DISSERTATION ON

"EVALUATION OF ANTI MULLERIAN HORMONE AS A PREDICTOR OF OVARIAN RESERVE IN INFERTILITY PATIENTS IN KARPAGA VINAYAGA MEDICAL COLLEGE HOSPITAL"

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University

In partial fulfillment of the regulations for the award of the degree of

M.S. (OBSTETRICS AND GYNAECOLOGY)

BRANCH –II

Submitted By

Registration Number: 221716752

KARPAGA VINAYAGA INSTITUTE OF MEDICAL SCIENCES

AND RESEARCH CENTRE

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY CHENNAI,

INDIA

MAY 2020

CERTIFICATE

This is to certify that the dissertation entitled, **"EVALUATION OF ANTI MULLERIAN HORMONE AS A PREDICTOR OF OVARIAN RESERVE IN INFERTILITY PATIENTS IN KARPAGA VINAYAGA MEDICAL COLLEGE HOSPITAL"** by **Dr.S.TAMILARASI**, Post graduate in Obstetrics and Gynaecology (2017-2020), is a bonafide research work carried out under our direct supervision andguidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University,Chennai, for M.S. Degree Examination in Obstetrics and Gynaecology, Branch VI, to be held in May 2020

Dr. Sufala Sunil ViswasRao, M.D

PRINCIPAL,

Karpaga Vinayaga Institute of Medical Sciences & Research Centre

Madhuranthagam Tk, Kanchipuram (Dt.), Tamilnadu – 603308

CERTIFICATE

This is to certify that the dissertation entitled, **"EVALUATION OF ANTI MULLERIAN HORMONE AS A PREDICTOR OF OVARIAN RESERVE IN INFETILITY PATIENTS IN KARPAGA VINAYAGA MEDICAL COLLEGE HOSPITAL"** by **Dr.S.TAMILARASI**, Post graduate in Obstetrics and Gynaecology (2017-2020), is a bonafide research work carried out under our direct supervision andguidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University,Chennai, for M.S. Degree Examination in Obstetrics and Gynaecology, Branch VI, to be held in May 2020**.**

Guide

Dr.S N S Minnalkodi
HOD & Professor,
Department of OBG
Karpaga Vinayaga Institute of
Medical Sciences & Research Centre,
Kanchipuram Dist. - 603308

DECLARATION BY THE CANDIDATE

I, hereby declare that this dissertation entitled **"EVALUATION OF ANTI MULLERIAN HORMONE AS A PREDICTOR OF OVARIAN RESERVE IN INFERTILITY PATIENTS IN KARPAGA VINAYAGA MEDICAL COLLEGE HOSPITAL "** submitted by me for the degree of M.S is the record work carried out by me during the period from August 2017 to August 2018 under the guidance of Dr.S N S Minnalkodi, Professor & Head of the Department of Obstetrics and Gynaecology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre and has not formed the basis of any Degree, Diploma or Fellowship titles in this or any other university or other similar institution of higher learning.

Place: Signature of the candidate

Date: **Dr.S.TAMILARASI**

Signature of the Guide

Dr.S N S Minnalkodi
MD (OG) DGO
HOD & Professor,
Department of OBG
Karpaga Vinayaga Institute of
Medical Sciences & Research Centre,
Kancheepuram Dist. – 603308

CERTIFICATE – II

This is to certify that this dissertation work titled **"EVALUATION OF ANTI MULLERIAN HORMONE AS A PREDICTOR OF OVARIAN RESERVE IN INFERTILITY PATIENTS IN KARPAGA VINAYA MEDICAL COLLEGE HOSPITAL"** of the candidate **DR.TAMILARASI.S** with registration Number 221716752 for the award of M.S. in the branch of OBSTETRICS & GYNECOLOGY. I personally verified the **urkund.com** website for plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 3 percentage of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

Prof. Dr. SNS MINNALKODI, Professor,
Department of OBSTETRICS & GYNECOLOGY,
Karpaga Vinayaga Institute of Medical Sciences and Research Center,
Madhuranthagam

URKUND

Urkund Analysis Result

Analysed Document:	dr.tamilarasi thesis amh.docx (D57379969)
Submitted:	10/21/2019 5:00:00 PM
Submitted By:	doc.tamil2015@gmail.com
Significance:	3 %

Sources included in the report:

SHWETHA THESIS (3).docx (D57297284)

Instances where selected sources appear:

7

INSTITUTIONAL ETHICAL COMMITTEE

KARPAGA VINAYAGA INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTRE

MADURANTHAGAM - 603 308

EC Ref. No: 106/2017

CERTIFICATE FOR APPROVAL

The Institutional Ethical Committee of Karpaga Vinayaga Institute of Medical Sciences & Research Centre, Maduranthagam reviewed and discussed the application for approval **"Evaluation of antimullerian hormone as predictor of ovarian reserve in infertility patients"** by **Dr.S.Tamilarasi, I PG**, Guided by **Dr. S. Viswanathan**, Professor and Head, Department of OBG, Karpaga Vinayaga Institute of Medical Sciences & Research Centre, Maduranthagam.

The proposal is **APPROVED**

The Institutional Ethics Committee expects to be informed about the progress of the study and any changes in the protocol / information / informed consent and asks to be provided a copy of the final report.

04/12/17

Chairperson, Ethics Committee

Date:04 /12/17

ACKNOWLEDGEMENT

I take this golden opportunity and immense pleasure to express my gratitude for the help rendered to complete my dissertation work with success. Firstly, I like to thank **ALMIGHTY** for blessing and giving me the health and ability for carrying out this work successfully.

I sincerely acknowledge and wish to express my thanks to Managing Director, **Dr. R. Annamalai, M.S.** and Principal **Dr. Sufala Sunil Viswas Rao, M.D** for providing all the necessary facilities, support and encouragement during the course of my research work.

I express my sincere thanks and gratitude to **Prof. Dr. S N S Minnalkodi**, Professor and Head, Department of OBG, Karpaga Vinayaga Institute of Medical Sciences & research center, for her constant encouragement and guidance during the entire period of my research work.

I sincerely thank all my Professors, Associate & Assistant professors of my department for their support and guidance.

It is a moment of great pleasure for me to express my deep sense of gratitude and Indebtedness towards Department of BIOCHEMISTRY for their support during entire period of my research work.

I am thankful to Ethical Committee of KIMS&RC for giving me permission to carry out this project.

Finally, I extend my wholehearted thanks to my beloved parents Mr.S.Sekar , Mrs.S.kannagi , my husband Dr .S. Kothandaraman , my sister Gayathri and my In laws for their silent sacrifices, support and words of encouragement which were a source of inspiration for the successful completion of this study.

Date:

CONTENTS

LIST OF ABBREVIATIONS

AFC	Antral follicle count
AMH	Antimullerian hormone
AMHR	Antimullerian hormone receptor
ART	Assisted reproductive technology
COH	Controlled ovarian hyperstimulation
E2	Estradiol hormone
ELISA	Enzyme linked immunosorbent assay
FSH	Follicle-stimulating hormone
GIFT	Gamete intrafallopian tube transfer
GnRH	Gonadotropin-releasing hormone
IVF	*In vitro* fertilization
LH	Luteinizing hormone
MIS	Mullerian-inhibiting substance
PRL	Prolactin hormone
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyroid-stimulating hormone
TVUS	Trans vaginal ultrasound
OV	Ovarian volume

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

Ovarian reserve is defined as the functional potential of the ovary and reflects the number and quality of the oocytes in the ovary at any given time. [1, 2]. With the understanding that chronological age alone is an inadequate predictor of the ovarian reserve, multiple tests have been developed to assess ovarian function (i.e., "ovarian reserve" tests). Some of these tests include basal FSH, basal inhibin B, the clomiphene citrate (CC) challenge test, basal E2, the GnRH challenge test, the ovarian antral follicle count (AFC) as assessed by transvaginal ultrasound examination, and serum levels of anti-Müllerian hormone (AMH) [3].

Although such tests are frequently labeled ovarian reserve tests, they are more accurately ovarian response tests. Anti-Müllerian hormone (AMH) is produced by the granulosa cells of preantral and small antral follicles and its levels can be assessed in serum. Since the number of ovarian follicles declines with increasing age, AMH levels might be used as a marker for ovarian aging [4, 5].

The human female serum contains measurable amounts of AMH during the reproductive life span. Since AMH is solely produced in the growing ovarian follicles, serum levels may be used as a marker for ovarian reserve, representing the quantity and quality of the ovarian follicle pool. [6, 7]. The ovarian reserve, constituted by the size of the ovarian follicle pool and the quality of the oocytes therein, declines with increasing age, resulting in the decrease of a woman's reproductive function [8].

Anti-Müllerian hormone seems to be the best endocrine marker for assessing the age-related decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan. The most established role for AMH measurements

is before in vitro fertilization is initiated, because AMH can be predictive of the ovarian response, namely poor and hyper- responses [9].

The human ovary function as to nurture, development and release of mature oocyte which is the need for the secretion of steroidal hormones and fertilization which stimulate the development and growth of secondary sexual characters. A female fetus contains 7 million oocytes at birth, (1- 2) million at puberty and 40,0000 oocyte1 at the onset of the menstrual cycle1.A fixed proportion of the remaining oocytes becomes recruited by the gonadotrophins from which one or two will achieve dominance and will progress to ovulation [10].

The quality and quantity of oocytes are determined by the term ovarian reserve.The reproductive potential is important and is measured by ovarian reserve. Ovarian reserve decline with age and the variations are significant in individual with the onset of decline in age and the assessment of the ovarian reserve is needed [11, 12].

Infertility refers to the inability of a woman to become pregnant after having unprotected intercourse for a specified amount of time, usually, within a year [2]. There are many factors that contribute to infertility, including, but not limited to the quantity and quality of the ovarian reserve [2]. However, a woman's age is not the only factor determining her ovarian reserve; decreases can occur at younger ages and be partially or totally responsible for infertility [3]. Couples struggling to become pregnant may consider fertility treatments, including multiple repeated tests, drugs and/or surgery [2] .Since treatments can be financially and emotionally draining, it is important to consider the patient's ability of becoming pregnant based on the limiting measures. Measurement of ovarian reserve is very important in predicting a woman's response to various fertility

treatments and helps us decide on appropriate fertility medication dosage levels for that treatment [2].

Follicle development is dependent on the interrelationship of many hormones, such as follicle stimulating hormone (FSH) and anti-Mûllerian hormone (AMH), secreted from the anterior pituitary gland and the ovaries respectively. Abnormal levels of these hormones may indicate a woman's diminished ability or inability of conception [2].

The woman's age and assays of serum FSH in the early follicular phase were among the earliest and most useful parameters used for evaluation of ovarian reserve. [4],[5] Various ultra-sound parameters are also used for evaluation of ovarian reserve, including ovarian volume [6],[7] and the antral follicle count (AFC), with varying degrees of reliability [8],[9].

Low levels of FSH are seen during follicle development and high levels during ovulation. The variation in levels of FSH is due to a feedback loop that exists between the hormones secreted from the ovaries and pituitary gland [3].

Follicle stimulating hormone level also depends on the estradiol (E2) level. As antral follicles develop in the ovaries, they excrete E2 and inhibin B. An increase in these hormones signals the gonadotropins in the pituitary gland to discontinue the release of FSH. Once ovulation occurs there is a decrease in E2, which signals for an increase of serum FSH, which helps to prepare for the next cohort of follicles in the growing pool. This accounts for variability of FSH during the menstrual cycle. Day three FSH has been the most used test of ovarian reserve and has been the standard way of determining ovarian reserve, providing greatest accuracy[2].

The measurement of serum AMH level is a relatively new method that is being considered for determination of the ovarian reserve, giving a more direct and accurate measurement. In women, AMH is produced by the granulosa cells (GC) of follicles. It is produced from the stage of the primary follicle to the initial formation of the antrum, thus is distinct from ovulation and is a step closer to being able to assess the true ovarian reserve [10] as AMH appears to accurately measure the active follicle pool, as active follicles only produce AMH. This is the basis of using AMH for determining ovarian reserve.

In recent decades, infertility has become a major global issue with medical, economic and psychosocial impact on infertile couples. A large number of infertile population is very anxious and eager to be treated. The prevalence of infertility worldwide is approximately 10-15% whereas in Asia it is around 8-12%.Infertility rate in Pakistan is about 21.9%.The rationale of the study was to assess the ovarian reserve in the infertile women by serum anti-mullerian hormone (AMH), and to opt for better fertility treatments and predict satisfactory outcome during assisted reproductive techniques [21,22] Ovarian reserve is the amount of oocytes present in both ovaries. The conventional tests to determine an individuals ovarian reserve include Day 3 serum follicle-stimulating hormone (FSH) levels and antral follicle count. Recently, another test for measuring AMH levels has also been included as a marker of ovarian reserve [24,25]. Mullerian inhibiting substance is the other name for AMH. It is a dimeric glycoprotein and a 140-kilodalton (kDa) hormone. It belongs to the super family of transforming growth factor beta. 26 Initially, synthesis of AMH occurs as a large precursor molecule. Later on, it is converted into the pre-prohormone and this forms the homodimers. The homodimer, or the mature

hormone, then undergoes glycosylation and produces two identical 70-kDa monomer subunits. These monomer subunits are bonded by disulphide-linkage by dimerisation, thus producing a 140-kDa dimer. Each monomer subunit contains two domains; an N-terminal domain and C-terminal domain. AMH acquires its full bioactivity by the N-terminal domain which causes the activation of C-terminal domain. 5 It is mainly produced and secreted by the granulosa cells of ovarian follicles. The levels of serum AMH are hardly detectable at birth, but remains stable throughout the reproductive period. With advancing age, AMH levels start declining. According to recent studies, serum AMH levels represent the quantitative aspect of ovarian reserve. Moreover, its levels strongly correlate with the size of the early growing follicle pool, and due to lack of inter-cyclic variability, it can be regarded as a diagnostic marker to assess ovarian reserve. The ovary secretes AMH directly into the blood; hence it is easy to measure AMH in serum [27].

AMH is involved in the process of folliculogenesis. Physiologically, there are two main functions of AMH, i.e. it inhibits the recruitment of primordial follicle cell and it also inhibits the selection of follicles that are under the influence of FSH for dominance.[28] Female reproductive hormones such as FSH, luteinising hormone (LH) and steroids are involved in late folliculogenesis i.e. during the formation of large antral follicles. Thus, they reflect the peri-ovulatory ovarian activity. On the other hand, AMH is produced by the granulosa cells of early antral follicles and provides information regarding ovarian reserve [8]. Diminished ovarian reserve (DOR) is regarded as one of the major causes of infertility [29,30]. AMH is regarded as an excellent marker of the ovarian reserve, a measure of the biological age of the ovaries and a diagnostic marker of ovarian dysfunction 10. Moreover, in DOR, serum AMH levels are found to be reduced earlier before any

increase in baseline FSH levels [11]. AMH testing is particularly beneficial for younger women because DOR is often overlooked in this age group, leading to an incomplete diagnosis of infertility [32,33]. Assessment of ovarian reserve by serum AMH levels can be determined with greater specificity and sensitivity in women of reproductive age rather than by determination of FSH together with other ovarian reserve markers. This is due to the fact that AMH acts in paracrine fashion and is not under the influence of negative feedback mechanisms of hypothalamic-pituitary-gonadal (HPG) axis. It can be measured on any day of the menstrual cycle [34,35]. This study was planned to include AMH as part of the ovarian reserve tests in our population besides the conventional markers like FSH and antral follicle count.

Measurement of ovarian reserve in a disease like Polycystic Ovarian Disease aid in mode of selection of mode of treatment. The clinical features of PCOS is a spectrum of disorder with a heterogeneous collection of signs and symptoms from mild to serve disturbance of metabolic functions and Reproductive Endocrine. The Polycystic Ovarian Syndrome pathophysiology appears to be polygenic and multifactorial. The most common endocrine disorder is PCOS of the Reproductive age group women. Polycystic Ovarian Syndrome is diagnosed based on the presence of any two of the following three criteria according to Durlinger AL *et.al.* [13]

They are

1. Oligo and Anovulation –menstrual cycle longer than 35 days or less than eight menstruation periods per year.

2. Hyperandrogenism – (clinical/biochemical) - as per the Ferrimann-Gallway scoring system designed to assess the clinical manifestation of Hirsutism 6. As per the

scoring system, the Masculine part of body hair growth is described in four degrees on different body places Viz. Upper lip, Chin, Chest, Lower leg, Upper back, Upper abdomen, Lower back, Arm, Lower abdomen, Forearm, and Thighs.Biochemical manifestations are Hyperandrogenism which is calculated by Free Androgen Index (FAI) from testosterone and sex hormone-binding globulin(SHBG)

Polycystic Ovaries- Where at least one of the ovaries should have follicles measuring 2-9 mm in diameter or should be of 12 follicles (or) an increase in Ovarian volume (>10cm3) [14, 915].

A wide variety of heterogeneity of signs and symptoms exist in PCOS women and in some PCOS subjects who can exist without any clinical signs and symptoms which can be expressed over time [12].

PCOS is prevalent in the young Reproductive age group where the distribution 20-30%. Presence of Insulin Resistance, Dyslipidemia and Central obesity which might lead to complications of Diabetes and Cardiovascular disease in PCOS women. Women with PCOS have been under the increased risk for Endometrial, Breast, and Ovarian Cancer [13].

The functional potential of the Ovary reflects the number and activity of the oocytes present within the Ovary which refers to ovarian reserve. The ovarian reserve constitutes the oocytes and the ovarian follicular pool size that it diminishes with increasing age. Ovarian reserve can be assessed by various markers Viz .serum FSH, serum Estradiol, Ovarian volume, antral follicle count, etc. [14]

Ovarian reserve is assessed by Biochemical parameter and ultrasound parameter. Biochemical parameters are measurement of Hormones such as FSH and E2.

