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Role of Serum Leptin Level in Primary Sub- Fertile Women

A Thesis

Submitted to the Council of the College of Medicine / University of Babylon as Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Physiology

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﴿وَٱمۡرَأَتُهُۥ قَآئِمَةٞ فَضَحِكَتۡ فَبَشَّرۡنَٰهَا بِإِسۡحَٰقَ وَمِن وَرَآءِ إِسۡحَٰقَ
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صدق الله ا علي العظيم

سورة هود: الأيات ٧١ ـ ٧٣

Supervisor Certificate

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We, the Examining Committee, after reading this thesis and examining the student Shema Zuhair Abdul Majeed Alkhwaja in its content, find it adequate as a thesis for the Degree of Master of Science in Medical Physiology.

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Dedication

To ….

My best home "My Lovely Iraq Country"

The Love support& power of my Life "Mom & 'Dad"

The sweetest thing& positive energy in my Life"My husband& family"

My support in this Life "My brothers"

My beautiful Smile& Nice "My Sisters"

The true thing in my Life "My friends"

The all sub-fertile women in Iraq to feel" hope &happy"

With all Love and respect, I can ever feel

Shema

Acknowledgement **Praise is to almighty Allah gracious for helping &giving me the strength &patience to achieve this work & finish what I started and see the day to present this work&study.*I would like to express my profound and appreciation to my supervisors Assist Prof Dr.Ban Jabir Edan and. Lecturer and Dr.Zeena Raad Muhi for their valuable scientific guidance,and continuous Help throughout the study that show me the light in my dark nights.*I am greatly indebted toProf.Dr.Safaa Sahib Naji Al-ghazali the dean of the college of medicine,andProf.Dr.SaadMerza,ProfDr.HaithemAli&Ms.Mysoon&Ms.Bashaer for their help and support to postgraduate students.*I would like to express my gratitude and thanks to all my teachers for making available facilities required for this research, all staff, Professors & Doctors in my department medical physiologybeinglikeafamilytome.*Mydeepest,thanks,with,respect,to,professor,Dr. HananAbdulJabarAltaee,forHervaluable supports, encouragement and the kind smilethatmakemesmile*Mydeepest,thanks,with,respect,to,head,of,department,of, medicalphysiologyProf.Dr.SamirSawadiHammoudforhisscientificadvices,support during,my,wok,hisadvice.give,me,hope*Mydeepe,thanks,with,respect,to,my,family (fatherProf.Dr.ZuhairAlkhwaja,&my,motherProf.Dr.suaadAlkhwaga&my,love,husbandDr.Husham,mohammed,&my,sisters&brothersDr.Shala,,Dr.Alyaa,Dr.Ahmed.Dr.Kadanfer,DrFiras,Dr.Drgham).fortheir,unceasing"Duaa",support,that,give,me,powerto,achieve,my,dream.*Iwouldliketo,express,mygratitude,toProf.Dr Hameed.K.AlHadrawy,forhis,help,in,statistical,analysis.*My,deepes,tthankswith respect to all members of fertility center of the Kamal Alsamurrai hospital&to all sub fertile &fertile women participants of the,study,who helped and gave consent to utilize their blood samples for the study during data collection& to People helped with nice word or by do something that matter to complete this work and reach where Iam stand today and ever.*

Shema

Summary

Subfertility represents one of the significant health problem worldwide, where millions of couples are infertile. It has an important impact on the couples and their families and denotes a real challenge to gynecologist and obstetricians in terms of treatment that may take long time. Leptin hormone has been widely studied in different types of infertility, however; the results of previous studies conflicting and the role of leptin in the process of fertility still under debate.

The aim of this study to evaluate serum leptin concentrations in women with primary subfertility, to investigate the relationship between serum leptin and some reproductive hormones to compare reproductive hormone with fertile women(control) and to evaluate the relationship between serum leptin level and endometrial thickness, antral follicle count in sub fertile women.

