 47 years old.[10,11]

Intermenstrual interval changing begins at early transition with symptoms: prolonged menstruation interval (40-50 days), change in bleeding pattern accompanied with a significant change of menstrual cycle such as skipped menstrual cycles, amenorrhea and the increasing frequency of an ovulation cycle. This stage called as the end of transition occurs for 1-3 years before menopause.[12]

Menopause/Final Menstrual Period (FMP)

Menopause or final menstrual period is a non-menstrual period of a female for 12 months.[9,10] Menopause is determined retrospectively, the average age of natural menopause is 51.4 years old. The menopause period is influenced by several factors including genetics and smoking habit.[3] Hormonal changes occurring in a menopause period are:

a. Follicle stimulating hormone, estradiol, and inhibin

Irregular menstruation period starting before menopause can cause a decrease of inhibin B level in the early follicular phase (STRAW stage 2) continued by the increase of FSH level.[10,12]

b. Progesterone and androgen

An anovulatory cycle is frequently increased by a low progesterone level during the menopause transition phase.[10,12] Androgen change is still controversial, but a study reported that the level of total testosterone may not change and Sex Hormone-Binding Globulin (SHBG) level is decreased at early transition menopause and early post-menopause, resulting in the increase of free androgen level.[3]

Post-Menopause

Post-menopause is a period between post-menopause until senium phase. It starts after 12 months of amenorrhea with a high level of FSH and LH, low level of estradiol and progesterone and also the steady level of testosterone (STRAW stage +1 and +2).[9,10]

Senium

Senium is a stage of life when a female enters the advanced post-menopause age of more than 65 years.

DIAGNOSIS AND CLINICAL SYMPTOMS OF MENOPAUSE

Clinical symptoms of menopause or estrogen deficiency syndrome are classified into short term and long term clinical symptoms. Short term clinical symptoms are menstrual change, vasomotor change, psychological change, and urogenital system.[10] Long term clinical symptoms are osteoporosis and cardiovascular disease.[11]

HORMONAL EXAMINATION IN MENOPAUSE

The duration of a female's menstrual cycle varies greatly, ranging from 21 days to 35 days. The 28-day cycle is defined as the average duration of menstruation.[8,9] Follicle stimulating hormone and LH are two hormones involved in the menstrual cycle, produced by the anterior pituitary (pituitary), their secretions are controlled by a gonadotropin-releasing hormone from the hypothalamus. Follicle stimulating hormone and LH stimulate the ovaries and cause the growth and development of ovarian follicles. Every month, a total 30-40 follicles grow in response to FSH stimulation.[10]

In the early follicular phase, the production of estradiol, progesterone, and inhibin A (produced by the corpus luteum from the previous cycle) are low, resulting in a negative feedback response from the GnRH pulsatile. Pulsatile GnRH increases and causes high stimulation of FSH and LH secretion by the anterior pituitary. Increased FSH secretion results in stimulation of ovarian follicle growth. FSH stimulates estrogen formation (estradiol and estrone).[10]

High levels of estradiol in the early follicular phase can also provoke a sudden increase in LH levels, which are thought to persist for 24 hours and a decrease in the luteal phase. A few hours after LH increases, estrogen production also decreases.[9]

In the final follicular phase, FSH concentration falls as well as inhibin secretion. Follicle stimulating hormone is also suppressed by inhibin B secreted by the follicle. In addition, the effects of negative feedback by steroid hormones produced by the ovaries (especially estradiol) turn to the effects of positive feedback and produce 'LH Surge. During this

phase, the dominant ovarian follicle ripens and is being ready to release the egg.[9,10]

A surge in LH stimulates ovulation within 36 hours. After ovulation, the follicular cells left in the ovary undergo luteinization and the corpus luteum secretes progesterone, and also inhibin A. This allows the clinical measurement of progesterone on the 21st day when progesterone levels are> 30 nmol/L (equal to 9.4 ng/mL), and very implies the occurrence of ovulation. Follicle Stimulating Hormone (FSH) and LH cause the remaining dominant follicles to turn into the corpus luteum. Corpus luteum secretes estradiol, which provides negative feedback on LH and FSH, and progesterone because of LH stimulation.[10]