Measurement of FSH and E2 on Day 312 has limitations in PCOS patients as it is very difficult to predict the appropriate time owing to irregular cycles. Antral follicular count by ultrasonogram is a better marker for assessing the ovarian reserve [15].

The characteristic of the Polycystic Ovary is variable and may be subtle and need lot of expertise to precisely determine the AFC count. Owing to the above-said limitations there is a need for a marker that correlates well with ovarian reserve.So as to ascertain the functional ability of Ovary and intervention of ovarian pathology for conception.AMH level decreases steadily with increasing age from 24 to 50 years of age13. Reports documented AMH as a predictor of age-related reductions in fecundability in the general populations [16].

AMH concentration in the serum is directly related to the antral follicle count and is a better indicator of ovarian reserve when compared to FSH and Estradiol level. As the concentrations of AMH are unaffected by gonadotrophins it is feasible to measure AMH throughout the cycle.With this background, the present study aims at measuring AMH levels in normoovulatory and Polycystic Ovarian Syndrome to assess the ovarian reserve [17].

Assessment of the status of an ovarian function is essential to evaluate and plan infertility interventions. In recent times, estimation of ovarian reserve (OR) is the most commonly used criteria to reflect the quality and quantity of oocytes, in turn imitating the fertility potential of a female. With advancing age, a drop in the extent of OR proportionately reflects the decline in a female's reproductive capabilities. Hence, its estimation provides an approximation of fertile years left for a woman. Several markers are used to reflect OR in infertility clinics that include patient's age, serum follicle-

stimulating hormone (FSH), luteinizing hormone (LH), anti-Mullerian hormone (AMH), estradiol levels, antral follicle count (AFC), and ovarian volume. AFC is considered as a gold standard for measurement of OR and is considered necessary before planning assisted reproduction support. [18, 19]. It is suggested that an optimum response to infertility assistance is reflected as retrieval of at least 5 oocytes on ovarian stimulation. Furthermore, an exaggerated AFC (>19) is linked to potential complications such as ovarian hyperstimulation syndrome (OHSS), rendering its evaluation as a better tool for optimization of protocol that may reduce the chances of cycle cancellation. However, it has its own drawbacks such as prerequisite of a skilled operator and latest machinery that reliably assess the count. In addition, its inability to reveal the quality of healthy oocytes results in counting even those follicles that may not act in response to treatment [20, 21, 22].

Oocyte numbers and quality are known to decline with age; however, large variations in oocyte reserve exist between individual patients, as do ovarian responses to gonadotrophin stimulation, even among women of the same age.Women with a low ovarian reserve are more likely to respond to ovarian stimulation with a modest degree of follicular development and may require greater management of their expectations for outcome success [23, 24, 25].

At the other end of the spectrum, women with a high ovarian reserve are at increased risk for excessive ovarian response that can lead to ovarian hyperstimulation syndrome (OHSS). Additionally, it has been suggested that some patients may benefit from one protocol over another, i.e. antagonist versus long agonist protocol, or from other protocol and FSH dose adjustments. Correspondingly, women predicted to have

excessive responses may benefit from a GnRH antagonist protocol, as comparative trials have shown they are associated with fewer developing follicles when using milder stimulation and allow for a GnRH agonist trigger instead of an HCG trigger in cases with a risk of OHSS [25, 26].

Women predicted to have poor response may benefit from a higher gonadotrophin dose for maximal stimulation in a GnRH antagonist protocol, long GnRH agonist protocol, or GnRH flare agonist protocol, which adds a burst of endogenous FSH and LH stimulation at the start of the protocol to enhance follicular recruitment. Therefore, accurate and reliable predictors of ovarian reserve are needed to identify patients likely to have poor response or hyper-response to treatment and to guide physicians in selecting the optimal dose of gonadotrophins for ovarian stimulation. [27, 28, 29].

AIM AND OBJECTIVES

PRIMARY OBJECTIVE

1. To study the level of AMH in infertility patients and to assess the levels of ovarian reserve.

SECONDARY OBJECTIVE

1. To study the level of AMH in infertility patients.
2. To determine the possibility of future conception in poor ovarian reserve patients.
3. It provides an improved ascertainment of ovarian reserve and thus helps in planning therapeutic interventions in couples seeking infertility treatment.

REVIEW OF LITERATURE

PHYSIOLOGY OVARY

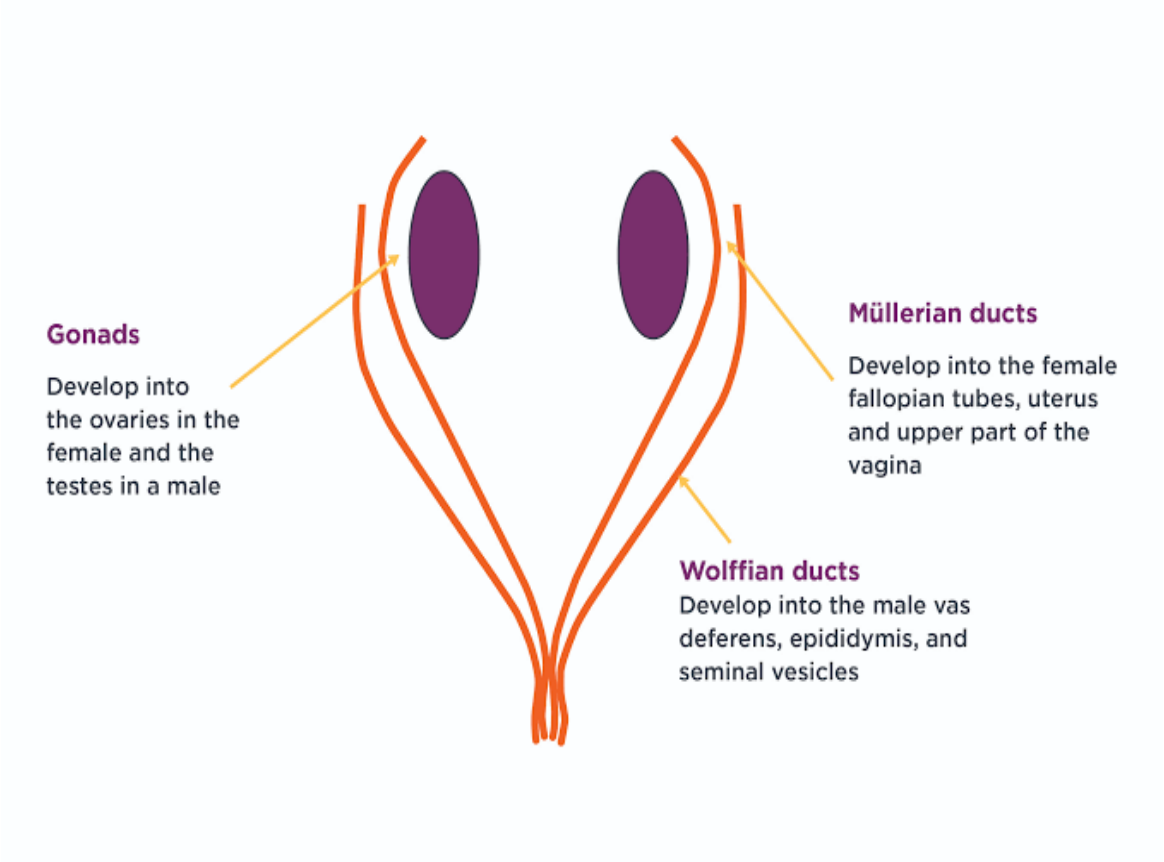
The ovaries form part of the female reproductive system. Each woman has two ovaries. They are oval in shape, about four centimeters long and lie on either side of the womb (uterus) against the wall of the pelvis in a region known as the ovarian fossa. They are held in place by ligaments attached to the womb but are not directly attached to the rest of the female reproductive tract, e.g. the fallopian tubes. Ovary undergoes through a number of stages as it passes from the stage of development [29, 30].

The development is initiated at 30 days after the post-conception by formation of a genital ridge, followed at about 35 days by an indifferent gonad, at 42 days developed into an embryonic ovary (6 weeks), and at about 8weeks an early fetal ovary is developed, at 16 weeks a late fetal ovary, and perinatal ovary develops by the end of 28 weeks. The functional ovary develops from the germ cells. The sex gonadal of an embryo becomes evident at about 6 weeks post-conception [31, 32].

Fetal early weeks span for about 8- 16 weeks. Primordial follicles are formed by the 12^{th} week when the epithelial cells surround ova and after 16 weeks of gestation they form the late fetal stage. The cuboidal epithelial cells emerge from the growing follicle by 28^{th} weeks of gestation.The stroma later differentiates into theca interna which demonstrates features of steroidogenesis, the presence of 3β-hydroxysteroid dehydrogenase [14, 33, 34].

Paired gonadal structures the ovaries which lie suspended between the pelvic wall and the uterus by the infundibulopelvic and uteroovarian ligaments. Adult ovaries are ovoid in shape and they measuring 5 X 3 X 3 cm. There is variation in dimension of the ovary with respect to hormonal changes during the menstrual cycle and also along with age. Normally small corpus luteum along with small cystic follicles are seen under the surface of the ovary. Each ovary consists of the outer cortex composed of specialized stroma and follicles of various sizes, the inner medulla occupies a small portion of ovary made of fibromuscular tissue and blood vessels. [35, 36]

SEX DETERMINATION AND DIFFERENTIATION



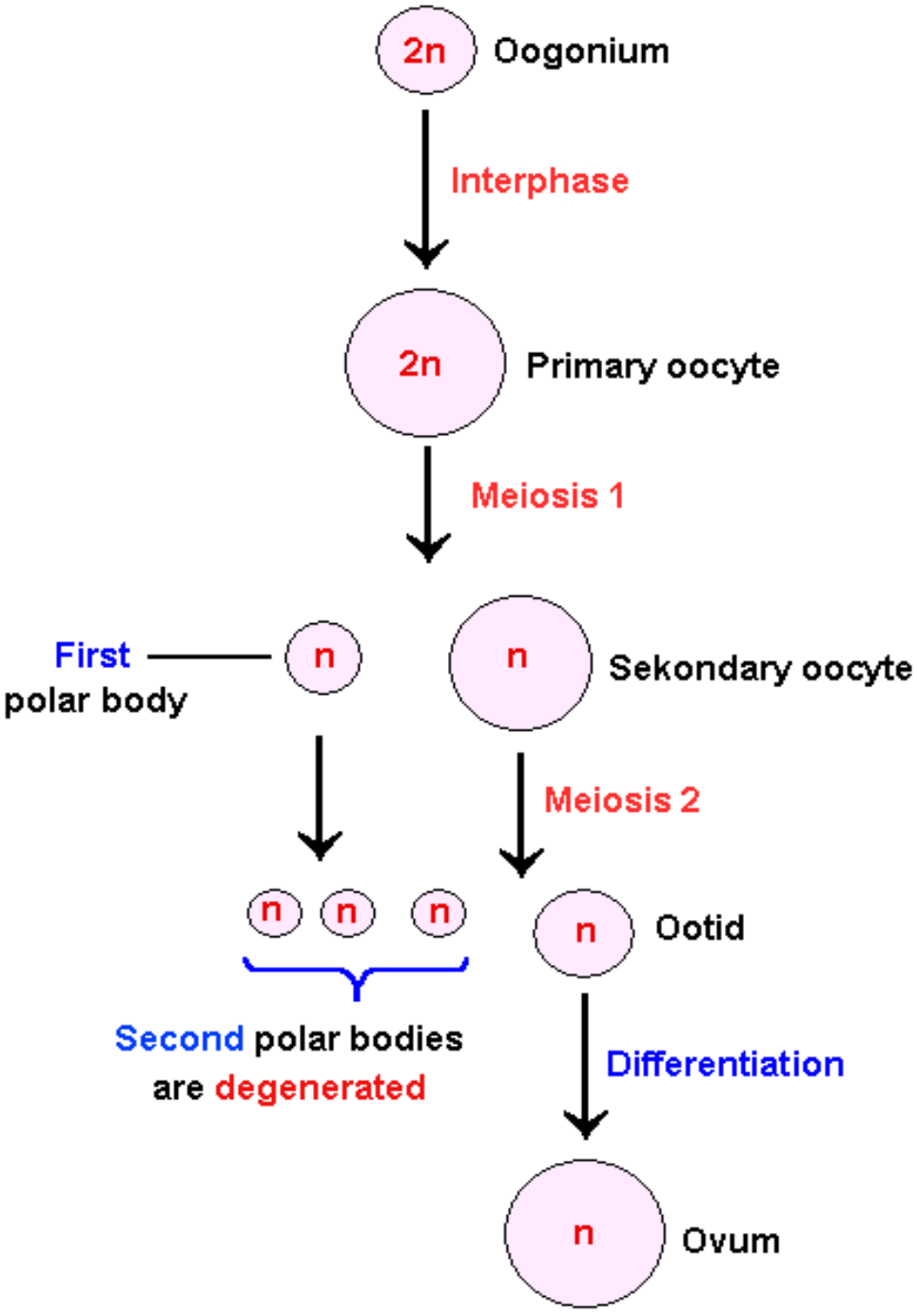
Ovarian function

The ovaries have two main reproductive functions in the body. They produce oocytes (eggs) for fertilization and they produce the reproductive hormones, estrogen and progesterone. The function of the ovaries is controlled by a gonadotrophin-releasing hormone released from nerve cells in the hypothalamus which sends their messages to the pituitary gland to produce luteinizing hormone and follicle-stimulating hormone. These are carried in the bloodstream to control the menstrual cycle.

The ovaries release an egg (oocyte) at the midway point of each menstrual cycle. Usually, only a single oocyte from one ovary is released during each menstrual cycle, with each ovary taking an alternate turn in releasing an egg. A female baby is born with all the eggs that she will ever have. This is estimated to be around two million, but by the time a girl reaches puberty, this number has decreased to about 400,000 eggs stored in her ovaries. From puberty to menopause, only about 400–500 eggs will reach maturity, be released from the ovary (in a process called ovulation) and be capable of being fertilized in the fallopian tubes/uterine tube/oviduct of the female reproductive tract.

The ovaries have two functions: the production of ova and the production of hormones. Both these functions are controlled through the hypothalamic-pituitary ovarian axis by endocrine, paracrine and autocrine pathways [37, 38, 39].

PRODUCTION OF OVA



The ovary of the female child at birth contains all the primary oocytes which are scattered amongst the mesenchymal stromal cells of the medulla and cortex. A female fetus contains 7 million oocytes at birth. It is further reduced

during childhood so that at puberty the figure is about 400000 oocytes at the onset of the menstrual cycle.Of these not more than 500 are designated to mature during the individual's lifetime and the remainder will be lost by degenerative process. During childhood, the ovary grows in size by an increase in stroma .Nevertheless, ova do attempt to ripen from time to time but they fail to complete the process and become blighted

In the ovary, all eggs are initially enclosed in a single layer of cells known as a follicle, which supports the egg. Over time, these eggs begin to mature so that one is released from the ovary in each menstrual cycle. As the eggs mature, the cells in the follicle rapidly divide and the follicle becomes progressively larger. Many follicles lose the ability to function during this process, which can take several months, but one dominates in each menstrual cycle and the egg it contains is released at ovulation [40,41].

As the follicles develop, they produce the hormone estrogen. Once the egg has been released at ovulation, the empty follicle that is left in the ovary is called the corpus luteum. This then releases the hormones progesterone (in a higher amount) and estrogen (in a lower amount). These hormones prepare the lining of the uterus for potential pregnancy (in the event of the released egg being fertilized). If the released egg is not fertilized and pregnancy does not occur during a menstrual cycle, the corpus luteum breaks down and the secretion of estrogen and progesterone stops. Because these hormones are no longer present, the lining of the womb starts to fall away and is removed from the body through menstruation [42, 43, 44].

After menstruation, another cycle begins. The menopause refers to the ending of a woman's reproductive years following her last menstruation. This is caused by the loss of all the remaining follicles in the ovary that contain eggs. When there are no more follicles or eggs, the ovary no longer secretes the hormones estrogen and progesterone, which regulate the menstrual cycle. As a result, menstruation ceases [45]

Risk factors

- **Hormonal problems:** These include taking the fertility drug clomiphene (Clomid), which is used to cause you to ovulate.
- **Pregnancy:** Sometimes, the cyst that forms when you ovulate stays on your ovary throughout your pregnancy.
- **Endometriosis:** This condition causes uterine endometrial cells to grow outside your uterus. Some of the tissue can attach to your ovary and form a growth.
- **A severe pelvic infection:** If the infection spreads to the ovaries, it can cause cysts.
- **A previous ovarian cyst:** If you've had one, you're likely to develop more.

Complications [46, 47]

Some women develop less common types of cysts that a doctor finds during a pelvic exam. Cystic ovarian masses that develop after menopause might

be cancerous (malignant). That's why it's important to have regular pelvic exams. Infrequent complications associated with ovarian cysts include:

Ovarian torsion. Cysts that enlarge can cause the ovary to move, increasing the chance of painful twisting of your ovary (ovarian torsion). Symptoms can include an abrupt onset of severe pelvic pain, nausea, and vomiting. Ovarian torsion can also decrease or stop blood flow to the ovaries.

Rupture. A cyst that ruptures can cause severe pain and internal bleeding. The larger the cyst, the greater the risk of rupture. Vigorous activity that affects the pelvis, such as vaginal intercourse, also increases the risk.