The study was designed as analytical case-control study, after it was approved by the Ethical committee of researches of College of Medicine, University of Babylon. The study conducted during the period from December 2020 to July 2021 at Kamal AL samurai Hospital (center of fertility, treatment infertility and In Vitro Fertilization(IVF).and in a private clinic, in Baghdad/ Iraq. The study included total of 100 women with primary subfertility, namely cases group, and100 fertile women as control group, who met the inclusion criteria, both groups were almost matched for their demographic characteristics. Verbal consent was obtained from all participants in both groups. Data collected through full history taking and thorough clinical examination. Blood samples collected from all participants and send to laboratory to perform the hormonal studies. Ultrasonography examination performed to assess the endometrial thickness and antral follicle count.

The results of the study showed that both groups were not significantly different in their demographic characteristics including age, education, job, family history of infertility or surgical history, (P.value > 0.05). Anthropometric measurements were also not significantly different between both groups, (P. value > 0.05). The mean values of luteinizing hormone Follicle stimulating hormone testosterone and prolactin were significantly higher in sub fertile women, (P<0.05) Anti-Müllerian hormone and antral follicle count was significantly lower in sub fertile women, (P<0.05). Estradiol and endometrial thickness were not significantly different between both groups, (P>0.05). the mean leptin level in sub fertile women was 26.8±15.2 ng/mL and it was significantly higher than that of control group which was 6.4 ± 2.3 ng/mL, (P. value < 0.001). among the sub fertile women,

female factor was the main cause of infertility it contributed for 49% followed by unexplained infertility in 39%. Leptin levels were not significantly different across the cause of subfertility, but in all subgroups of sub fertile women the mean leptin level was much higher than that in controls, (P. value < 0.001). It had been significantly found that leptin level increased with higher body mass index where the mean leptin level of obese sub fertile women was 34.7 ± 8.9 kg/m^2 compared to 26.3 ± 6.7 kg/m^2 in overweight group and 19.4 ± 5.6 kg/m^2 in normal weight sub fertile women, (P.value < 0.001). No similar association between leptin and body mass index was found in control group, (P. value > 0.05).

High leptin level found to be significant predictor of subfertility where women with higher leptin levels of 11 ng/ml or higher about 2.8 fold more likely to be sub fertile, (odds ratio = 2.793). Leptin level showed a high sensitivity, specificity and accuracy of 96%, 98% and 96.9%, respectively as predictor of subfertility.

In conclusion higher leptin level was the stronger, excellent and valid predictor of subfertility with a high sensitivity specificity, accuracy, positive predictive value and negative predictive value. So it is recommended to be used in clinical practice for the assessment of sub fertile women in addition to other traditional parameters.

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List of Abbreviation

Abbreviation	Full text
AFC	Antral follicle count
AMH	Anti mullerian hormone
ART	Assisted Reproductive Technology
AUC	Area under the curve (statistics)
BMI	Body mass index
C.I. (95%)	95% confidence interval
DNA	Deoxyribonucleic Acid
E2	Estradiol
ELISA	Enzyme-linked immunosorbent assay
et al	Eta lia (Latin) , and others (English)
FSH	Follicle stimulating hormone
GIFT	Gamete intrafallopian Transfer
GnRH	Gonadotropin releasing hormone
HCG	Human chorionic gonadotropin
ICSI	Intracytoplasmic sperm injection
IL-6	Interleukin-6
IVF	In Vitro Fertilization
kDa	kilo Dalton
LH	Luteinizing hormone
mIU	Milli-international unit
mL	Milliliter
Ng	Nano-gram
Ns	Not significant

OD	**Optical density**
OR	**Odds ratio (statistics)**
PCOS	**Polycystic ovary syndrome**
Pg	**Picogram**
QC	**Quality control**
r value	**Correlation coefficient (statistics)**
ROC	**Receiver Operating Characteristics (statistics)**
SD	**Standard deviation (statistics)**
SPSS	**Statistical Package for Social Sciences**
TMB	**Tetramethylbe nzidine**
TNF-α	**tumor necrosis factor alpha**
TSH	**Thyroid-stimulating hormone**
USA	**United States of America**
WHO	**World Health Organization**
ZIFT	**Zygote intlafrllopian transfer**
ZP	**Zona pellucida**