On the 25th day, LH also decreases with estradiol and progesterone, there by eliminating negative feedback from the pituitary, but a continuous stimulus to GnRH results in FSH and LH secretion and then the next cycle begins.[9,10]

The ovulation phase generally occurs for 28 days but it varies. Variations in the ovulation cycle reflect variations in the speed of preparing eggs (follicular phase). The luteal phase is relatively fixed, which occurs for 14 days. Physiology in these phases determines the timing of clinical hormone measurements, i.e. day 1 is the first day of vaginal bleeding from the previous cycle, days 2-5 is the time to measure FSH, LH, estradiol, and AMH, and day 21 is the time to measure progesterone and inhibin.[9,10]

Follicular Stimulating Hormone (FSH)

Follicle stimulating hormone is a glycoprotein produced by the anterior pituitary that acts to stimulate ovarian follicle growth. Follicle stimulating hormone level varies during the menstrual cycle and reaches the highest peak before ovulation.[6,13] Follicle stimulating hormone analysis result in pre-menopausal phase shows that FSH is slightly increased. So that, it can lead to excessive ovarian stimulation resulting in a very high increase in estrogen levels. Symptoms can occur in the premenopausal phase, both at normal or increased hormone levels, while complaints that appear in the post-menopause phase are generally caused by increased hormone levels.[3,13,14]

Follicle stimulating hormone levels > 25 IU/L are characteristic of the late menopause transition period (late peri-menopause), but measurements of serum FSH during the late menopause transition period are not routinely recommended due to their variability.[13] Increasing serum FSH levels continue until the FMP period, then increase for several years to reach 70-100 IU/L levels, followed by decreased levels according to age. After entering menopause, FSH levels are usually high (> 35 mIU/mL) and estradiol levels are low (<30 pg/mL). However, in the early days of menopause, it is sometimes found to have low estrogen levels. Follicle stimulating hormone levels in the postmenopausal phase (STRAW level +1 and +2) is high (> 35 mIU/mL).[3,13,14]

Follicle stimulating hormone levels are as follows: pre-puberty about < 3 IU/L; follicular phase (2nd-13th day) 3.8-8.8 IU/L; the mid of the cycle (14th day) 4.5-22.5 IU/L; luteal (15th-28th day) 1.8-5.1 IU/L; and post-menopause 16.7-113.6 IU/L. Follicle stimulating hormone levels about 40 IU/L or more are used as a marker of menopause transition end phase.[6]

Anti-Mullerian Hormone (AMH)

Anti-mullerian hormone is a hormone directly secreted by the ovary, especially granulosa cells that surround the ovarian follicles. AMH is a useful marker for ovarian reserve. Anti-mullerian hormone is the best endocrine marker to assess ovarian pool conditions in healthy females. Thus, it has the potential ability to predict the reproductive period. Anti-mullerian hormone is a dimeric glycoprotein transformation from growth factor-β super family involved in growth and differentiation. Anti-mullerian hormone levels are almost undetectable at birth, but increase significantly during puberty and slowly decrease during the reproductive period until they become undetectable at menopause. Anti-mullerian hormone secretion increases during follicular development. The highest level of AMH secretion is mediated by antral follicles, preantral and small antral. The anti-mullerian hormone has two main mechanisms of action in the

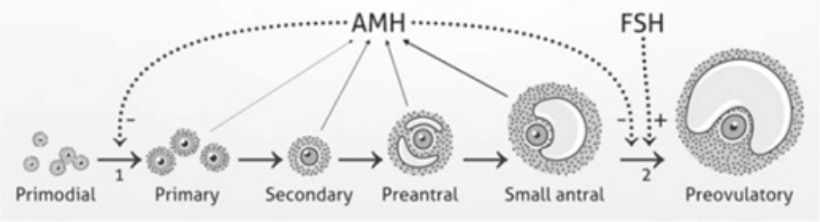

Figure 3. Anti-Mullerian Hormone (AMH) and folliculogenesis[14]

ovary. Firstly, it inhibits the initial recruitment of primary follicles from the pool of primordial follicles. Secondly, it inhibits the sensitivity of antral follicles to Follicle Stimulating Hormone (FSH) during the cycle period (Figure 3).[3,11,14]