The Menstrual Cycle

The menstrual cycle is the regular natural change that occurs in the female reproductive system (specifically the uterus and ovaries) that makes pregnancy possible. The cycle is required for the production of oocytes, and for the preparation of the uterus for pregnancy. The menstrual cycle occurs due to the rise and fall of hormones [3]. This cycle results in the thickening of the lining of the uterus, and the growth of an egg, (which is required for pregnancy). The egg is released from an ovary around day fourteen in the cycle; the thickened lining of the uterus provides nutrients to an embryo after implantation. If pregnancy does not occur, the lining is released in what is known as menstruation [48, 49, 50].

Up to 80% of women report having some symptoms during the one to two weeks prior to menstruation. Common symptoms include acne, tender breasts, bloating, feeling tired, irritability and mood changes. These symptoms interfere

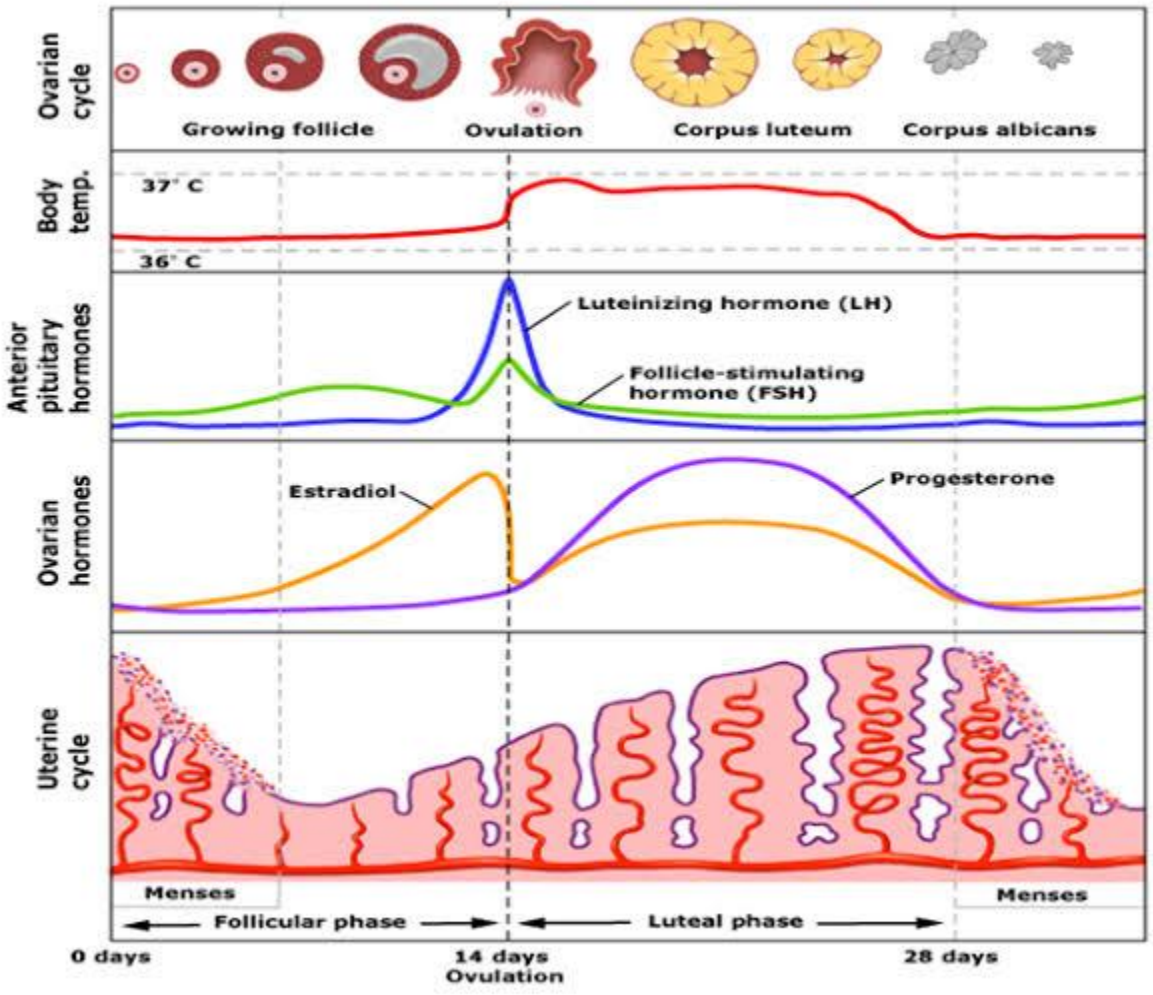
with normal life and therefore qualify as premenstrual syndrome in 20 to 30% of women. In 3 to 8%, they are severe. The first period usually begins between twelve and fifteen years of age, a point in time known as menarche. They may occasionally start as early as eight, and this onset may still be normal. The average age of the first period is generally later in the developing world and earlier in the developed world. The typical length of time between the first day of one period and the first day of the next is 21 to 45 days in young women and 21 to 35 days in adults (an average of 28 days. Menstruation stops occurring after menopause which usually occurs between 45 and 55 years of age [50]. Bleeding usually lasts around 3 to 7 days.

The menstrual cycle is governed by hormonal changes. These changes can be altered by using hormonal birth control to prevent pregnancy. Each cycle can be divided into three phases based on events in the ovary (ovarian cycle) or in the uterus (uterine cycle). The ovarian cycle consists of the follicular phase, ovulation, and luteal phase whereas the uterine cycle is divided into menstruation, proliferative phase, and secretory phase [51,29.33].

Stimulated by gradually increasing amounts of estrogen in the follicular phase, discharges of blood (menses) flow stop, and the lining of the uterus thickens. Follicles in the ovary begin developing under the influence of a complex interplay of hormones, and after several days one or occasionally two become dominant (non-dominant follicles shrink and die). Approximately mid-cycle, 24–36 hours after the luteinizing hormone (LH) surges, the dominant follicle releases an oocyte, in an event called ovulation. After ovulation, the

oocyte only lives for 24 hours or less without fertilization while the remains of the dominant follicle in the ovary become a corpus luteum; this body has a primary function of producing large amounts of progesterone. Under the influence of progesterone, the uterine lining changes to prepare for potential implantation of an embryo to establish a pregnancy. If implantation does not occur within approximately two weeks, the corpus luteum will involute, causing a sharp drop in levels of both progesterone and estrogen. The hormone drop causes the uterus to shed its lining in a process termed menstruation [53, 55].

PHASES OF MENSTRUAL CYCLE



Normal Menstrual Cycle [56,57]

The ovarian and the uterine cycles are the two-phase of menstrual cycle. The uterine cycle is divided into the proliferative and secretory phases while the ovarian cycle is divided into follicular and luteal phases. The Follicular phase is the growth and recruitment of a dominant follicle that proceeds to the process of ovulation.The entire process is under the control of hormones. The Normal duration of the menstrual cycle is from 21 to 35 days cycle.This includes the follicular phase with a duration of 10 to 14 days which is, in turn, has varied depending on the individuals. Luteal phase duration is of 14 days which starts from the day of ovulation to the onset of menses. The menstrual flow is of about two to six days and with an average menstrual blood loss about approximately 20 to 60 ml per cycle. Variations from these would lead to an irregular cycle [37]

Follicular development

Primordial follicle

Follicular epithelial cells are of a single layer and they surround the oocytes measuring about 25-30 μm in diameter. In young individuals there are more primordial or primary follicles of about 38,000. As these individuals reach the age of 40 their follicular number is reduced to about 8000. At the time of menopause there are only a few follicles or even reduced extremely to one or two in later period of life. A follicle grows up to the antral stage all along the period of fetal life and also during the period of infancy [38]

Growing follicle

At 28 wks of gestation the follicular growth begins and continues up to menopause. Only about 10% of these follicles are in the active growing period the rest being inactive. These inactive follicles are called as Primordial follicles or Primary follicles. The active growing follicles are called secondary follicles and the tertiary the preovulatory follicles[16].The secondary follicles are further divided into preantral, antral and preovulatory follicles.In the preantral-the follicles grow up to size of 0.4mm and antral follicles grow up to 1-2mm. It requires about 50 days to grow to a preovulatory stage. Only one or two follicles attain the preovulatory stage during each menstrual cycle. The pool of primordial follicle is formed in the neonatal period and is considered infinite.[39]

The oocytes continue to grow to the antral stage. The selected follicles compete with each other for growth-inducing FSH.In response to rise of FSH the preantral follicles begin to secrete estrogens. This has negative feedback on FSH. Follicles with fewer FSH receptor will not be able to develop further, they will show retardation of their growth and become atretic. Eventually only one follicle will be recruited and is called the dominant follicle. This dominant follicle will grow up to 20mm in diameter to become the preovulatory follicle which stimulates the estrogen hormone to a high level for a period of 48 hrs[49,50]

Ovulation

By the end of the follicular phase or phase of the 13th day of menstrual cycle.The preovulatory follicle releases a single oocyte and it is called ovulation. This needs the midcycle LH surge and it is responsible for the ovulation [20].

Atretic follicles

The primordial follicles are about one to two million at the time of birth and only four hundred ovulate among them. The rest of the follicles undergoes a process called atresia and disappears. This follicular atresia is more pronounced in pregnancy, menarche, and PCOS women.The androstenedione is produced by the theca internal cells in the absence of granulosa which cannot be converted to estradiol and they are responsible for the androgenic activity in PCOS

Corpus luteum

Post ovulation the oocytes are discharged from the follicles and the ruptured follicles tend to collapse.This leads to a scared theca interna called corpus luteum which exists for 14 days after ovulation and begins to degenerate if fertilization does not occur. Then menstrual flow is regulated by estrogens .In the absence of fertilization, post-ovulation on the 14 days the corpus luteum disappear

Ovarian hormones

Estradiol

Estradiol (E2) is a C18 steroid with the presence of phenolic hydroxyl group at C-3 on an aromatic ring, and either a hydroxyl group (estradiol) or a ketone group (estrone) at C-17, which synthesized from its precursor cholesterol. The site of secretion is primarily from the ovary, the placenta, and also in also from adrenal cortex. The secretion is very low after menopause. About 89% of the estrogen is bound to sex hormone-binding globulin in bloodstream. It is bound to albumin also to a lesser extent. Estrogen activity is affected by the estradiol-receptor complexes which are situated in the target site like the uterus, breast, hypothalamus and pituitary gland

Luteinizing hormone [51**]**

Luteinizing hormone (LH) is a glycoprotein with a molecular weight of 30 Kilo Daltons [18]. The gonadotropin-releasing hormone (GnRH) releases the Luteinizing Hormone from the anterior pituitary and the hormone is taken to the targeted site via the blood. LH then stimulates that follicle until the stage of corpus luteum. Lutenizing Hormone is under the control of the Gonadotrophin Releasing hormone which in turn stimulates the secretion of sex steroids Hormones from the gonads. This operates by a negative feedback loop mechanism on the sex steroids which in turn inhibits the secretion of GnRH which in turn has a negative effect on gonadotrophs.

Prolactin hormone [57]

Prolactin hormone (PRL) is made of a single polypeptide chain containing about two hundred amino acid residues and three disulfide bridges and has considerable structural similarity to the human growth hormone. The half-life of

PRL, like that of growth hormone, is about 20 minutes.PRL is secreted from the anterior pituitary and its target site of action is on the mammary glands. The main action of the prolactin hormone is the growth and induction of milk secretion during pregnancy and reaching a peak at paturation, PRL secretion is increased by stimulation of the nipples.

Thyroid-stimulating hormone [58]

Thyroid-stimulating hormone (TSH) is a glycoprotein which is of about 28 to 30 KDa and is secreted from the anterior pituitary. Its target site of action is on the thyroid follicular cells present in the thyroid gland. TRH, in turn, stimulates the anterior pituitary and leads to the secretion of the TSH, which in turn stimulates the release of T4 and T3.The secretion of thyroid-releasing hormone, and in turn the TSH, is inhibited by classical negative feedback loop mechanism. Free or unbound T_3 and T_4 also play an important role by acting via this negative feedback mechanism by maintaining the circulating thyroid hormone levels.

Poly Cystic Ovarian Syndrome [60]

PCOS is a collection of the spectrum of disorder with heterogeneous collection of signs and symptoms which causes changes in the metabolic functions and Reproductive Endocrine in women. PCOS is characterized by irregular menstrual cycles or period of amenorrhoea with or without excess hair growth on face and body (Hirsutism), increase in weight, acene, thinning of the hair on the scalp and associated with ovarian cysts.

The other factors associated with PCOS are obesity, type 2 diabetes, cardiovascular disease, and obstructive sleep apnoea, impaired fertility which increases the risk of diabetes, cardiovascular risk and endometrial cancer which are the short and long term complications of PCOS in women. In PCOS women the identity, mental health, and health-related quality of life are becoming stigmatizing conditions

Prevalence

The prevalence of Poly Cystic Ovarian Syndrome was estimated to be about 6.6% in adult women aged 18–45 years. However, the prevalence of PCOS was found to be higher in other countries than the U.S based on the diagnostic criteria. Estimated PCOS according to the phenotypes, morbidities, and prevalence associated with PCOS due to the lack of large-scale and epidemiological studies on PCOS [62,63].

The prevalence of other androgens excess disorder using the new diagnostic criteria suggested for diagnosis of PCOS and the most common classic feature of PCOS was an androgen-excess disorder which was about 30% of patients with clinical hyperandrogenism.

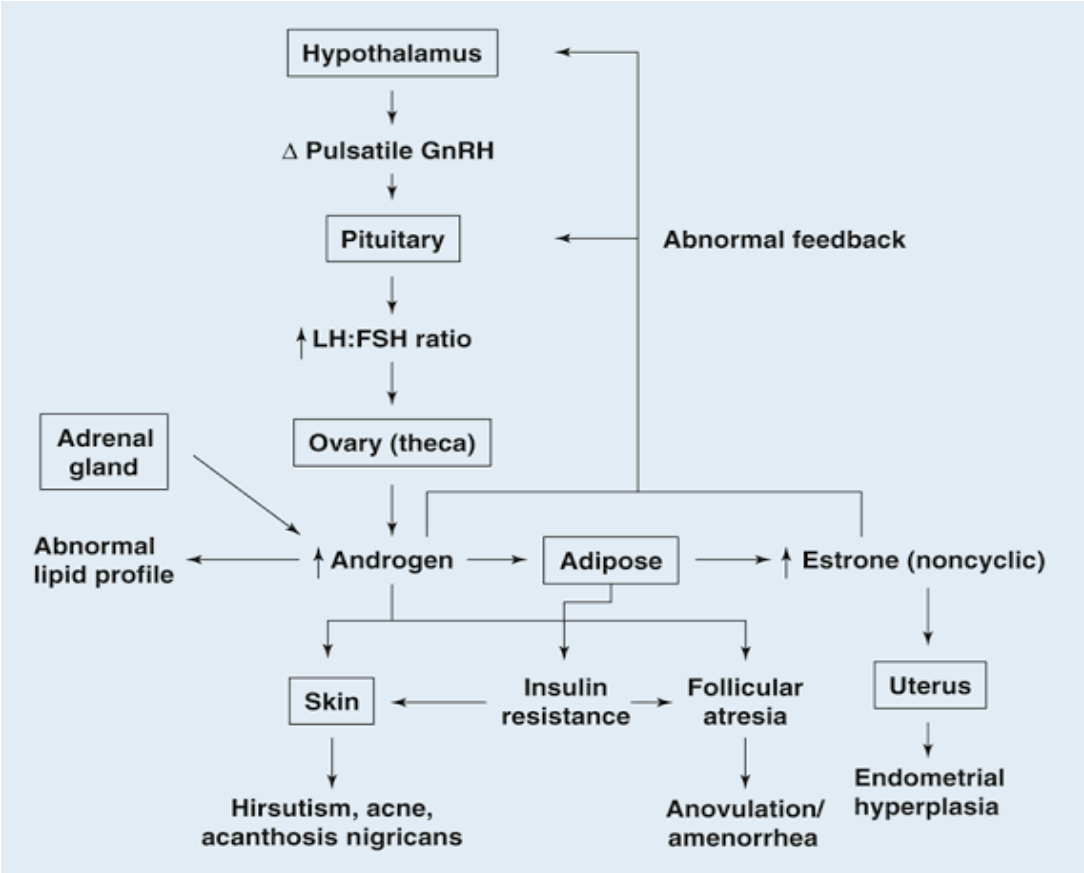
There is a necessity for the estimation of proportion of women who are affected by PCOS in the population. Earlier studies support the percentage of 2.2- 2.6%.Using different available data of three different countries the prevalence is 4.0-11.9%.but in India there is paucity [49,50].

Clinical features of PCOS

The common clinical features of PCOS are Oligomenorrhoa and amenorrhoea are signs of anovulation and hirsutism is a sign of hyperandrogenism. Menstrual cycles longer than 35 days or less than eight periods of menstruation per year is applied for oligo/amenorrhoea. Hirsutism is a sign of androgens excess being the underlying endocrine abnormality. In PCOS the presence of hirsute must be evaluated by clinical examination and other etiological abnormalities must be excluded. Although hirsutism is a frequent feature in PCOS the identification of the etiology needs a systemic approach for evaluation and earlier management [26]. The Ferriaman-Gallwey scoring was designed to assess the severity of hirsutism.

The masculine pattern of body hair growth is described in four degrees on eleven different body parts; upper lip, chin, lower back, chest upper back, lower abdomen, upper abdomen, forearms, arms, thighs and the lower part of the legs. A score greater than 8 was considered hirsute.Ovary and adrenal glands produce the androgens. Androgen excess which is the hallmark feature of PCOS and contributor to the stromal hyperplasia, antral follicle formation, and hypervascularity and this androgen excess which is necessary to measure the negative impact on fertility and subfertility its regulatory benefits in replacement with androgen therapy [53].