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Chapter One
Introduction

1. Introduction

Subfertility is the inability of a sexually active couple, not using any contraceptive techniques in a year or more, to get pregnant. There is paucity of data from countries of Asia to Latin America, according to the WHO, the prevalence of infertility in these regions is between 8% and 12% in couples of reproductive ages (Hussain *et al.*, 2017; Akhondi *et al.*, 2019; Farquhar *et al.*, 2019).Primary infertility refers to couples without any previous pregnancy after at least 1 year having sex without using birth control methods(Zhou *et al.*, 2018).

Leptin, a product of the 0b gene which is primarily produced by adipose tissues, helps to regulate the hypothalamic-pituitary-gonadal axis and plays a vital role in obesity, hyperinsulinemia, and increased insulin resistance which may effect on the reproductive function (Lainez and Coss, 2019).

Leptin positively influences the reproductive system from the onset of puberty to pregnancy, establishing a close link between energy homeostasis and fertility However, animal studies have clearly shown that excessive leptin secretion may have adverse effects on female fertility. Observations made in obese women of reproductive age has partly confirmed this hypothesis, as the prevalence of infertility is higher in this subgroup of women than in the general population (Ramos-Lobo and Donato Jr, 2017; Lainez and Coss, 2019). In addition to its role as a central signal to the brain conveying information on the amount of energy stores to regulate energy homeostasis; leptin has been found to exert equally important effects on gonadal organs. The endocrine and/or direct paracrine effects of leptin on the gonads are implied by the expression of functional leptin receptors on the surface of ovarian follicular cells, including granulosa, theca, and interstitial cells (Pérez-Pérez *et al.*, 2015).

Also it is expressed in the glandular and luminal tissues of the endometrium throughout the menstrual cycle (Pérez-Pérez *et al.*, 2015; Kalaitzopoulos *et al.*, 2021). Leptin, is primarily produced by adipose tissues, helps to regulate the hypothalamic-pituitary gonadal axis and plays a vital role in obesity, hyperinsulinemia, and increased insulin resistance in the polycystic ovary syndrome (PCOS) patients (Anwar *et al.*, 2021). An association of leptin with obesity, infertility, and other endocrine functions have been reported (Park and Ahima, 2015; Silvestris *et al.*, 2018; Kargasheh *et al.*, 2021). Circulatory leptin levels are considered to be a predictor of menstrual function. however, the regulatory loop by which leptin concentrations vary during the menstrual cycle is so far, not clear (Salem, 2021) . Leptin does not only modify gonadotropin-releasing hormone and gonadotrophin production, but it also plays an important role in the functioning of the ovary and endometrium and takes part in the development of an embryo (Kawwass, Summer and Kallen, 2015; Sylvia *et al.*, 2018). It seems that the central and peripheral signals of the hypothalamic - pituitary - gonadal axis play an important role in the action of leptin in the menstrual cycle(Salem, 2021). Although the physiological variation during the menstrual cycle is uncertain it emerges that the lowest threshold of the circulating leptin is essential for a normal ovulatory function (Childs *et al.*, 2021). Therefore, leptin clearly appears to be linked to the reproductive system, probably coupling information regarding the adequacy of body energy homeostasis to the prospective event of pregnancy, demonstrating the importance of nutritional resources to a successful pregnancy, higher leptin levels are considered to be the cause of infertility and a predictive marker in unexplained infertility (Pratibha Kumari *et al.*, 2017; Childs *et al.*, 2021). On the other hand, leptin stimulates the release of gonadotropins, follicle stimulating hormone (FSH) and luteinizing

hormone (LH), from the pituitary glands, and its administration has resulted in the resumption of fertility in experimental animals (Baig *et al.*, 2019a). The leptin receptors have been discovered on gonads, which show that leptin may have a direct impact on the ovaries in females (Elizabeth and Peery, 2021).