The number of ovarian follicles decreases with age, so do AMH levels. Anti-mullerian hormone peaks at puberty decrease there after and end in the menopause transition period. Anti-mullerian hormone decreases even earlier than the increase of FSH during late reproduction. In the peak and final reproductive periods, AMH is lower in females who enter the menopause transition period earlier than the remaining during the reproductive period. Comparison of AMH levels during the menopause and reproductive period show that AMH is significantly lower during the menopausal transition period than during the reproductive period. However, AMH levels were also below the testing detection threshold in subjects with menopause and post-menopause transition periods. Some cross-sectional data showed that AMH was the first indicator for the detection of the menopause transition period when compared with FSH, inhibin B, and estradiol.[3,11]

The international standard reference for AMH level has not been defined yet, but several guidelines stated that an AMH level of< 1.0 ng/mL is indicating baseline ovarian reserve, probably limited amount of ovum during the sampling process; AMH level 1.0-3.5 ng/mL indicating a good response toward ovarian stimulation; AMH level >3.5 ng/mL indicating a strong response and should be handled carefully to avoid ovarian hyperstimulation syndrome.[14]

Luteinizing Hormone (LH)

Luteinizing Hormone (LH) is a glycoprotein produced by the anterior pituitary with a molecular weight about 30,000 Dalton. LH has an important role in follicle growth and oocyte maturation. Follicle stimulating hormone and LH are measured on the third day of the menstrual period.[3,11]

The post-menopause phase (STRAW stages +1 and +2) is characterized by higher LH other than FSH levels, low estradiol and progesterone levels while testosterone remains.[3,13,15] The levels of LH, FSH, and estrogen vary greatly in the peri-menopause phase. So that, climacteric symptoms appear not only at low hormone levels.[15,16]

Luteinizing hormone levels are as follows: pre-puberty females<1 IU/L; follicular phase (2^{nd}-13^{th} day) 2-11 IU/L; the middle of the cycle (14^{th} day) 19-103 IU/L; luteal phase (15^{th}-28^{th} day) 1-3 IU/L; and post-menopause 11-59 IU/L.15

Inhibin B

Inhibin B is a hormone measured in the transition phase of early menopause (around 5 years before menopause), about 41% not detected in two years before menopause and 71% not detected in one year before menopause (menopause transition end).[6,10]

The level of inhibin B changes slightly until the age of 40 years when there is a decrease inversely proportional to the increase of FSH level. Inhibin B is the main regulator of FSH levels in the follicular phase of the female normal cycle. Thus, reproductive aging is characterized by a decrease of inhibin B secretion.[3]

The results of the hormonal analysis show that inhibin B levels begin to decrease in pre-menopause and progressively decrease in the peri-menopause.[3,13,15] Menstrual irregularities begin before the cessation of menstruation and initial changes are found as a decrease of inhibin B levels in the initial follicular phase (STRAW stage -2).[15,16]

Estradiol

Decreasing levels of estradiol occur mostly at 1-2 years at the end of the menopause transition period, while in pre-menopause phase show the fixed estradiol levels. About 50% of estradiol levels in the follicular phase during the reproductive period are produced and continue to decrease after the last 2-3 years of final menstruation. At this time, estradiol levels have decreased by 90% or more.[15,16]

Levels of estradiol may not significantly differ

Table 2. Reproductive hormones according to STRAW criteria[2]

	Peak Reproductive	Late Reproductive	Early MT	Late MT	FMP	Post menopause
FSH	Normal	↑	↑	↑		↑
AMH	Normal/↓	↓	↓	Undetectable		Undetectable
Inhibin B	Normal	↓	↓	Undetectable		Undetectable
Estradiol	Normal	Normal	Normal	↓		↓

between reproductive and post-menopause period, the peak level of estradiol secretion in menstrual until post-menopause period may not decrease. There is no estradiol cut-off point to predict the time of menopause transition phase or menopause period.[6,7]

EXAMINATION METHOD OF HORMONES IN MENOPAUSE

Hormone examination method for menopause (FSH, AMH, LH, Inhibin B, Estradiol) are using Enzyme-linked Immunosorbent Assay (ELISA), Radioimmunoassay,Electrochemiluminescence Immunoassay (ECLIA) Competitive Inhibition (Competitive Reaction), and Double Antibody Sandwich methods.[16,17] The importance of timing to examine the hormones should be considered carefully.