PATHOPHYSIOLOGY OF PCOS



The pathophysiology for PCOS is multifactorial and polygenic.The phenotype of women with polycystic ovaries (PCO) and polycystic ovarian syndrome is variable.In understanding the pathophysiology the nature of ovarian dysfunction has to be considered.There is uncertainty in considering the association of inappropriate gonadotropin secretion that is LH and FSH in PCOS.Hence , twenty-four hours pulsatile parameter for serum LH and pituitary gonadotrophin response to I.V bolus GnRH was given and the results showed that BMI does not gonadotrophin in normal cycling women. Taking LH levels and LH/FSH ratio along with BMI is considered beneficial [54, 55].

It is indicated with higher androgen levels, increased prevalence of insulin resistance and more often CVD in first-degree relatives.The correlation of LH / FSH ratio, BMI and clinical manifestations of PCOS was conducted at institution of Infertility treatment and Embryo research Iraq. The conclusion was negative and no significant correlation was found and the belief that obesity plays a role in pathophysiology of PCOS which was a factor year back [56,57].

The ovarian morphology is distinct and pathognomonic for its major marker the hyperandrogenemia which is from the theca cells. The entire process from the follicular development to the period of primordial to pre-ovulatory is dependent on the gonadotropins. Polycystic ovaries of the anovulatory cycle had increased number of preantral follicles. This is thought to reduce the rate of recruitment from resting follicles. LH is considered to be the cause of hyperandrogenism of PCOS.Along with steroidogenic enzymes in the synthesis of ovarian androgens [58,59]

There is a possible explanation of the working mechanism of ovarian cautery by laparoscopy as a treatment in PCOS.They established that in PCOS there was thickened ovarian capsule along with dense hyperplastic ovarian stroma and multiple subcapsular cysts of the ovary. In PCOS endocrine disorder associated with chronic anovulation, an imbalance of LH and FSH results in abnormal estrogen and androgen production [60].

The serum LH level is high and the serum FSH is low an altered LH/FSH ratio is characteristic. The combination of enlarged polycystic ovaries and obesity, oligomenorrhea and hirsutism show an increased risk for the

development of endometrial and possibly breast carcinoma.There is large influence of genetic factors in the pathogenesis of PCOS and justified the search for the susceptibility of the gene location [61,62].

Diagnosis of PCOS

The Revised criteria of 2003 in diagnosing Polycystic Ovarian Syndrome based on the clinical or biochemical evidence of hyperandrogenism, chronic anovulation clinical trial showed that the evidence two out of the three criteria is necessary for the diagnosis.

1. Oligo or amenorrhea
2. Biochemical signs or clinical features of hyperandrogenemia
3. Transvaginal ultrasonography establishing polycystic ovaries.

There is a wide distribution of heterogeneity of signs and symptoms among women with PCOS and in some subjects PCOS can be expressed over time and can exist without clinical signs and symptoms at present.

PCOS is prevalent in the young Reproductive age group where the distribution is about 20-30%. Polycystic ovaries have several times more primary, secondary and antral follicles compared to non-PCO women. The biological mechanisms behind PCOS were explained less, the condition behind this seems to be two-factorial. Mainly the intra-ovarian hyperandrogenism which leads to the early follicular growth promotion and leads to excess of follicle with of size 2-5 mm follicle. Studies have also revealed a positive correlation between follicle number and serum Testosterone/Androstenedione concentration in

PCOS women. A low aromatase activity due to an insufficient FSH stimulation affected by the synthesis of Estrogens which interferes the selection and growth of a dominant follicle Seconds the hyperandrogenism, Insulin resistance, secondary to both genetic and lifestyle factors, is associated with anovulation, but probably not its primary cause.

The ESHRE/ASRM sponsored for PCOS consensus workshop which was aimed at the diagnosis and the management of infertility publication in 2004, 2008 respectively. They had discussions on hirsutism and acne include adolescence, the menstrual irregularities, the pregnancy complication, then on the contraception and quality of life, latter with long-term metabolic and cardiovascular health and cancer risks associated with PCOS women [13,16,19].

The criteria needed for the diagnosis of PCOS differ from adolescents to the reproductive age group women and the risk group with obesity, hirsute and irregular menses which should be classified and treated accordingly in a word of caution with overdiagnosis in PCOS. The individual PCOS manifestation in adolescents should be taken into account and treated accordingly.The ovarian volume as the diagnostic criteria for PCOS included 154 women with PCOS under NIH criteria as cases and 57 normal ovarian women as control. They concluded that ovarian volume 10 cm3 is a good diagnostic criterion for PCOS but even a threshold of 7cm3 with greater than 12 follicles appears to be the best diagnostic criteria and ovarian volume can be used as a surrogate for ovarian volume difficult situations [20,21,22].

Ovarian dysfunction

Women of about one-quarter and one-third of all women with anovulation or menstrual dysfunction have PCOS. The number of antral follicles increases in the ovaries leads to the risk of anovulation. In contrast, the number of ovulatory cycles increases if the number of antral follicles decreases, no matter if it is due to increased age (before menopause) or after ovarian parenchyma-reducing surgery. Women with PCOS usually have increased levels of luteinizing hormone (LH) relative to follicle-stimulating hormone (FSH), i.e. the circulating LH to FSH ratio is elevated. The normal LH to FSH ratio which is 1:2 is altered to 2:1 or 3:1. This increase in the LH levels stimulates the theca cells to express the enzyme essential for the production of androgens to a higher extent, thus contributing to androgen excess [62,43].

Also, insulin stimulates theca cells to secrete androgens. The accumulation of the antral follicle along with failure for the selection of the dominant follicle is the characteristic of PCOS. Oligoamenorrhea influences ovarian follicle in PCOS in two different ways.The sensitivity of the follicles to the FSH is excess and leads to more follicular development of small-sized follicle 2-5mm diameter. The selection of the dominant follicle from the follicular poll is lost which is under the influence of LH. This explains the follicular arrest in PCOS ovaries.

The excessive stimulation of follicular cells by hormones insulin, LH, or both which contributes to the hyperandrogenism leading to follicular arrest, environment. However, exact mechanisms of follicular arrest are not fully understood.In PCOS women with oligo ovulation exhibit ovarian dysfunction in

milder phenotype pattern compared to anovulatory and are favorable in response to the treatment with ovulation induction with clomiphene citrate. But in with recombinant FSH treatment the oligo ovulatory responds less than the anovulatory PCOS [45,46].

Ultrasound description of polycystic ovary

Transabdominal ultrasound is greatly replaced by transvaginal ultrasound. It was demonstrated that polycystic ovaries were defined as the presence of ≥10 cysts (2-8 mm diameter), ovarian volume ≥12cm^3 and bright echogenic stroma (23 books). Increased stromal echogenicity assessed transvaginally appeared to be exclusively associated with PCOS although this was a subjective appearance rather than a quantifiable measurement. Women with amenorrhoea had similar ultrasound features to those with oligomenorrhea.

The appearance of polycystic ovaries is less important and there is a need to measure the follicular size in PCOS. Polycystic ovaries in premenopausal women are larger than the postmenopausal women. The multiple cysts in the ovary are, with a diameter of 4-10 mm and with normal echogenicity of the stroma. Although during puberty this is the characteristic appearance of the ovary and also in women recovering from the hypothalamic amenorrhoea- in the above situations it is the follicular growth which continues without recruitment of a dominant follicle. There is a need to differentiate between multicystic ovaries from polycystic ovaries for the diagnosis and proper management [56, 57].

In all young women immediately after menarche, irregular menses are common in these years. PCO is a common feature in women with irregular

cycles when ultrasonography is done. There is no consensus of PCOS diagnostic criteria during adolescence, emphasizing a polycystic ovary from a multicystic or multi follicular. In adolescents, the ultrasonographic examination is often performed transabdominally, rather than transvaginally, even though the resolution is better transvaginally. This problem is further magnified by the inferior ultrasound resolution in obesity, and by the changes of ovarian findings with age. There was significant intra- observers and inter-observer variability also using these criteria among inexperienced ultrasonographers, radiologists and gynecologists, hence there is a need for careful consideration of clinical picture and a supportive biomarker in diagnosing PCOS [58].

The three-dimensional ultrasound scanning and pulsed Doppler ultrasonography as a research tool in infertile women. In polycystic ovaries the measurement of ovarian stromal blood flow is an additional and useful parameter. The three- dimensional ultrasound technology can facilitate as a routine in clinical practice. In women undergoing IVF the prediction of ovarian reserve and antral follicular count is of paramount importance in predicting the outcome of pregnancy. The overall performance was assessed by a summary with ROC curve. The conclusion was that ovarian response was similar in the IVF patients with the use of one single test cannot be used in the assessment of ovarian volume. The stage of the disease and the management is largely based on ultrasound findings. Evaluation of ultrasound features of PCOS is dependent on the fact that experience of the specialist and necessary to standardize the description probability [59,60].

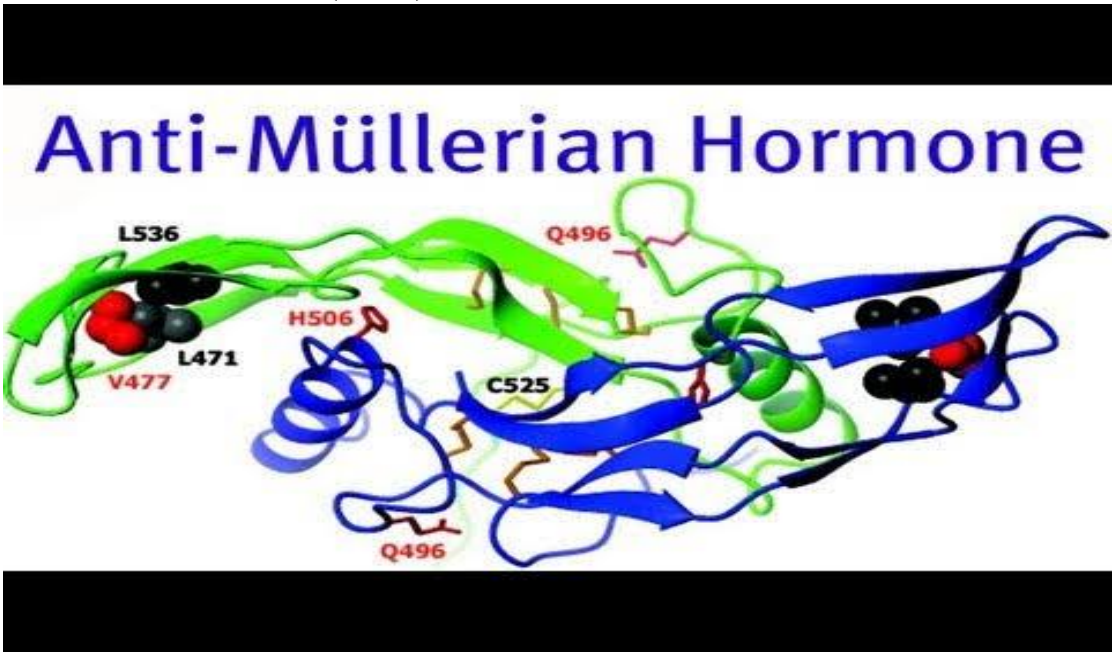
A reproductive consequence of PCOS

The most common cause of PCOS is anovulatory cycles of infertility. In majority of women with PCOS anovulation, oligomenorrhoea or amenorrhoea is the main cause and it is due to the irregularities in the menstrual usually. This may be associated with hyperandrogenism either clinical and /or biochemical [61]

The metabolic consequence of PCOS [17,18,19]

The condition "the diabetes of bearded women" is a disorder associated with metabolism of carbohydrate and hyperandrogenism. In women with hyperandrogenism and diabetes mellitus skin lesions like acanthosis Nigerians frequently occurred.Additional features include the lipoatrophic diabetes syndromes, pineal hypertrophy, dental precocity, nails are thickened and associated ovarian enlargement.The metabolic evidence is there to justify the inclusion of both non- NIH group PCOS as Polycystic ovarian subgroup in predicting the risk factor of CVD and T2DM.There is a strong genetic relationship to the etiology of PCOS. Considerable progress is made in identifying the susceptibility of gene and mapping studies with TGF and insulin signaling, T2DM and obesity susceptibility.A retrospective cohort study to show the effect of BMI on the outcome of patients with PCOS. Concluded that PCOS is a broad syndrome with obese and lean PCOS as two distinct population and have a difference in the IVF outcome and there is a need to differentiate them for the purpose of management and treatment in infertility.

Anti Mullerian Hormone (AMH)



Mullerian-inhibiting substance (MIS) was the initial name given for Anti-Mullerian Hormone (AMH).It is a glycoprotein and its molecular weight of AMH is of 140 kD. This belongs to a family of Transforming growth factor β (TGF-β).It is expressed in the gonads and has the main role in sex differentiation. During sex differentiation in the male fetus the AMH is expressed in the Sertoli cells. The testosterone is produced by the induction of the Müllerian ducts and the Leydig cells. The Wolffian duct is differentiated into epididymis, Vas deference, and the seminal vesicle. The absence of this hormone is responsible for the development of the female reproductive tract, which in turn leads to the differentiation of the Mullerian duct into the oviduct, the uterus and the upper part of the vagina in female fetus. Only from the 36 weeks of gestational age the AMH is, expressed in the ovary from long after the Müllerian ducts female fetus. The pre-antral and antral follicles produce AMH from the granulosa cells and involve in the follicular growth and development. Anti Mullerian Hormone in

female serum is low at birth but later increase by puberty until adulthood and finally ceases at the period of menopause.The expression of AMH in the follicular fluid is highest in pre-antral and antral follicles sized 2-9 mm, followed by the stage of follicle growth through the next stages of follicle development and is lost in the FSH-dependent stages as well as in atretic follicles. The primordial follicle pool expresses AMH, not from the dominant follicle or atresia cells. In granulosa cells the up-regulation of AMH at the mRNA levels depending on the stages of development of the oocytes. The signaling pathway for AMH has been identified in the gonads and the gonadal cell lines. The main role of AMH on the follicle is inhibition of initial follicle recruitment and reduction of FSH sensitivity on the growing follicles. Remarkably few reports refer that AMH is a predictor of fecundity in women with normal fertility. AMH levels can predict the poor response in ART and are unrelated to pregnancy outcome. Serum AMH seems to represent a good quantitative measure of the ovarian pool of the primordial follicles, however, whether it represents a quality measure is less founded. Obviously, many aspects of AMH are still unknown and needs to be further explored.[33,34,35]

Several studies have recognized the connection between antral follicle count and AMH. When the follicle-count diminishes fewer granulosa cells are available for production of AMH, which does not imply a direct relationship. As age advances there is a decrease in antral follicles and also decrease in both the levels of inhibin and sex steroids the reduced negative feedback acts in and leads to a rise in levels of serum Follicular Stimulating Hormone. Studies looking into the

relationship between FSH and AMH under hormonal stimulation in women undergoing IVF. AMH shows a is in negative relationship between serum levels AMH and FSH.However, extension of the FSH-window that occurs during such gonadotropin-stimulation implies real supra-physiological levels of FSH over a prolonged time.This, in turn, causes increased recruitment of antral follicles with FSH- induced accelerated growth that at a certain size loses their AMH expression. Hence, the AMH level must drop until a new cohort of follicles in line reach a stage where the AMH production again increases. For a limited amount of time, the amount of granulosa cells able to produce AMH is reduced [36,37,38,39].

Anti-Mullerian hormone as a marker of ovarian response in assisted reproductive techniques

ART have become a standard treatment of care in indicated cases of male and female infertility. Age and ovarian reserve help in determining the success of ART. AMH especially along with antral follicle counts (AFCs) has been shown to correlate with the number of oocytes retrieved as well as cycle cancelations. AMH helps in identifying both poor responders as well as the hyper-responders prior to initiating *in vitro* fertilization cycle [7],[8],[9]. Thus, it aids the clinicians in counseling patients and providing a realistic prognostication before embarking on an expensive and physically and emotionally taxing procedure of ART. It also helps in individualizing stimulation protocols and dosage of gonadotropins to optimize results with minimum cycle cancelations and complications, most dreaded being ovarian hyperstimulation syndrome [7],[8] .However, AMH does not

correlate well with pregnancy rates probably because it is an indicator of the quantity rather than the quality of oocytes [10].

Anti-Mullerian hormone and polycystic ovarian syndrome

PCOS is the most common cause of chronic anovulation and hyperandrogenism affecting around 5–10% of women. It is characterized by an increase in the number of ovarian follicles at all growing stages, particularly the preantral and antral follicles which are accepted to produce AMH. The levels of AMH in PCOS women are 2–4 times higher than that in the healthy women, being 75-fold higher in anovulatory PCOS and 20-fold higher in normo-ovulatory PCOS suggesting an intrinsic granulose cell dysfunction [11] .Different studies have displayed that AMH levels highly correspond with the number of follicles in polycystic ovaries and further suggested a strong positive correlation of severity of symptoms including hyperandrogenism and oligo-anovulation with the levels [12]. Thus AMH has been proposed as a surrogate marker for AFC in the diagnosis of PCOS. But this diagnostic transition has not materialized or been accepted in defining disease, as there is no uniformity and international standards in AMH lab assay. Further, the threshold of AMH across various populations worldwide has not yet been determined. One study has proposed a cutoff of 35 pmol/L (4.9 ng/mL) using the enzyme immunoassay technique by Beckmann Coulter, which needs to be validated by similar studies [13]. AMH levels, therefore, are of utmost help in deciding the treatment protocols and in defining the best strategy for ovulation induction in such patients. It can also help in assessing the response to treatment such as ovarian drilling and predict hyperstimulation. More studies are needed to clearly define these thresholds that predict response.