Aim of study:

1. To evaluate serum leptin level in women with primary subfertility.

2. To investigate the relationship between serum leptin level and some reproductive Hormones.

3. To compare reproductive hormones with control fertile women.

4.To evaluate the relationship between serum leptin level and endometrial thickness, antral follicle count in sub fertile women.

Chapter Two
Literature Review

2.1.Female Reproduction

2.1.1 anatomy

Reproduction can be defined as the process by which an organism continues its species. the female reproductive organs figure (2.1) include ovaries, uterus, fallopian tubes, cervix, and upper vagina (Rimon-Dahari *et al.*, 2016).The ovaries are female gonads the site of gametogenesis and secretion of sex hormones ,Fallopian tubes provide a passage way for oocyte to travel from the ovaries in to the uterine cavity The fallopian tubes communicates with the upper part of the uterus, while the lower part of uterus is closest through the narrow canal of the cervix with the vagina and external organs, which are collectively called the vulva(Ganong ,*et al*.,2010).and so the mammary glands are considered a part of the female reproductive system (Guyton and Hall,2012).the female reproductive system is designed to carry out several functions. It produces the female egg cells necessary for reproduction, called the ova or oocytes. The reproductive system is designed to transport the ova to the site of fertilization (Waters, 2007; Hammond, 2009; Rendi *et al.*, 2012 ; Hunter,2012).female gametes derive from germ cells in utero(Wray, 2007; Taylor and Gomel, 2008). oogonia rapidly divide until approximately 7 million germ cells form by the 7th month of gestation. The number of germ cells then rapidly declines; most oogonia perish while the remaining cells, primary oocytes begin the first meiotic division. These cells arrest in prophase I and remain dormant as such until menarche (Channing, Hillensjo and Schaerf, 1978; Channing *et al.*, 1980; Rimon-Dahari *et al.*, 2016; Machaty, Miller and Zhang, 2017).

The anterior vagina abuts the posterior bladder wall while the posterior vagina abuts the anterior rectum (DeLancey, 1999; Rosner J, Samardzic T, 2021) Bartholins glands open lateral to the vaginal opening (Stocco, Telleria and Gibori, 2007).