CONCLUSION

Menopause is a permanent end point of the menstrual cycle that occurs naturally or by induction of surgical procedures, chemotherapy, or radiation. Clinical symptoms of menopause are menstrual change, vasomotoric change, psychological change and urogenital system, osteoporosis and cardiovascular disease. Follicle stimulating hormone level about 40 IU/L or more is used as a marker of menopause transition end phase. Follicle stimulating hormone, AMH, LH, inhibin B, and estradiol are examined by Enzyme-linked Immunosorbent Assay (ELISA), Radioimmunoassay, Electrochemiluminescence Immunoassay (ECLIA), Competitive Inhibition (Competitive Reaction), and Double Antibody Sandwich methods. The importance of timing to examine the hormones should be considered carefully.

REFERENCES

1. Edwards BJ, Li J. Endocrinology of menopause. Periodontol 2000, 2013; 61(1): 177-94.
2. Su HI, Freeman EW. Hormone changes associated with the menopausal transition. Minerva Ginecol, 2009; 61(6): 483-9.
3. Edmonds DK. Dewhurst's textbook of obstetrics and gynecology. 7th Ed., London, Blackwell Publishing, 2007; 47: 479-493.
4. Beck-Peccoz P, Persani L. Premature ovarian failure. Orphanet J Rare Dis, 2006; 1:9.
5. Holt IG, Richard HNA. Essential endocrinology and diabetes. 6th Ed., London, Willey Blackwell, 2012; 127-164.
6. Hall JE. Endocrinology of the menopause. Endocrinol Metab Clin N Am 44, 2015; 485-496.
7. Burger HG. Physiology and endocrinology of the menopause. Medicine (Baltimore), 2006; 34(1): 27-30.
8. O'Neill S, Eden J. The pathophysiology of menopausal symptoms. Obstet Gynecol Reprod Med, 2017; 27(10): 303-10.
9. PriorJC, Hitchcock CL. The endocrinology of perimenopause: Need for a paradigm shift Front Biosci. Frontiers in Bioscience S3, 2011; 474-86.
10. Buckler H. The menopause transition: Endocrine changes and clinical symptoms. J Br Menopause Soc, 2005; 11(2): 61-5.
11. Casper RF. Clinical manifestations and diagnosis of menopause. Up To Date [Internet]. 2018; Available from https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-menopause/print. Accessed on October 9, 2018.
12. Giulia P, Spina S, Bernacchi G, Cecchi E,Bello SD, *et al*. Therapeutic management of the menopausal transition. Front Gynecol Endocrinol ISGE Ser, 2014; 191-200.
13. Release P, Set D, This I, Sas L, Follicle L, *et al*. Follicle Stimulating Hormone (FSH) in Serum - NHANES 2001-2002 . 2002; 1-9.
14. Grynnerup AGA, Lindhard A, Sørensen S. The role of anti-müllerian hormone in female fertility and infertility - An overview. Acta Obstet Gynecol Scand. 2012; 91(11): 1252-60.
15. NHANES. Luteinizing Hormone (LH) in refrigerated serum. Collab Lab Serv LLC. Available from: https://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l40_b_met_luteinizing_hormone.pdf. Accessed on October 9, 2018.
16. Asihara Y,Asihara KY. Immunoassay and immunochemistry. In: Jhon BH (eds).21st Ed., clinical diagnosis and management by laboratory methods. Philadelphia, WB Saunders Company, 2001; 869-870.
17. Richter MM. Electrochemiluminescence (ECL). Chem. Rev. 2004; 104: 3003-3036.