Anti-Mullerian hormone beyond infertility

Anti-Mullerian hormone and assessment of iatrogenic ovarian damage. The measurement of AMH levels have found utilization in assessing damage to the ovarian reserves caused by iatrogenic sources such as gonadotoxic chemotherapy, pelvic irradiation, uterine artery embolization, and ovarian surgery (cystectomy, stripping, fulguration) and may, therefore, guide in devising strategies to preserve fertility [5] . The option of cryopreservation of oocytes or embryos before chemotherapy or radiotherapy should be considered based on the AMH levels. Studies have shown declining AMH in young adults exposed to chemotherapy in childhood cancers and breast cancers [14] . In addition to reflecting post treatment ovarian damage AMH can also predict ongoing ovarian activity useful to counsel women as regards menstruation besides fertility issues. The impact of ovarian surgery (particularly for endometrioma) has been elucidated in two systematic reviews; both of which have demonstrated a decline in AMH levels post procedure indicating a significant attenuation of ovarian reserve despite surgical expertise [15],[16]. All these should be considered when planning surgery in a female desirous of future pregnancy.

PITFALLS OF ANTI-MULLERIAN HORMONE

Though AMH has emerged as a promising marker with a wide array of clinical applications, it is still shrouded with shortcomings. There is still confusion as regards the most ideal and acceptable AMH assay [21]. In the last 20 years, there has been a constant evolution of the assay from single laboratory versions, through to the more recent commercially available Diagnostic Systems Lab (DSL) and Immunotech (IOT) (also

branded as the Immunotech Beckman Coulter) assays. However, these assays utilized two different primary antibodies against AMH and different standards and consequently the crude values reported by authors and between papers can differ substantially, with the IOT assay giving values for AMH that are higher than those obtained with the DSL assay. But with the recent consolidation of these two companies by Beckman Coulter, there is finally a single commercially available assay − the AMH Generation II assay (AMH Gen II assay). Enzyme linked immunosorbent assay initially developed has evolved from IOT to the present Gen II assay. It is not recommended to adapt clinical cut-off values from the IOT assay to the Gen II assay, because a different antibody pair is used.

It is important to develop an international standard for AMH to allow harmonization of current and potential new AMH assays, thereby eliminating the need to establish assay-specific normative and cut-off values. To resolve these issues on AMH assay, currently the fully automated assay, Elecsys® AMH assay is indicated for the quantification of AMH, which (in conjunction with other clinical and laboratory findings) can help to determine ovarian reserve [22]. The assay is highly sensitive and precise, with a broad linear range, and correlates well with manual AMH assays and transvaginal sonographic assessment of AFC, but has the benefit of being less variable. The Elecsys® AMH assay also has a shorter testing time than some other AMH assays and was shown to provide a reproducible measure of ovarian reserve in the women of reproductive age in a large prospective cohort study, demonstrating its usefulness in this setting.

There is also high inter-individual variability of AMH. Ethnic variation has been shown in few studies with African-American and Hispanic women having lower levels of AMH as compared to Caucasians. In one study, it was concluded that Asians have faster

ovarian aging as compared to their European counterparts [23]. Also body mass index (BMI) may affect the ovarian reserve and subsequently the AMH levels, and a negative correlation between the two has been proposed [24]. Intra-individual variability with different AMH levels at different times has also been studied. Majority of the studies indicate that AMH levels remain relatively stable throughout the menstrual cycle, yet a recent small study found a reduction in AMH levels in the luteal phase [25]. Also the use of prolonged oral contraceptive pills or gonadotropin releasing hormone agonists leads to a reduction in AMH. In relation to pregnancy, it has been seen that AMH levels fall significantly in the second and third trimesters as compared to the first trimester.

Therefore, it is important to establish valid nomograms of AMH levels in diverse populations across the world. Jamil Z, *et al.*[26] and Nelson *et al.*[27] have created nomograms for the European and the American population, but a uniform international standard is the need of the hour. Currently, in the reproductive aged women (25–40 years), studies in terms of fertility recommend, levels of 1.0–3.0 ng/mL AMH as "normal," 0.7–0.9 ng/mL as "low normal," and 0.3–0.6 ng/mL as "low" and <0.3 ng/mL to be a "very low" range, but whether it can be extrapolated to all ethnic groups is debatable.

Potential future usage

AMH has been synthesized. Its ability to inhibit growth of tissue derived from the Müllerian ducts has raised hopes of usefulness in the treatment of a variety of medical conditions including endometriosis, adenomyosis, and uterine cancer. Research is underway in several laboratories. If there were more standardized AMH assays, it could potentially be used as a biomarker of polycystic ovary syndrome [53].

In mice, an increase in AMH has been shown to reduce the number of growing follicles and thus the overall size of the ovaries. This increase in AMH production reduces primary, secondary and antral follicles without reducing the number of primordial follicles suggesting a blockade of primordial follicle activation. This may provide a viable method of contraception which protects the ovarian reserve of oocytes during chemotherapy without extracting them from the body allowing the potential for natural reproduction later in life [54].

MATERIALS AND METHODS

STUDY DESIGN: Hospital-based observational study

STUDY SETTING : Karpaga Vinayaga Institute of Medical Sciences and Research Center – OBG OPD & IVF OPD

STUDY PERIOD : One year 2018-2019

STUDY POPULATION: females with infertility

SAMPLE SIZE: 100 Females with infertility

SAMPLING: Purposive sampling

INCLUSION CRITERIA:

1. Healthy infertile women less than 40yrs
2. Patients with ovarian causes of infertility
3. Patients who are willing to study
4. Patients with infertility of more than 2years

EXCLUSION CRITERIA

1. Patients with other acute infections like pelvic inflammatory disease, endometriosis, and history of ovarian surgery.
2. Patients who are not willing for the study.
3. Post Menopausal
4. Thyroid dysfunction
5. Cushing syndrome

ETHICAL CONSIDERATIONS

The researcher obtained the necessary approval to conduct the study Women were

explained about the purpose of this study and informed written consent was obtained, confidentiality about their results was assured. Their participation was optional.

METHODOLOGY

BLOOD SAMPLE COLLECTION AND STORAGE

About 5 ml of blood samples were drawn from the median cubital vein from each woman on day three of the cycle or progesterone-induced cycle into a plastic pyrogen-free disposable syringe. Transferred into a plastic tube and left for 20 minutes to allow it to clot. After centrifugation at 3500 rpm for 10 minutes clear serum is obtained or stored at a -20°C until use.AMH, E2, FSH, LH, Ft3, Ft4, TSH, PRL and estradiol analyses were carried out at our central clinical Laboratory.

BMI: The body mass index (BMI) was determined by weight and height calculations using the following equation:

BMI = Weight in Kg / Square of height in meters.

According to Indian guidelines, a BMI from 23 to 24.9 is overweight, if the BMI is to be greater ≥ 25 is moderate obesity, and a BMI ≥ 30 is severe obesity.

Hirsutism

Ferriman - Gallway score was used to assess the score for hirsutism, any score value >8 was considered to be positive and the presence of hirsutism is confirmed.

Ultrasonography

Ultrasound analysis was performed using a transvaginal US (TVUS) probe on each patient with a 6.5 MHz probe. In unmarried patients a transabdominal ultrasound was performed.The ultrasound measurement was then obtained by a real-time B scan and was done by a single physician according to the standardized protocol. A simple formula for

prolate ellipse is 0.5XlengthXwidthXthickness was used to calculate ovarian volume. The average of the OV of both ovaries is defined as Ovarian volume per ovary.The antral follicular count was made by a 3-dimensional view and looking for follicle size and number in each dimension.

Uterine size

Uterine diameters are measured in the sagittal plane: maximum length from cervix to fundus X maximum anteroposterior diameter to provide the cross-sectional area.

The follicle number

The follicle number, size and total volume of ovary should be considered the number of follicles per ovary or single slice of ovary in two diameters number ≥10 or≥12 or≥15 is considered polycystic. Normal ovaries do not have more than 9 follicles in number.

Size of the follicle

Follicle size 2-6, 2-8, >10mm are taken, as good discriminator between normal and polycystic ovaries.The mean diameter of ovarian volume and the number of follicles with a diameter of 2.0-8.0 mm were used for statistical analysis.

Hormone assays

In clinical practice, apart from AMH measurement, the assessment of ovarian reserve includes the following tests:

- FSH level measurement on the 3rd day of the cycle – single FSH measurement is characterized by low reliability due to its significant intra- and inter-cycle

variability. It is assumed that FSH levels > 10 IU/l indicate a reduced ovarian reserve.

- The measurement of FSH and estradiol (E_2) levels on the 3rd day of the cycle,
- Ultrasonographic determination of the number of antral follicles (AFC) sized 2-10 mm in diameter during the early follicular phase.
- The measurement of ovarian volume.

AMH is considered a good and reliable parameter in the assessment of ovarian reserve, the best of the above-mentioned ones. Its reduced levels may indicate reduced ovarian reserve, even if the woman has regular menstrual cycles and the levels of FSH and E_2 are still normal. A good correlation has been found between AMH levels and age and also between AMH levels and the number of antral follicles. The diversity of results depending on the type of laboratory test used and the absence of a universal calculation method to facilitate the comparison of results obtained via different tests is a significant limitation associated with the use of AMH as a test for ovarian reserve. The interpretation of the result and the cut-off values should be individually determined for each test.It is usually assumed that AMH values of 1 ng/ml and lower may translate into reduced ovarian reserve.

RESULTS

TABLE 1: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO THEIR AGE IN YEARS

Age_Group	Frequency	Percent
22-25 Years	10	10.0
26-29 Years	40	40.0
30-33 Years	26	26.0
Above 33 Years	24	24.0
Total	**100**	**100.0**

GRAPH 1: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO THEIR AGE IN YEARS

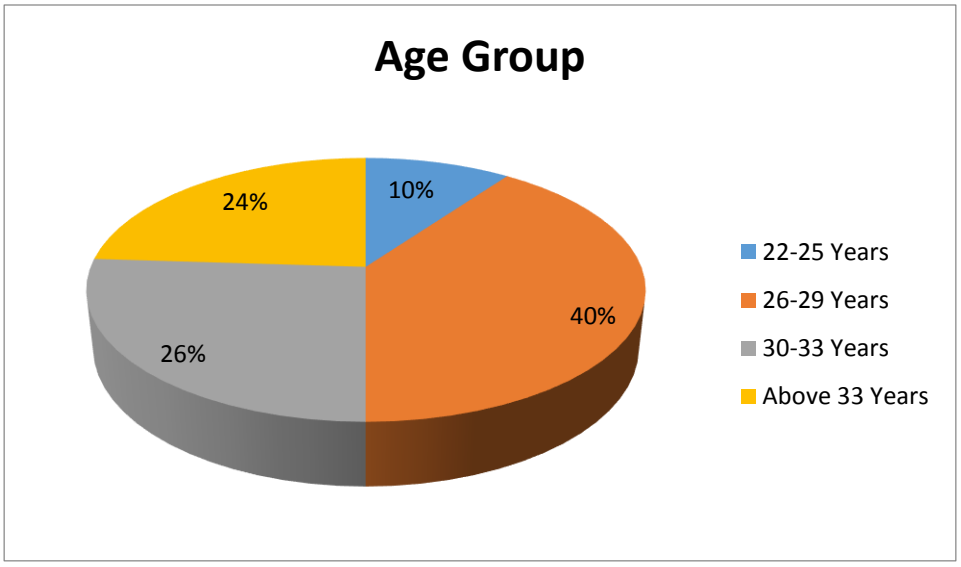


Table: 1 & Graph: 2 Totally 100 females were included in the study. 40 cases were between 26-29 years which is the majority group in our study. 24 cases were above 33 years. 6 cases were above 26 years. 10 cases were between 22-25 years.

TABLE 2: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO THEIR BMI

BMI_Class	Frequency	Percent
Normal	23	23.0
Overweight	36	36.0
Obese	41	41.0
Total	**100**	**100.0**

Table: 2 & Graph:2 23 cases were in normal BMI, 36 were overweight, 41 were obese.

GRAPH 2: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO THEIR BMI LEVEL

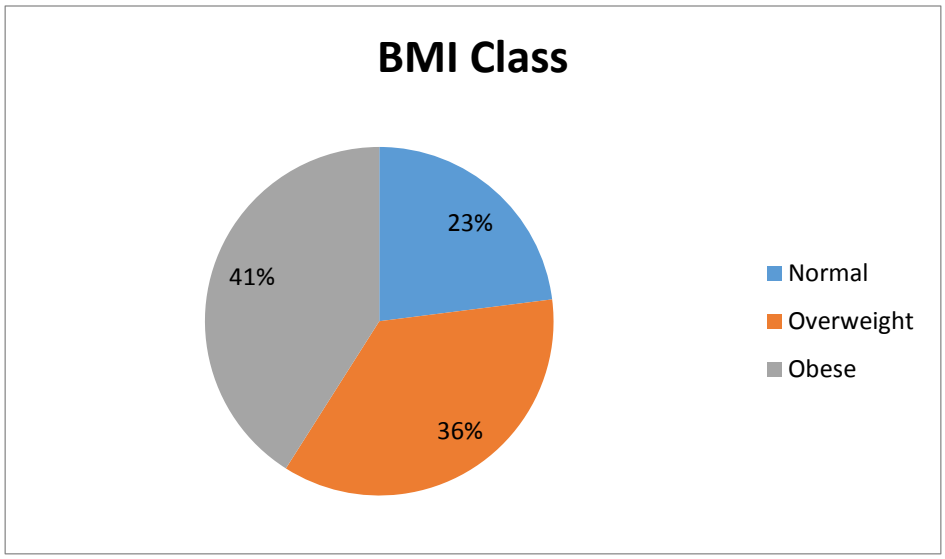


Table: 2 & Graph:2 23 cases were in normal BMI, 36 were overweight, 41 were obese.

TABLE 3: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO THEIR AMH LEVEL

AMH_Class	Frequency	Percent
<0.5 (Very Low)	4	4.0
0.5-1.0 Low	6	6.0
1.01-1.5 (Low Normal Range)	6	6.0
1.5- 4.0 (Normal)	38	38.0
Above 4.0 (High)	46	46.0
Total	**100**	**100.0**

Table :3 & graph :3 shows the AMH values differentiation among cases. 46 cases had a high level of AMH Above 4.0 (High).38 cases had 1.5- 4.0 (Normal) 6 cases had 0.5-1.0 Low levels of AMH. 6 cases had 1.01-1.5 (Low Normal Range)

GRAPH 3: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO THEIR AMH LEVEL:

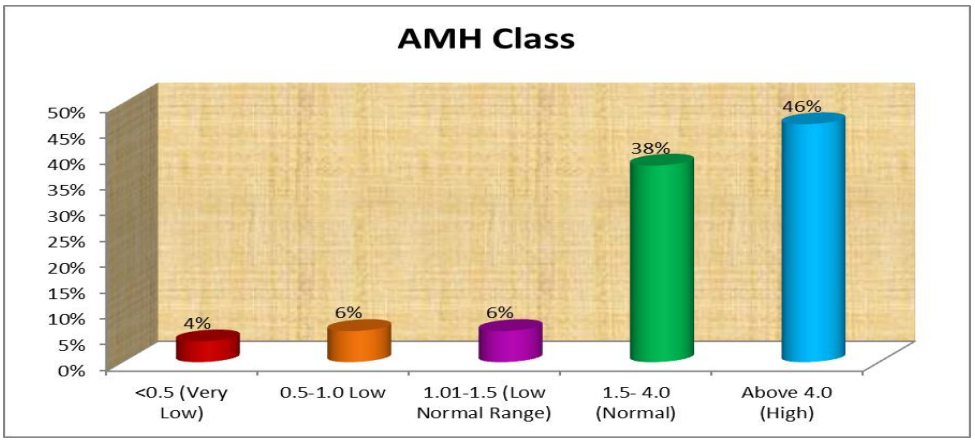


TABLE 4: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO TYPE OF INFERTILITY

Type of Infertility	Frequency	Percent
Primary	77	77.0
Secondary	23	23.0
Total	**100**	**100.0**

GRAPH 4: SHOWS FREQUENCY DISTRIBUTION OF THE CASES ACCORDING TO TYPE OF INFERTILITY :