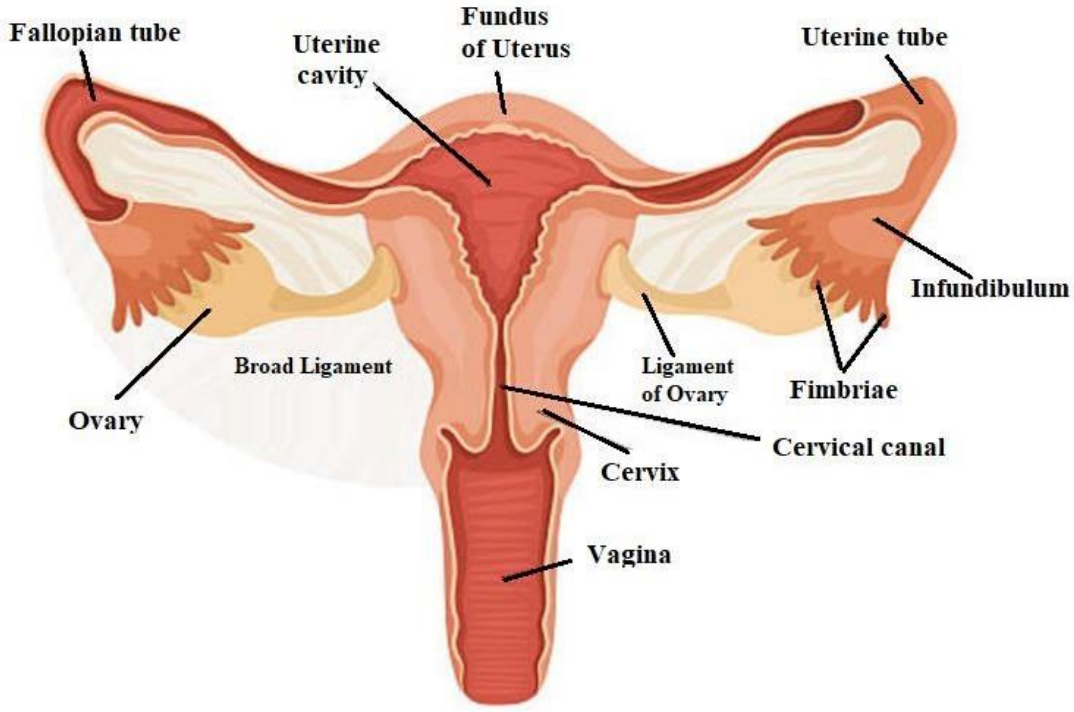

Figure 2.1. Part of female reproductive system (Rendi *et al.*, 2012)

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2.1.2. The ovarian cycle

The ovarian cycle describe the changes occurring in the follicles tiny fluid filled cavities ,each of which holds an immature egg cell the oocyte (Wilcox, 2010) this cycle consist of three phases; the early**:-**

1.(*follicular phase): corresponds to the menstrual bleeding phase and is expressed by low levels of both sex hormone (Farage, Osborn and MacLean, 2008) and this stimulate the hypothalamus to secret gonadotropin releasing hormone (GnRH).prompts the anterior pituitary gland to secretion both Follicle-stimulating hormone (FSH) luteinizing hormone(LH) the increasing levels of both these gonadotropin-hormones triggers not only follicle growth in the ovary but also the secretion of estrogen (Sherwood, 2018).under normal circumstances during each cycle time interval limited pool of follicles is mobilized in the ovary from which only one will continue through the maturation process, released and ovulate. The follicle appears randomly on either the right or left ovary (Goodman, 2009). As these follicles grow, they produce a type of estrogen called estradiol. An increasing amount of estradiol stimulates the lining of the uterus to prepare a possible pregnancy (Wilcox, 2010). Estrogen levels start rising rapidly peaking in the late follicular phase one day before ovulation (Farage, Osborn and MacLean, 2008) so peaking around this is time is LH, the physiological signal for ovulation, Its concentration in blood rise sharply and reaches a peak about sixty before ovulation (Goodman,2008).

2.(*ovulation stage): is characterized by the maturation of the follicle as an effect of stimulation from increased blood levels of FSH, well as the first spike estrogen secretion (Wilcox, 2010) This first triggers a spike in LH levels. which in turn causes the follicle to rupture and the egg to be released. Initiation

ovulation (Dyer, 2013). The ovum has less than 24 hours to be fertilized after ovulation If fertilization does not occur , dies and a new follicle must be prepared Coordination of these event requires two-way communication between pituitary and the ovaries, and between the ovaries and the reproductive tract (Goodman,2008).

3.(*luteal phase): follows ovulation and encompasses the life cycle of the corpus luteum, a temporary structure in female ovaries involved in the production of sex hormones. This is accompanied by a rapid decline in estrogen levels, ending at the beginning of the next menses. Under the influence of the gonadotropin, the collapsed follicle is transformed into a small swelling of yellowish tissue called the corpus luteum: "yellow body" in Latin. The corpus luteum main task is to produce progesterone, the hormone that alters the uterine lining and prepares it for pregnancy (Wilcox, 2010).In this phase, there is a constant rise in levels of progesterone, which peaks in the mid-lutealphase in parallel with a second estrogen peak. If fertilization doesn't occur, the corpus luteum degenerates and the end of the luteal phase sease in both estrogen and levels, This loss of the proliferated endometrium the internal lining of the uterus by bleeding; this cyclic vaginal discharge is called menstruation (Goodman,2008) both estrogen and progesterone reach baseline shortly before (Farage et al., 2008).

These changes occur regardless of whether conception has occurred, (Maner and Miller, 2014) energy costs of preparing for possible pregnancy in the absence of conception are by the reproductive costs of falling to generate the necessary environment for the growth of a fertilized egg thus, during the luteal phase of each menstrual cycle, a woman's body prepares itself for possible pregnancy whether or not an egg has been fertilized. Although the rhythmic release of GnRH is controlled by the hypothalamus, the timing for this is coordinated by the ovary, The corpus luteum has a programmed life cycle of about twelve days, Anew follicles cannot arise so long as the corpus leutum remains functional when it dies it appears to allow follicular growth and FSH secretion in the blood once again stimulating the growth of the next cohort of follicles So, the ovary determines the interval the LH surge and the emergence of the new cohort of follicles, length or the follicular phase may somewhat variable. influenced by events happening, outside the ovaries, but timing of the LH Surge resides in the ovary (**Goodman,2008**). It is only when the developing follicle signals of it is ready to ovulate with increasing blood levels of estradiol that the pituitary secretes the ovulatory spike or gonadotropin. Throughout the cycle, it the ovary that notifies the pituitary hypothalamus of its readiness to proceed to the next stage. Compared with the follicular phase, and the luteal phase is less variable, due to the programed death of the corpus luteum which will involate despite continued stimulation with LH (Wilcox,2010).

2.1.3. Ovulation

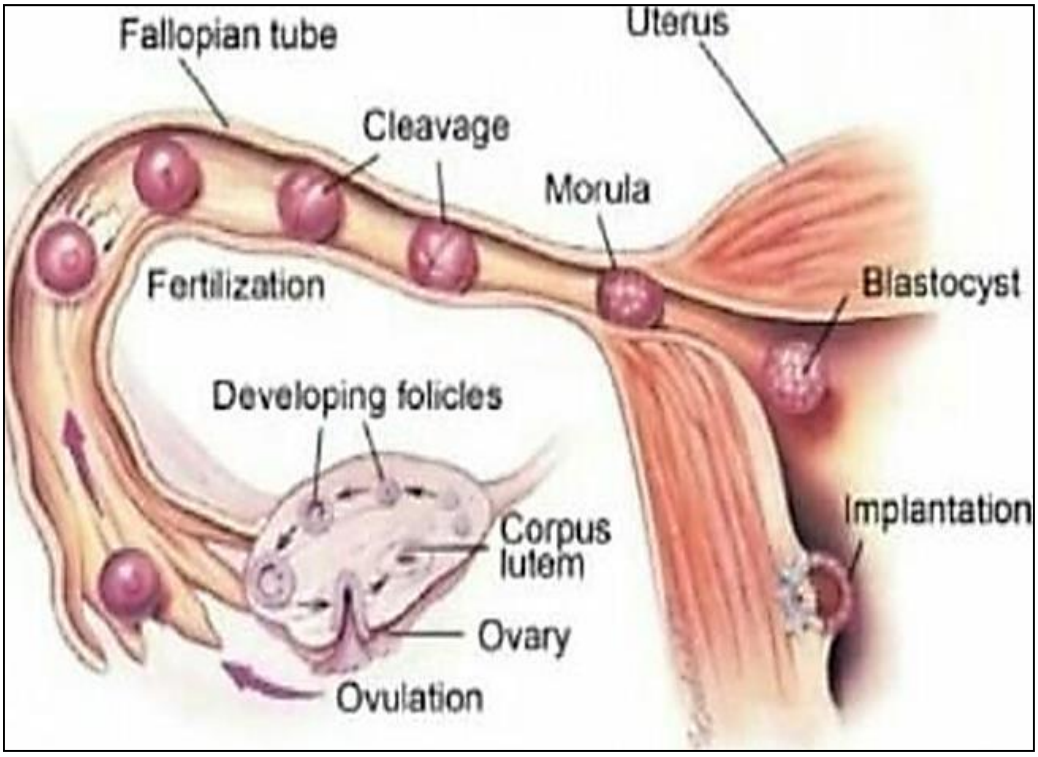


Figure 2.2. Ovulation & Fertilization (Rendi et al., 2012)