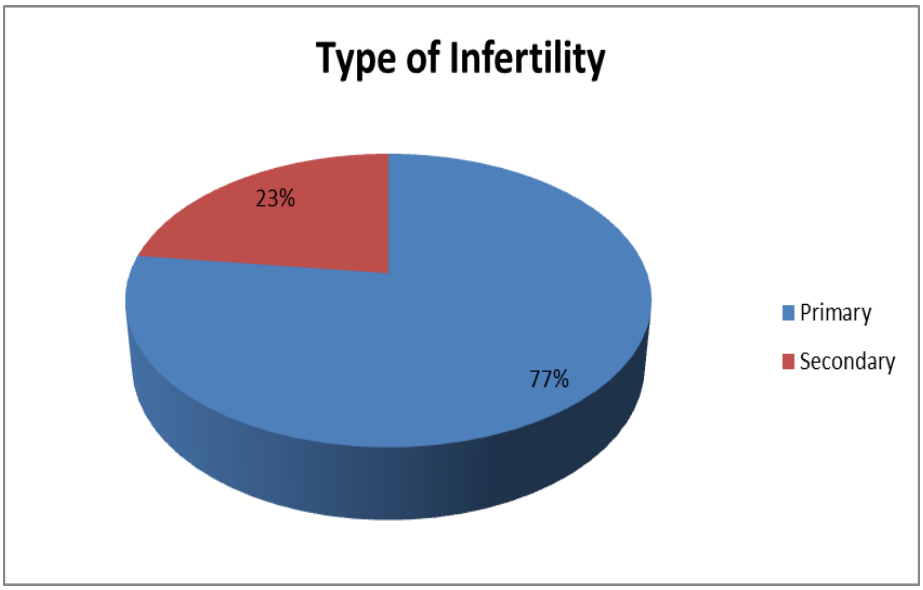


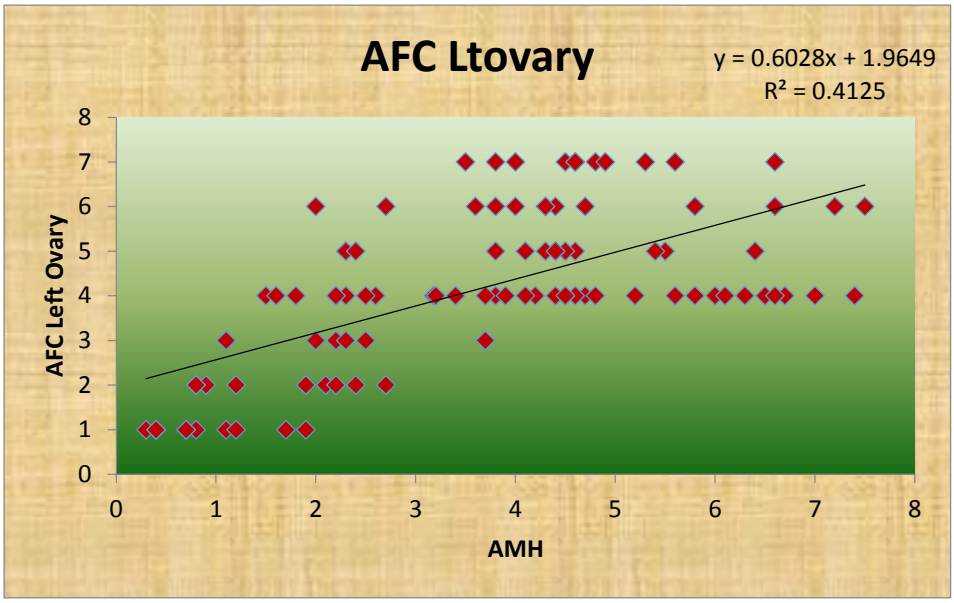
Table: 4 & graph: 4 shows In present study, 77% of patients had primary infertility while 23% of patients had secondary infertility

TABLE 5: AMH IN CORRELATION WITH OTHER HORMONES

CORRELATIONS										
		AMH	BMI	LH	FSH	TSH	Prolactin	AFC_left ovary	AFC_right _ovary	estradiol
AMH	Pearson Correlation	1	.058	.073	-031	.000	162	.642**	.619**	-.192
	Sig. (2-tailed)		.565	.469	.759	1.000	107	.000	.000	.056
	N	100	100	100	100	100	100	100	100	100
**. Correlation is significant at the 0.01 level (2-tailed).										
*. Correlation is significant at the 0.05 level (2-tailed).										

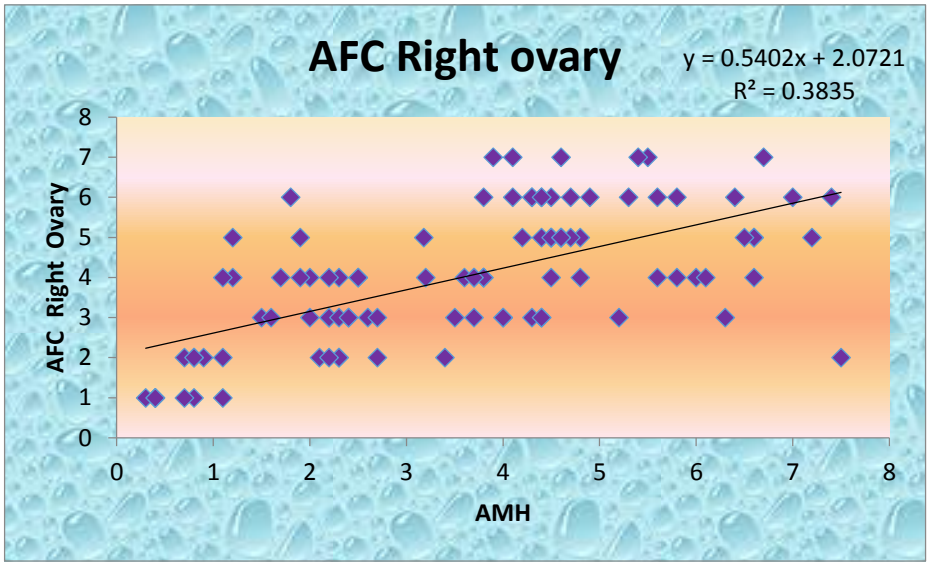
TABLE 5: Negative linear relationships with FSH levels and this correlation was not statistically significant.In subjects with PCOS, AMH levels had a negative linear relationship with FSH levels and this correlation was found to be statistically significant with AMH levels.Except AFC Left and Right Ovary None was correlated with AMH.

GRAPH 5: ANTRAL FOLLICULAR COUNT IN LEFT OVARY



Graph 5 Predictors: (Constant), estradiol, prolactin, LH, TSH, Type_of_Infertility, Age, Fsh, AMH, BMI 1.28788of p-value <0.005 which is statistically significant

GRAPH 6: ANTRAL FOLLICULAR COUNT IN RIGHT OVARY



Graph 6 Predictors: (Constant), estradiol, prolactin, LH, TSH, Type_of_Infertility, Age, Fsh, AMH, BMI 1.38491of p-value <0.005 which is statistically significant.

Linear Regression for Predicting AFC Left Ovary

Model Summary				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.661a	.437	.381	1.38491
a. Predictors: (Constant), estradiol, prolactin, Lh, Tsh, type_of_infertility, age, Fsh, Amh, Bmi				

ANOVA[a]					
Model	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Regression	134.131	9	14.903	7.770	.000[b]
Residual	172.619	90	1.918		
Total	306.750	99			
a. Dependent Variable: AFC_Ltovary					
b. Predictors: (Constant), estradiol, prolactin, Lh, Tsh, type_of_infertility, age, Fsh, Amh, Bmi					

Coefficients[a]					
Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	3.805	1.819		2.091	.039
Age	-.065	.041	-.132	-1.592	.115
Bmi	.027	.040	.058	.671	.504
Type_of_infertility	.014	.342	.003	.042	.967
Lh	.041	.113	.030	.365	.716
Fsh	-.041	.055	-.062	-.751	.455
Tsh	-.028	.153	-.015	-.180	.857
Amh	.593	.078	.632	7.569	.000
Prolactin	-.020	.024	-.072	-.850	.398
Estradiol	.000	.002	-.006	-.073	.942
a. Dependent Variable: AFC_Ltovary					

CORRELATION OF ANTRAL FOLLICULAR COUNT IN RIGHT & LEFT OVARY

1. If we fix the AMH level of 5.595 (~6) as a cut-off, sensitivity was 100% in diagnosing all cases of PCOS but 3.3% cases reported false positivity.

2. If we fix the AMH level of 5.7 as a cut-off, sensitivity was 96.7% in diagnosing cases of PCOS but 3.3% cases reported false positivity.

3. If we fix the AMH level of 5.9 as a cut-off, sensitivity was 96.7% in diagnosing cases of PCOS with a specificity of 100% (no false positives).

4. If we fix the AMH level of 6.4 as a cut-off, sensitivity was 93.3% in diagnosing cases of PCOS with a specificity of 100% (no false positives).

5. Based on the current study findings and ROC data table, the cut-off for AMH level between 5.59 to 5.9 units seems appropriate in delineating PCOS subjects because it has a high level of sensitivity and specificity.

DISCUSSION

The presumed linkage in the relationship between baseline FSH and random AMH is that both hormones are indicators of ovarian reserve. The present study was meant to infer the variability of AMH during the menstrual cycle and that, if there is a direct feedback mechanism between these two hormones; rather, to find a relationship between their levels and with AFC, and we believe that they are independent indicators of ovarian reserve.

Baseline FSH level increases in infertility and our study also shows mean FSH level is 9.1 ± 2.51 mIU/ml, which is on the higher side. These Baseline FSH levels have for many years been used to predict a patient's response to ovulation induction and success with IVF. [17] Determinations of FSH, however, are characterized by many difficulties. One quite obvious problem is the inconvenience of a required blood draw on the day 2 or 3 of menses. The second issue of concern is the degree of cycle-to-cycle fluctuation in baseline FSH levels, at least partially caused by the dependency of FSH levels on the negative feedback from E2 levels. [2]

Anti-Müllerian hormone does not exhibit these difficulties. It is relatively stable throughout the cycle [11],[18],[19],[20] and therefore can be drawn at random. This is also evident by our study results which show AMH levels are not statistically different on day 3 and day 14 of the menstrual cycle. This makes serum AMH a more reliable test, as it is not constrained to a time frame for measurement. It is also said not to be affected by other hormonal variations, including the use of oral contraceptives. [21],[22]

In our study, we have noted a significant inverse correlation between serum AMH and subsequent baseline FSH on day 3 within the same menstrual cycle ($r = -0.488$, $P <$

0.001), which is similar to as previously noted in some other studies. [23] Serum FSH level on the third day of the menstrual cycle ensures the greatest accuracy possible. [2] AMH null mice with low FSH levels was seen to present with a large number of growing follicles. This fact led to the hypothesis that, in the absence of AMH, follicles show a tendency to become more sensitive to FSH action. [24] Further, it has been reported that FSH and E2 down-regulate AMH gene expression in GC of rat follicles. [25] Thus, serum AMH level can, therefore, be used as a reliable biomarker of ovarian reserve (similar to serum FSH levels). [26] Another study similar to our study also reported that serum AMH concentration shows a negative linear correlation with basal FSH levels in women who have a poor response to controlled ovarian stimulation with human gonadotropins. [27]

Ovarian reserve is a complex clinical phenomenon influenced by age, genetics, and environmental variables. The decline in a woman's ovarian reserve with time is irreversible and the rate at which women lose primordial follicles varies considerably, with wide variation regarding the onset of sterility and timing of the menopausal transition [12].

Although it is challenging to predict the rate of decline of individual's ovarian reserve, clinicians are often asked for advice about fertility potential and/or recommendations regarding the pursuit of fertility treatment options. Over the past few years, there have been several comprehensive reviews on ovarian reserve tests that focused mainly on ovarian response prediction in the context of assisted reproductive technology (ART) [13,14].

In women anti-Müllerian hormone (AMH) is produced in the ovary by granulosa cells of antral follicles. Then, it is released into the follicular fluid and blood vessels. In clinical practice, its levels are measured in peripheral blood.This hormone plays a

significant role in the development of reproductive organs in both sexes during the embryonic period. In an adult woman its role probably consists in the regulation of folliculogenesis, predominantly in the mechanism of inhibiting primordial follicle recruitment and decreasing the sensitivity of small antral follicles to follicle-stimulating hormone (FSH) activity [23, 24].

To date, the main clinical application of AMH determination in women has been the assessment of ovarian reserve in the diagnosis of infertility, premature ovarian failure, and hypogonadotropic hypogonadism. Recently published studies highlighted the value of AMH determination in polycystic ovary syndrome (PCOS). Seemingly, AMH may also be a very useful predictive marker of the time of menopause [25, 26].

The use of early follicular phase (basal) FSH as a marker of the ovarian reserve was proposed almost 30 years ago, as a tool to predict ovarian response to in vitro fertilization (IVF). This test is an indirect assessment of ovarian reserve and is based on the feedback inhibition of FSH pituitary secretion by ovarian factors [27, 28].

At the beginning of the menstrual cycle, estradiol and inhibin B levels reach a nadir, offering a glimpse of the unsuppressed hypothalamus-pituitary-ovarian axis before levels of these ovarian hormones rise and inhibit FSH secretion. Women with normal ovarian reserve have sufficient production of ovarian hormones at this early stage of the menstrual cycle to maintain FSH levels within normal range. In contrast, elevation of FSH at this stage of the menstrual cycle indicates poor production of ovarian hormones by diminishing the ovarian follicular pool consistent with DOR. [31,32,33].

However, basal FSH testing has several major limitations including significant intercycle and intracycle variability that limits its reliability, it requires a functional hypothalamus-pituitary ovarian axis, and it is not adequately sensitive for clinical utility only elevations carrying significance [54, 55].

The latter limitation is the reason that the basal FSH test must be combined with estradiol which enhances the sensitivity of early follicular FSH testing. Combining basal FSH with estradiol is more meaningful since even normal-range FSH can imply DOR in the setting of elevated basal estradiol. In women with declining ovarian reserve, premature elevations in FSH in the early follicular phase drive estradiol levels higher, which, in turn, may lead to increased negative feedback on pituitary FSH production thus masking abnormal FSH elevation, which would otherwise reveal DOR [23, 19].

Measurement of both FSH and estradiol on cycle day 3 may, therefore, help decrease the incidence of false-negative testing. Despite its limitations, FSH is commonly used as an ovarian reserve test, and high values have been associated with both poor ovarian response and failure to achieve pregnancy. FSH has particularly high specificity (45-100%) for predicting poor response to ovarian stimulation (usually defined as 4 retrieved oocytes) using multiple cut-off points >10 IU/L (10-20 IU/L), but its sensitivity is generally poor (11-86%) and decreases with increasing FSH cut-off points. In terms of predicting failure to conceive, FSH testing is still specific (50-100%) but much less sensitive (3-65%) using similar cut-offs.This test is still clinically useful since an abnormally elevated FSH result is almost synonymous with late DOR (high positive predictive value), but the majority of women who are tested (including those with DOR) will have a normal test result (low negative predictive value). Moreover, a

single abnormal FSH value in a woman.A gradual increase in AMH levels is observed in girls from the first day of life, with maximum levels observed in women at around the age of 25. [14, 15]. After puberty AMH is produced by granulosa cells of primary, preantral and small antral (diameter: 2-4 mm) follicles, in which the highest levels of AMH were reported. AMH expression is not found in atretic follicles. In an adult woman AMH levels gradually decrease until they reach values below detectable limits in postmenopausal women [16,17].

AMH is considered a stable hormone as regards its levels over a menstrual cycle and demonstrates low variability in subsequent cycles, as it is a marker of non-cyclic ovarian sensitivity. According to studies on AMH fluctuations, the lowest levels were observed during the very early luteal phase, directly after ovulation. These findings were reported with regard to young women. In the case of older women, the range of AMH level fluctuation over a menstrual cycle was very low [55, 56, 57].

In clinical practice, apart from AMH measurement, the assessment of ovarian reserve includes the following tests:

- FSH level measurement on the 3rd day of the cycle – single FSH measurement is characterized by low reliability due to its significant intra- and inter-cycle variability. It is assumed that FSH levels > 10 IU/l indicate a reduced ovarian reserve.
- The measurement of FSH and estradiol (E_2) levels on the 3rd day of the cycle.
- Ultrasonographic determination of the number of antral follicles (AFC) sized 2-10 mm in diameter during the early follicular phase.

- The measurement of ovarian volume.

AMH is considered a good and reliable parameter in the assessment of ovarian reserve, the best of the above-mentioned ones. Its reduced levels may indicate reduced ovarian reserve, even if the woman has regular menstrual cycles and the levels of FSH and E_2 are still normal. A good correlation has been found between AMH with age and also between AMH levels and the number of antral follicles. The diversity of results depending on the type of laboratory test used and the absence of a universal calculation method to facilitate the comparison of results obtained via different tests is a significant limitation associated with the use of AMH as a test for ovarian reserve. [58, 59]. The interpretation of the result and the cut-off values should be individually determined for each test. It is usually assumed that AMH values of 1 ng/ml and lower may translate into reduced ovarian reserve.

The age of 51 is the average time of menopause, defined as the last menstrual period in a woman's life. Physiological menopause may occur between 40 and 60 years of age, but the decrease in the natural fertility of women starts 10-13 years prior to menopause. It was estimated that during perimenopause the number of ovarian follicles decreases below several thousand and the progression towards menopause and onwards is characterized by a very low number of follicles – estimated to be below 1000. It is suggested that the age of menopause is highly heritable [41,42,43].

Research is conducted to find factors, including biochemical and hormonal markers, which would facilitate the determination of the period of fertility and the time of menopause in women. The determination of predictors of the period of fertility in women is gaining importance because of society's aging and later motherhood. Another problem

which is tackled is better prophylaxis as regards diseases which occur significantly more commonly after menopause. These include osteoporosis, cardiovascular disorders and also hormone-related neoplasms, e.g. breast cancer and endometrial cancer. Parameters that indirectly indicate the time of menopause are the ovarian volume and the number of antral follicles, which is correlated with AMH levels [45,46]. As regards hormonal factors, the determination of FSH and inhibin B was found valuable, but the predictive value was lower than AMH because its levels decreased earlier than in the case of the above-mentioned hormones [47, 48].

Several authors reported that AMH determination was a valuable predictor of menopause. It is considered that the level of AMH is very low, or even below detectable limits approximately 5 years before menopause. According to some research the time of menopause may be predicted via a mathematical model on the basis of a single AMH measurement and the age of the patient. The obvious value of this study is the age of study group participants (20-49 years), which enables the implementation of the model in very young women and predicts the time of menopause even several decades earlier. The authors observed an even reduction in AMH levels in all age groups over 6 years of observation of the study cohort. Beyond the age of 38 years the control women had very low serum AMH levels than with women with PCOS. AMH levels were not statistically significant with age and showed a negative relationship. As age increases in control subjects, AMH level tend to decrease [49, 50].