Ovulation is a physiologic process defined by the rupture and release of the dominant follicle from the ovary into the fallopian tube where it has the potential to become fertilized (Richards and Pangas, 2010). The ovulation process is regulated by fluxing gonadotropic hormone (FSH/LH) levels. Ovulation is the third phase within the larger uterine cycle (Menstrual Cycle).The follicular release follows the Follicular phase (dominant follicle development) and precedes the Luteal phase (i.e. maintenance of corpus luteum) that progresses to either endometrial shedding or implantation. Follicular release occurs around 14 days prior to menstruation in a cyclic pattern if the hypothalamic-pituitary-ovarian axis function is well regulated (Tsutsumi and Webster, 2009). Through a signal transduction cascade initiated by LH, proteolytic enzymes are secreted by the follicle that degrades the follicular tissue at the site of the blister, forming a hole called the stigma. The cumulus-oocyte complex leaves the ruptured follicle

and moves out into the peritoneal cavity through the stigma, where it is caught by the fimbriae at the end of the fallopian tube (also called the oviduct).

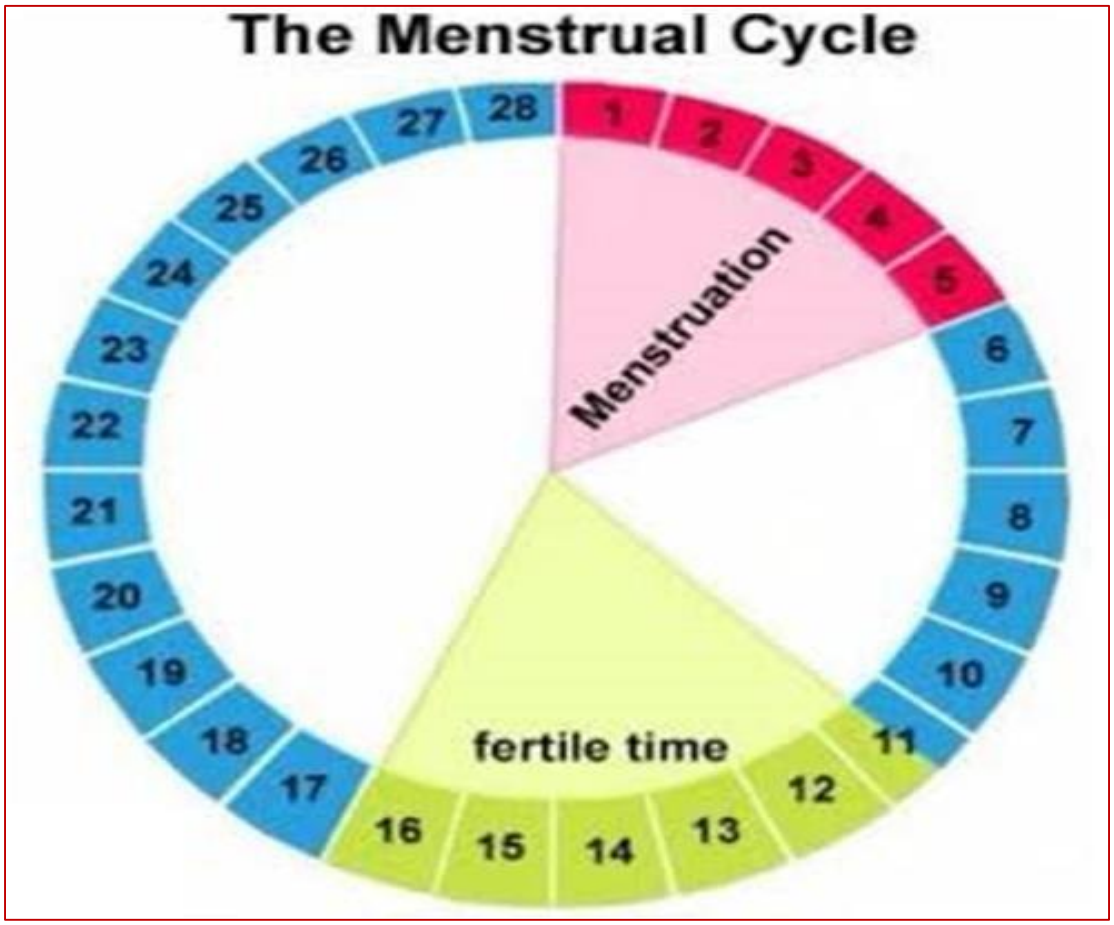


Figure 2.3.Fertile day and menstrual cycle day (Tsutsumi and Webster, 2009).

2.1.4. Fertilization:

Is a sequence of coordinated molecular events involving the merging of the sperm with the egg, the fusion of the pro nuclei and the intermingling of the maternal and paternal chromosomes. The first form of human life is the zygote (a diploid cell) from which the new organism will result during sexual Intercourse, millions of sperm are deposited into the vagina. number of these will die in the acidic environment. However, many will survive due to the protective elements provided in the fluids surrounding them soon afterwards, the sperm have to swim through the cervical mucus, towards to the uterus and then on to the fallopian tubes. As they swim towards these, they decrease in number, in an attempt to make it through the mucus. Inside the uterus, the contractions of the uterus assist the journey of the sperm towards the egg (Wassarman, 1997; Okabe, 2013). Fertilization takes placed the ampulla of the oviduct. If the oocyte is not fertilized here, It slowly passes to the uterus, where it becomes degenerated and is absorbed Achieving fertilization requires the activation of sperm and oocyte maturation However, the oocyte and sperm interaction depends on a number of changes occurring the egg and the sperm The egg complex following ovulation enters the oviduct consisting of three components**:**