Seifer DB *et al.* also demonstrated that AMH measurement and the use of a statistical model precisely predicted the time of menopause. However, no young women were included in their study [51]. Sharara FI *et al.* reported that the levels of AMH < 0.2 ng/ml

occurred on average 5.99 years prior to menopause in women aged 45-48 years and 9.94 years in women aged 35-39. As regards AMH values over 1.5 ng/ml, menopause occurred on average after 6.23 years in the older group and after more than 13 years in the younger group [52]. A prospective study by Tal R, *et al.* included over 250 women who underwent tests at baseline (age 21-46) and after 11 years. It was demonstrated that the time of menopause may be precisely determined on the basis of AMH level and the patient's age. Totally 100 females were included in the study. 40 cases were between 26-29 years which is the majority group in our study. 24 cases were above 33 years. 6 cases were above 26 years. 10 cases were between 22-25 years [55,56].

Kwee J, et al found that even after correcting for chronological age, two of the tests, the ovarian AFC and serum levels of AMH, were still significantly correlated with the number of ovarian primordial follicle. In addition, approximately 74% of the variation in ovarian primordial follicle count could be explained with only two of the parameters, chronological age, and the ovarian AFC [34].

Majumder K et al found that plasma AMH levels remained relatively static (20–25 pmol/L) from 18 to 29 years of age. By 30 years of age, plasma AMH levels start to drop rapidly, reaching only10 pmol/L by 37 years [39].

In the present study, 77% of patients had primary infertility while 22% of patients had secondary infertility. 93% of patients had their first cycle of ICSI, 3% of patients had their second cycle of ICSI, 2% of patients had third cycle of ICSI and 2% of patients had their fourth cycle of ICSI. In present study, 36% patients had normal AMH, 18% patients were in low normal range, 5% patients had low values and 2% patients had very low values. 41% of patients had values in high range suggestive of PCOS. Amongst this, 21%

had values between 4 to 8 ng/ml where we got good AFC count and good result in terms of pregnancy [40,41].

Among all ovarian reserve tests, AMH is considered the earliest and most sensitive. It correlates strongly with the primordial follicle pool, has an inverse correlation with chronologic age,35,36 reliably predicts ovarian response in ART[37,38] and is predictive of the timing of the onset of menopause 39-41 years . In a systematic review of studies in women undergoing controlled ovarian stimulation with gonadotropins, low AMH cutoff points (0.1-1.66 ng/mL) have been found to have sensitivities ranging between 44-97% and specificities ranging between 41-100% for prediction of poor ovarian response.4 In a meta-analysis that included 28 studies, AMH was found to have good predictive ability for poor ovarian response, with an area under the curve (AUC) of 0.78.42 Moreover, AMH has remarkable utility in predicting ovarian hyperstimulation to gonadotropin stimulation, with sensitivities ranging from 53-90.5% and specificities ranging from 70-94.9% when using cut-off values of 3.36- 5.0 ng/mL.4 However, despite its strong correlation with ovarian response to stimulation in ART, AMH is a poor predictor of nonpregnancy with sensitivities between 19-66%, and specificities between 55-89% when using cut-offs ranging [45,46].

AMH levels also had a positive linear relationship with combined ovarian volume but this correlation was not statistically significant. In subjects with PCOS, AMH levels had a positive linear relationship with combined ovarian volume and this correlation was statistically significant.While AMH levels in control subjects had a positive and a linear relationship with E_2 levels but this was also not statistically significant In subjects with

PCOS, AMH levels had a negative linear relationship with E_2 levels but this was also not statistically significant [50,51].

Ovarian reserve is very crucial part in predicting assisted reproductive treatment, a combination of clinical endocrinal and ultrasonic measures is used for this (55), and our study was designed to see whether or not ultrasound can replace the endocrinal ways in follow-up. In our study there was marked decrease in ovarian reserve reflected on both hormonal and ultrasonic measurements, this goes with who reported decrease in ovarian Antral follicle count after cancer therapies [56], [57]. Cancer itself can impair ovarian reserve. Hormone and ultrasound measures of ovarian reserve suggest decreased underlying ovarian reserve comparing before and after therapy [58]. So we used this decline in function to measure the percentage of change and whether this decline reflected on ultrasound parameter as well as hormones in the same percentage.

Anti-Müllerian Hormone (AMH) is a very sensitive indicator of the ovarian follicular content. Müllerian hormone is the most informative serum marker of ovarian reserve currently available and should be considered an important part of any contemporary reproductive medicine practice. It is more convenient and informative than basal FSH and can be assessed at any point in the cycle. It is the most useful serum method of determining ovarian reserve, which guides pretreatment counseling, choice of infertility treatment, and avoidance of ovarian hyperstimulation [8,11,29].

Ovarian reserve refers to the pool of fertilizable eggs that remain within the ovary, a pool that naturally declines with age. It has long been recognized that as women approach menopause, low anti-Müllerian hormone (AMH) levels decrease and follicle-stimulating hormone levels increase. However, the significance of these biomarkers in predicting

conception in both fertile and infertile women is unknown. Despite this uncertainty, AMH has gained popularity among OB/GYNs and fertility specialists as a "fertility test." It is not uncommon to see a new patient who is scared and confused about her potential to have a child based on the finding of a low AMH.

This is the first large, well-designed prospective analysis of the significance of ovarian reserve markers in predicting pregnancy in women aged 30 to 44 years without a prior infertility diagnosis. Women who were enrolled had just begun to try naturally for pregnancy within the last 3 months. Interestingly, the researchers found that low ovarian reserve/low AMH does not predict fecundability — monthly chance of conceiving — even in women aged up to 44 years. This lack of effect was noted for up to 12 months of trying to conceive.

CONCULSION

1. A single AMH measurement is more valuable in the assessment of ovarian reserve than measuring estradiol, FSH or inhibin B.
2. Compared to female age and FSH alone, AMH has a superior role in projecting accurate antral follicle pool, especially in setups where technological expertise to assess AFC is not available.
3. The determination of AMH levels appears to be a good and reliable parameter which may accelerate the development of such a model.
4. Incorporation of AMH along with other biomarkers constitutes a better model for the prediction of ovarian response. Further prospective studies are required to ascertain its role in predicting the outcomes of ART in such patients.
5. When basal AMH levels and the number of antral follicles were correlated, high AMH levels correlate with low cancellation rates, retrieval of more eggs, higher live birth rates and a high chance for freezing of embryos.
6. Low AMH levels (alone) do not predict low success rates in women less than 35 years of age.
7. Couples should not be excluded from attempting assisted reproductive cycles due to low AMH values alone because live birth success rates were reasonable in these cases.
8. Measurement of AMH can provide a high specificity and sensitivity (92% and 67% respectively) by which it can act as a marker for PCOS.

9. The determination of the predictors of the time of menopause and developing a model which would facilitate the precise prediction of the time of menopause in a particular woman is still a challenge
10. In situations where accurate ultrasonography data are not available this based on the above findings can be proposed that, in the diagnostic criterion for PCOS than the follicular count.
11. The ideal ovarian reserve test should be reproducible, with limited inter- and intra-cycle variability, and demonstrate high specificity to minimize the risk for incorrectly categorizing women as having decreased ovarian reserve.
12. Composite measures that incorporate both methods could potentially be used to provide a comprehensive assessment of ovarian reserve, although AMH has been shown to be a better predictor of oocyte yield in patients with discordant AFC and AMH results.
13. Further research is necessary, including population studies, which will help to specify reference values, standardize AMH measurement and develop a reliable model to calculate the time of menopause. However, it seems that AMH measurement will be the most important element in this model.

LIMITATIONS OF THE STUDY

This study has several limitations. First, conception, not live birth, was the primary outcome. Fecundity, the capacity to reproduce, is comprised of both the ability to conceive and to carry a fetus to viability. Diminished ovarian reserve could affect fecundity by increasing the risk of miscarriage- perhaps through an effect on egg quality. Prior studies to date have failed to show such an association.25,26 Second, not all women remained in the study for 12 cycles of attempt. This was anticipated, given the older reproductive-age cohort.

Current recommendations advise women over the age of 35 to obtain an infertility evaluation after 6 months of attempt. The median attempt cycle at which women started infertility treatment in the study was 8 cycles. For this reason conception by 6 cycles of attempt was calculated and Cox models, which allow participants who initiate fertility medications to contribute time to the analysis until they are censored for their fertility medication use, were constructed. Third, ovulation was not assessed. This information would have allowed us to evaluate the strictest definition of fecundability (the probability of conceiving in a given *ovulatory* menstrual cycle). Fourth, male partners did not provide a semen sample for analysis. However, there is no reason to think that women with diminished ovarian reserve would be more or less likely to be partnered with a male with abnormal semen parameters. Fifth, not all women were enrolled in their first, second, or third cycle of attempt; however, when the <10% of women who entered after their third cycle of attempt were excluded, the findings did not differ. Sixth, while various AMH cut-off values were explored, the study was not powered to look at very low (0.1ng/ml or lower) AMH values, which reflect diminished ovarian reserve more consistent with the late

perimenopause transition. It is possible that in such advanced stages, fecundability may be affected, especially if it results in frequent anovulation.

SUMMARY

1. Ovarian reserve is defined as the functional potential of the ovary and reflects the number and quality of the oocytes in the ovary at any given time.Although such tests are frequently labeled ovarian reserve tests, they are more accurately ovarian response tests.
2. Anti-Müllerian hormone (AMH) is produced by the granulosa cells of preantral and small antral follicles and its levels can be assessed in serum. Since the number of ovarian follicles declines with increasing age, AMH levels might be used as a marker for ovarian aging
3. To study the level of AMH in infertility patients and to assess the levels of ovarian reserve.
4. This Hospital-based observational study was conducted in Karpaga Vinayaga Institute of Medical Sciences and Research Center – OBG OPD & IVF OPD from 2018-2019.100 cases were included.
5. Totally 100 females were included in the study. 40 cases were between 26-29 years which is the majority group in our study. 24 cases were above 33 years. 6 cases were above 26 years. 10 cases were between 22-25 years.
6. 23 cases were in normal BMI, 36 were overweight, 41 were obese.AMH values differentiation among cases.
7. 46 cases had a high level of AMH Above 4.0 (High).38 cases had 1.5- 4.0 (Normal) 6 cases had 0.5-1.0 Low levels of AMH. 6 cases had 1.01-1.5 (Low Normal Range)
8. In present study, 77% of patients had primary infertility while 23% of patients had secondary infertility.

9. Negative linear relationships with FSH levels and this correlation was not statistically significant.In subjects with PCOS, AMH levels had a negative linear relationship with FSH levels and this correlation was found to be statistically significant with AMH levels.
10. Except AFC Left and Right Ovary None was correlated with AMH.
11. Predictors: A (Constant), estradiol, prolactin, LH, TSH, Type_of_Infertility, Age, Fsh, AMH, BMI 1.28788of p-value <0.005 which is statistically significant
12. Predictors: B (Constant), estradiol, prolactin, LH, TSH, Type_of_Infertility, Age, Fsh, AMH, BMI 1.38491of p-value <0.005 which is statistically significant
13. If we fix the AMH level of 5.595 (~6) as a cut-off, sensitivity was 100% in diagnosing all cases of PCOS but 3.3% cases reported false positivity.
14. If we fix the AMH level of 5.7 as a cut-off, sensitivity was 96.7% in diagnosing cases of PCOS but 3.3% cases reported false positivity.
15. If we fix the AMH level of 5.9 as a cut-off, sensitivity was 96.7% in diagnosing cases of PCOS with a specificity of 100% (no false positives).
16. If we fix the AMH level of 6.4 as a cut-off, sensitivity was 93.3% in diagnosing cases of PCOS with a specificity of 100% (no false positives).
17. Based on the current study findings and ROC data table, the cut-off for AMH level between 5.59 to 5.9 units seems appropriate in delineating PCOS subjects and control subjects as it has a high level of sensitivity and specificity.
18. The ideal ovarian reserve test should be reproducible, with limited inter- and intra-cycle variability, and demonstrate high specificity to minimize the risk for incorrectly categorizing women as having decreased ovarian reserve.

19. Composite measures that incorporate both methods could potentially be used to provide a comprehensive assessment of ovarian reserve, although AMH has been shown to be a better predictor of oocyte yield in patients with discordant AFC and AMH results
20. Further research is necessary, including population studies, which will help to specify reference values, standardize AMH measurement and develop a reliable model to calculate the time of menopause. However, it seems that AMH measurement will be the most important element in this model.

Bibilography

1. Abdul H Zargar,Vipin K Gupta,Arshad I Wani et.al.Presence of ultrasonography proved polycystic ovaries in north Indian women with type 2 diabetes mellitus.Reproductive biology and endocrinology .2005,3:35.pg.1-7.
2. Alexandra L.L.Durlinger ,Jenny A.Visser ,Axel P.N.Themmen.Regulation of ovarian function :the role of anti-Mullerian hormone.2002.Reproduction 124,601-609.
3. Amelia P.Baliey,Leah.K.Hawkins.A.Missmer.et.al.Effect of body mass index in vitro fertilization outcomes in women with polycystic ovary syndrome.2014.Am J Obstet Gynecol;211:163,e1-6.
4. Antonio La Marca,Stefania Malmusi,Simone Giulini.Anti-Mullerian hormone plasma levels in spontaneous menstrual cycle and during treatment with FSH to induce ovulation .2004.Human Reproduction .Vol 19,No.12 pp.2738-2741.
5. Behringer RR, Finegold MJ, Cate RL. Mullerian-inhibiting substance function during mammalian sexual development. Cell 1994;79: 415-25
6. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: Practical recommendations for better standardization. Fertil Steril. 2010;94:1044–51
7. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685-718.
8. Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014;20: 688-701.
9. Broer SL, Eijkemans MJ, Scheffer GJ, et al. Anti-mullerian hormone predicts

menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab 2011;96: 2532-9.

10. Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimüllerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril 2009;91:705-14.

11. Broer SL, van Disseldorp J, Broeze KA, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update 2013;19:26-36.

12. Coccia ME, Rizzello F. Ovarian reserve. Ann N Y Acad Sci. 2008;1127:27–30.

13. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 2001;142:4891-9.

14. Durlinger AL, Kramer P, Karels B, et al. Control of primordial follicle recruitment by antimullerian hormone in the mouse ovary. Endocrinology 1999;140:5789-96

15. El-Toukhy T, Khalaf Y, Hart R, Taylor A, Braude P. Young age does not protect against the adverse effects of reduced ovarian reserve – an eight year study. Hum Reprod. 2002;17:1519–24

16. Esposito MA, Coutifaris C, Barnhart KT. A moderately elevated day 3 FSH concentration has limited predictive value, especially in younger women. Hum Reprod 2002;17:118-23.

17. Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J. High reproducibility of serum anti-mullerian hormone measurements suggests a multi-staged follicular secretion

and strengthens its role in the assessment of ovarian follicular status. Hum Reprod 2005;20: 923-7.

18. Freeman EW, Sammel MD, Lin H, Boorman DW, Gracia CR. Contribution of the rate of change of antimüllerian hormone in estimating time to menopause for late reproductiveage women. Fertil Steril 2012;98:1254-9.
19. Freeman EW, Sammel MD, Lin H, Gracia CR. Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab 2012;97:1673-80.
20. Garcia-Velasco JA, Moreno L, Pacheco A, et al. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. Fertil Steril 2005;84:82-7.
21. Grossman MP, Nakajima ST, Fallat ME, Siow Y. Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. Fertil Steril 2008;89(Suppl):1364-70.
22. Hadlow N, Longhurst K, McClements A, Natalwala J, Brown SJ, Matson PL. Variation in antimüllerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response. Fertil Steril 2013;99:1791-7.
23. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 2011;95:170-5.
24. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 2006;91:4057-63.
25. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of

anti-müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update. 2015;21:698–710.

26. Iliodromiti S, Nelson SM. Ovarian response biomarkers: physiology and performance. Curr Opin Obstet Gynecol 2015;27:182-6.

27. Jamil Z, Fatima SS, Ahmed K, Malik R. Anti-mullerian hormone: Above and beyond conventional ovarian reserve markers. Dis Markers 2016. 2016:5246217.

28. Jayaprakasan K, Campbell B, Hopkisson J, Clewes J, Johnson I, RaineFenning N. Establishing the intercycle variability of three-dimensional ultrasonographic predictors of ovarian reserve. Fertil Steril 2008;90:2126-32.

29. Kalaiselvi V, Saikumar P, Prabhu K, Krishna G. The anti mullerian hormone – A novel marker for assessing the ovarian reserve in women with regular menstrual cycles. J Clin Diagn Res. 2012;6:1636–9.

30. Kelsey TW, Anderson RA, Wright P, Nelson SM, Wallace WH. Data-driven assessment of the human ovarian reserve. Mol Hum Reprod 2012;18:79-87.

31. Kissell KA, Danaher MR, Schisterman EF, et al. Biological variability in serum anti-mullerian hormone throughout the menstrual cycle in ovulatory and sporadic anovulatory cycles in eumenorrheic women. Hum Reprod 2014;29: 1764-72.

32. Köninger A, Koch L, Edimiris P, Enekwe A, Nagarajah J, Kasimir-Bauer S, et al. Anti-mullerian hormone: An indicator for the severity of polycystic ovarian syndrome. Arch Gynecol Obstet. 2014;290:1023–30.

33. Kunt C, Ozaksit G, Keskin Kurt R, Cakir Gungor AN, Kanat-Pektas M, Kilic S, et al. Anti-mullerian hormone is a better marker than inhibin B, follicle stimulating hormone,

estradiol or antral follicle count in predicting the outcome of *in vitro* fertilization. Arch Gynecol Obstet. 2011;283:1415–21.

34. Kwee J, Schats R, McDonnell J, Lambalk CB, Schoemaker J. Intercycle variability of ovarian reserve tests: results of a prospective randomized study. Hum Reprod 2004;19:590-5.

35. La Marca A, Sighinolfi G, Radi D, et al. Antimullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16:113-30.

36. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006;21:3103-7.

37. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice. Hum Reprod Update. 2014;20:124–40.

38. Lee TH, Liu CH, Huang CC, Hsieh KC, Lin PM, Lee MS. Impact of female age and male infertility on ovarian reserve markers to predict outcome of assisted reproduction technology cycles. Reprod Biol Endocrinol. 2009;7:100.

39. Majumder K, Gelbaya TA, Laing I, Nardo LG. The use of anti-mullerian hormone and antral follicle count to predict the potential of oocytes and embryos. Eur J Obstet Gynecol Reprod Biol 2010;150:166-70.

40. Muasher SJ, Oehninger S, Simonetti S, et al. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. Fertil Steril 1988;50:298-307.

41. Nelson SM, La Marca A. The journey from the old to the new AMH assay: how to avoid getting lost in the values. Reprod Biomed Online 2011;23:411-20.
42. Nelson SM, Pastuszek E, Kloss G, et al. Two new automated, compared with two enzymelinked immunosorbent, antimüllerian hormone assays. Fertil Steril 2015;104:1016-21.e6.
43. Nelson SM. Biomarkers of ovarian response: Current and future applications. Fertil Steril. 2013;99:963–9.
44. Overbeek A, Broekmans FJ, Hehenkamp WJ, et al. Intra-cycle fluctuations of anti-mullerian hormone in normal women with a regular cycle: a re-analysis. Reprod Biomed Online 2012;24:664-9.
45. Peluso C, Fonseca FL, Rodart IF, Cavalcanti V, Gastaldo G, Christofolini DM, et al. AMH: An ovarian reserve biomarker in assisted reproduction. Clin Chim Acta. 2014;437:175–82.
46. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril 2015;103: e9-17.
47. Roberts JE, Spandorfer S, Fasouliotis SJ, Kashyap S, Rosenwaks Z. Taking a basal follicle-stimulating hormone history is essential before initiating in vitro fertilization. Fertil Steril 2005;83:37-41.
48. Scott RT Jr, Hofmann GE, Oehninger S, Muasher SJ. Intercycle variability of day 3 folliclestimulating hormone levels and its effect on stimulation quality in in vitro fertilization. Fertil Steril 1990;54:297-302.

49. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. Fertil Steril 1989;51:651-4.

50. Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril 2002;77:468-71.

51. Seifer DB, MacLaughlin DT, Penzias AS, et al. Gonadotropin-releasing hormone agonistinduced differences in granulosa cell cycle kinetics are associated with alterations in follicular fluid mullerian-inhibiting substance and androgen content. J Clin Endocrinol Metab 1993;76:711-4.

52. Sharara FI, Scott RT Jr, Seifer DB. The detection of diminished ovarian reserve in infertile women. Am J Obstet Gynecol 1998;179: 804-12.

53. Sowers M, McConnell D, Gast K, et al. Antimullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril 2010;94:1482-6.

54. Tal R, Seifer DB. Potential mechanisms for racial and ethnic differences in antimüllerian hormone and ovarian reserve. Int J Endocrinol 2013;2013:818912.

55. Tal R, Tal O, Seifer BJ, Seifer DB. Antimüllerian hormone as predictor of implantation and clinical pregnancy after assisted conception: a systematic review and meta-analysis. Fertil Steril 2015;103:119-30.e3.

56. Toner JP, Philput CB, Jones GS, Muasher SJ. Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril 1991;55:784-91.

57. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: A sea change in determining ovarian reserve using antimüllerian hormone. Fertil Steril. 2013;99:1825–30.

58. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimüllerian hormone. Fertil Steril 2013;99: 1825-30.

59. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-mullerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod 2007;22:1837-40.

60. Ubaldi F, Vaiarelli A, D'Anna R, Rienzi L. Management of poor responders in IVF: Is there anything new? Biomed Res Int 2014. 2014:352098.

61. van Disseldorp J, Lambalk CB, Kwee J, et al. Comparison of inter- and intra-cycle variability of anti-mullerian hormone and antral follicle counts. Hum Reprod 2010;25:221-7.

62. van Helden J, Weiskirchen R. Performance of the two new fully automated anti-mullerian hormone immunoassays compared with the clinical standard assay. Hum Reprod 2015;30: 1918-26

63. Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002;8:141–54. [

64. Wunder DM, Bersinger NA, Yared M, Kretschmer R, Birkhauser MH. Statistically significant changes of antimüllerian hormone and inhibin levels during the physiologic menstrual cycle in reproductive age women. Fertil Steril 2008;89:927-33.

65. Yates AP, Rustamov O, Roberts SA, et al. Anti-mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod 2011;26:2353-62.
66. Zarek SM, Mitchell EM, Sjaarda LA, et al. Is anti-mullerian hormone associated with fecundability? Findings from the EAGeR trial. J Clin Endocrinol Metab 2015;100:4215-21.

PROFORMA

Name of patient : Name of husband:

Age : Age:

Occupation: Occupation:

Address:

Married for : Trying to conceive for:

Living together for: Undergoing treatment for:

Type of previous treatment:

MENSTRUAL HISTORY: Regularity: Flow: Associated with dysmenorrhoea previous use of contraception/ pelvic / pelvic surgery /previous pregnancy if any – describe

MARITAL& SEXUAL HISTORY: Awareness on fertile period/ coital frequency/timing/dyspareunia/coital problems.

Excessive intake of coffee/tea Allergies Vigorous exercise Medications Exposure to chemicals

PAST MEDICAL HISTORY: Diabetes/Hypertension/Hepatitis/Tuberculosis/others

PAST SURGICAL HISTORY:

FAMILY HISTORY: Relevant

GENERAL PHYSICAL EXAMINATION

Height:

Weight:

BMI:

Breast:

Thyroid:

Acne

Hirsutism:

Galactorrhoea:

Vitals monitoring: pulse rate/blood pressure/respiratory rate/temperature

Anemia :

Pedal edema :

Cardiovascular system:

Heart sounds : S1 and S2

Respiratory system:

Breath sounds :

Bimanual pelvic examination:

FACTOR ASSESMENT

Ovulation:

Endocrine status: FSH/LH/TSH/Estradiol/prolactin

Tubes:

Uterus/ pelvis/ovaries by USG:

Ovarian volume/ antral follicular count

Cervix:

Male factor:

PATIENT CONSENT FORM

Study Detail	:	Evaluation of Anti-mullerian hormone as a predictor of ovarian reserve in infertility patients in Karpaga Vinayaga Medical College Hospital
Study Centre	:	Karpaga Vinayaga Medical College Hospital
Patient's Name	:	
Patient's Age	:	
Identification Number	:	

Patient may check (√) these boxes

a) I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction.	☐
b) I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected.	☐
c) I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.	☐
d) I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well-being or any unexpected or unusual symptoms.	☐
e) I hereby consent to participate in this study.	☐
f) I hereby give permission to undergo complete clinical examination and hematological tests.	☐

Signature/ thumb impression

Patient's Name & Address:

Signature of the Investigator

Study Investigator's Name

Dr. S. TAMILARASI

சுய ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு:

Evaluation of Anti-mullerian hormone as a predictor of ovarian reserve in infertility patients in Karpaga Vinayaga Medical College Hospital

பெயர்: வயது: தேதி: உள்நோயாளி எண்.

இடம்: கற்பக விநாயகா மருத்துவக் கல்லூரி, சின்னக்கோளம்பாக்கம், மதுராந்தகம் தாலுகா, காஞ்சிபுரம் மாவட்டம்.

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் ஆய்வாளரால் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வின் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்படாது என அறிந்து கொண்டேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்று தெரிவிக்கிறேன் என்று புரிந்து கொண்டேன். இச்சுய ஒப்புதல் படிவத்தில் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்றும் தெரிந்து கொண்டேன்.

பங்கேற்பாளர்/பாதுகாவலர் கையொப்பம் தேதி:

ஆய்வாளர் கையொப்பம் தேதி:

MASTER CHART

S.No	Age	Bmi	Type of infertility	Lh	Fsh	Tsh	Amh	Prolactin	AFC Lt ovary	AFC rt ovary	estradiol
1	26	22.5	Primary	6.93	4.5	2.1	3.18	12.3	4	5	233.6
2	25	25.4	Primary	4.4	11.2	1.5	7.5	14	6	2	123.8
3	27	24.5	Primary	5.4	6.5	3	2.6	10.6	4	3	118.4
4	29	30.5	Primary	3.2	10.1	4.1	1.1	30.4	1	1	432.8
5	31	23.5	Primary	6.1	5.3	3.3	4.5	16.7	5	5	154.6
6	29	27.6	Primary	5.3	6.4	2.7	2.5	18.8	4	4	178.9
7	28	32.9	Primary	3.2	7.2	1.9	0.4	20.4	1	1	234.8
8	22	29	Primary	4.1	8.3	2.5	3.7	12.9	3	4	346.7
9	24	24.4	Primary	2.6	2.3	3.2	4.6	13.7	5	7	432.8
10	37	32.6	Secondary	5.6	9.1	3.9	2.5	25.6	3	4	322.6
11	26	23.2	Primary	6.5	11	2	4	12	7	3	316.6
12	31	29.6	Primary	6.5	5.4	2.1	4.7	12.5	4	6	183.4
13	27	28.7	Primary	6.6	8.7	4.2	1.9	26.1	2	5	261.3
14	35	23.6	Primary	4.8	6	3.3	3.7	21.2	3	3	313
15	35	21.9	Secondary	6.1	9.3	3.4	1.7	20.1	1	4	366.6
16	26	23.4	Primary	5.9	5.5	2	2.1	13.5	2	2	313.1
17	28	21.8	Secondary	5.3	10.9	3.6	3.8	21.7	5	4	267.9
18	28	21.7	Secondary	4.9	4.2	4.4	3.8	24.3	4	4	303.6
19	26	34.2	Primary	2.6	8.8	2	1.2	29.4	1	4	161.1
20	26	31.5	Primary	5.9	11.2	1.8	4.8	17.3	4	4	373.6
21	32	32.8	Primary	6.1	9.6	1.8	4.5	10.7	7	5	362.5
22	25	25.9	Secondary	3.6	7.3	4.2	4.1	16.2	5	7	367.1
23	29	28.9	Primary	5.7	10.6	4.1	4.4	28.8	6	3	257.2
24	27	28.5	Primary	3.3	6.6	2.3	2.3	18.4	4	2	383.1
25	27	27.7	Primary	2.7	5.9	3.8	5.8	15.6	4	6	391.5
26	27	32.4	Primary	5.4	4.4	1.7	0.9	24.6	2	2	385.4
27	26	27.1	Secondary	3.7	6.3	2	4.5	11.2	5	6	196.1
28	35	30.7	Primary	2.6	2.9	4.7	3.2	16.9	4	4	207.5
29	26	23.6	Primary	5.2	5	4	2.3	20.3	5	4	331.9
30	34	32.6	Secondary	3.9	5	2.4	6.4	17.7	5	6	397.5
31	25	32.3	Primary	6.7	8.6	4.7	3.8	18.9	7	6	182.5
32	33	32.5	Primary	5.9	9.7	4.4	5.5	27.3	5	7	203.5
33	31	28.1	Primary	5.9	2.8	3.1	2.2	10.4	3	3	410.5
34	29	26.7	Primary	3.5	4.7	4.7	1.1	11.1	3	4	203
35	28	26.2	Primary	4.6	2.8	4.5	1.5	28.2	4	3	261.1
36	27	30.4	Primary	6.1	10.7	3.4	3.9	15.5	4	7	265.9
37	27	22.5	Primary	3.8	10.1	3.7	2.4	21.2	2	3	218.5
38	30	27	Primary	6	5.2	3.9	3.5	11.9	7	3	321.7
39	36	28	Primary	4	6.1	3.8	4.6	22.6	4	5	258.6
40	33	29	Primary	5.3	11.3	3.7	0.3	16.2	1	1	341.7

41	29	33.7	Primary	3	4.7	2.9	4.5	26.7	4	4	370.9
42	30	33	Primary	2.9	3.8	4.4	5.2	30.4	4	3	380.6
43	33	28.6	Primary	3.1	5.4	4.7	2.7	13.2	6	2	410.8
44	32	28.2	Primary	3.1	10.2	4.4	4	12.7	6	3	213.6
45	34	30.7	Primary	6.4	3.8	2.5	4.8	23.7	7	5	270.4
46	25	27.7	Secondary	4.1	9.9	3.3	6.6	15	6	5	195.7
47	30	30.3	Secondary	5.8	11	4.7	1.2	28.4	2	5	123.8
48	33	27.4	Primary	3.9	6.3	4	2.7	13.3	2	3	363.8
49	25	24.1	Secondary	5	5.1	3.4	4.2	18.9	4	5	140.3
50	27	27.6	Primary	3.1	5.3	4.2	0.8	21.9	2	1	409.8
51	35	33.4	Primary	4.3	7.3	1.6	0.4	17.4	1	1	162.7
52	27	28.3	Secondary	3.8	9	2.5	4.3	29	6	3	269.6
53	28	30.5	Primary	4.9	5.9	4.5	1.1	29.5	3	2	400.6
54	34	33.7	Secondary	4	3.6	2.3	5.4	17.2	5	7	200.2
55	26	22.7	Primary	3.9	5.8	3.9	1.8	27.9	4	6	410.7
56	30	30.8	Primary	4.6	5.4	4.3	4.7	17.1	6	5	204.5
57	32	31.9	Secondary	4.8	6.9	2.2	4.4	26.1	5	5	140.9
58	33	32.3	Primary	5.6	8.9	3.6	0.4	26.3	1	1	346
59	28	30.8	Primary	3.3	6.4	1.8	1.6	30	4	3	395.3
60	27	27.1	Primary	4	6.8	3.7	4.4	29.6	4	6	162.7
61	29	26.5	Primary	5.9	7.1	3.3	4.5	26.9	4	5	271.5
62	34	31.9	Primary	3.4	8.7	2.3	2.3	21.5	5	2	145.9
63	27	32.7	Primary	5.5	3.1	2	5.6	27.7	4	6	340.8
64	34	25.5	Primary	2.7	10.8	2.7	4.3	17.7	6	6	324.8
65	25	33.3	Primary	4.3	3.2	2.3	6.6	18.6	7	5	124.2
66	28	29	Secondary	4.9	7.9	3.6	4.4	21.5	5	3	424
67	35	30.1	Primary	3.4	11.7	3.6	6.5	12	4	5	205.5
68	35	23.3	Primary	4.1	6.9	3.2	4.1	14.3	4	6	127.3
69	35	31	Secondary	4.9	4.3	3.8	5.6	16.5	7	4	182.5
70	29	25.4	Primary	2.8	11.4	4.4	7	18.2	4	6	178.8
71	31	27.2	Primary	6.4	3.9	1.8	2.2	29.3	4	3	396.8
72	35	24	Secondary	6.7	8.2	3.6	2.3	22.9	3	3	255.5
73	35	25	Secondary	3.7	9.7	1.8	0.8	12.8	1	2	358.3
74	29	27.7	Secondary	3.9	9.4	4.7	6.7	27.1	4	7	223.2
75	36	32.5	Primary	5.7	8.2	2.2	2	24	6	4	283
76	33	34.3	Secondary	4.6	9.8	3.2	3.4	28.3	4	2	193.3
77	34	24	Primary	4.8	6	2.4	4.3	25.1	5	6	149.1
78	30	32.6	Primary	3.8	10	2.6	5.3	29.9	7	6	266.3
79	31	29.3	Primary	4	3.1	3.9	4.9	10.8	7	6	430
80	30	21.5	Primary	6.2	4.8	4.3	0.7	23.5	1	2	431
81	25	22.1	Primary	2.7	6.5	4.5	2.2	24.8	4	4	207.8
82	28	32.4	Primary	3.4	5.1	3.6	4.6	17.1	7	5	357.7
83	31	25.6	Primary	2.6	5	2.9	2.2	18.8	2	2	304.1
84	34	34.2	Secondary	5.4	9.3	4.2	7.4	12.4	4	6	277
85	28	23.5	Primary	5.2	10.5	4.4	3.8	18.9	6	4	319.6

86	30	23.8	Primary	5.5	4.6	2.7	7.2	11.5	6	5	379.1
87	35	27.7	Primary	6.7	6.8	2.6	6	27.5	4	4	181.8
88	27	33.6	Primary	4.7	11.3	3.5	0.8	28.1	2	2	419.7
89	34	32.7	Primary	3.5	6.6	4.2	6.3	18.2	4	3	310.5
90	30	31.3	Secondary	3.5	10.4	2.4	3.6	25.4	6	4	394.3
91	25	31	Primary	5.6	2.6	3.2	2	29.3	3	3	214.6
92	36	26.7	Primary	5.4	5.1	4.5	6.1	21	4	4	356.6
93	30	34.4	Primary	5.8	7.2	3.4	5.8	30.3	6	4	312.2
94	32	23.1	Primary	6.1	7	3.1	1.9	16.6	1	4	401.7
95	29	27.8	Secondary	2.9	11.5	2.1	4.4	28.5	5	6	120.4
96	29	30.4	Primary	2.6	8.4	4	3.7	17.1	4	4	359.8
97	27	31.6	Secondary	4.1	5.2	2.9	2.4	23.8	5	3	356.2
98	30	22.5	Primary	3	6	3.9	4.6	10.8	7	5	306.8
99	36	34.1	Primary	6.7	11.3	4.4	0.7	12.7	1	1	158.8
100	34	28.3	Primary	4.5	3.1	3.9	6.6	24.3	4	4	286