1. The oocyte (egg) arrested at metaphase of meiosis II.

2. The extracellular matrix of the egg or zona pellucida consisting of three glycoproteins (ZPI: ZP2 and ZP3): synthesized and secreted by the oocyte;

3. The cumulus oophorus ,consisting of granulosa cells enriched with hyaluronic acid (Wassarman, 1997; Okabe, 2013).Cumulus cells support fertilization, and in vitro fertilization can be achieved more efficiently with than without them (Jin *et al.*, 2011) .The journey of the sperm is facilitated by ovarian hormones that affect the structure, composition and activity of the secretory epithelia of the cervix: the uterus and the fallopian tubes and the contractility of these elements. The

estrogen hormone favor these factors while progesterone does not. Oxytocin, which is secreted during intercourse by stimulation of the posterior pituitary, causes the contraction of the uterus and the fallopian tubes, as well as by the prostaglandins that affect the contractility of the uterus and fallopian tubes. During the ovulation period the uterus becomes more sensitive to prostaglandins oocytes acquire the ability to fuse with sperm when they reach 20 mm diameter and they are rested at the prophase of meiosis II (Zuccotti *et al.*, 1995). Spermatozoa undergo a series of events during maturation in order to acquire motility and fertility, as they move from the proximal towards the distal end of the epididymis Only spermatozoa that have passed through the epididymis are mature enough to be capable of motility. Moreover, via deposition of new proteins the nucleus, the DNA becomes more condensed, The sperm head decreases and becomes more compact The above is crucial for the subsequent correct decondensation of the paternal DNA the maternal oocyte The ability for motility is achieved. finally the structure of the plasma membrane is altered by the addition of glycoproteins and other proteins. This is affects the motility, the capacitation ability and Sperm is the male gamete (Nelson, 2012)

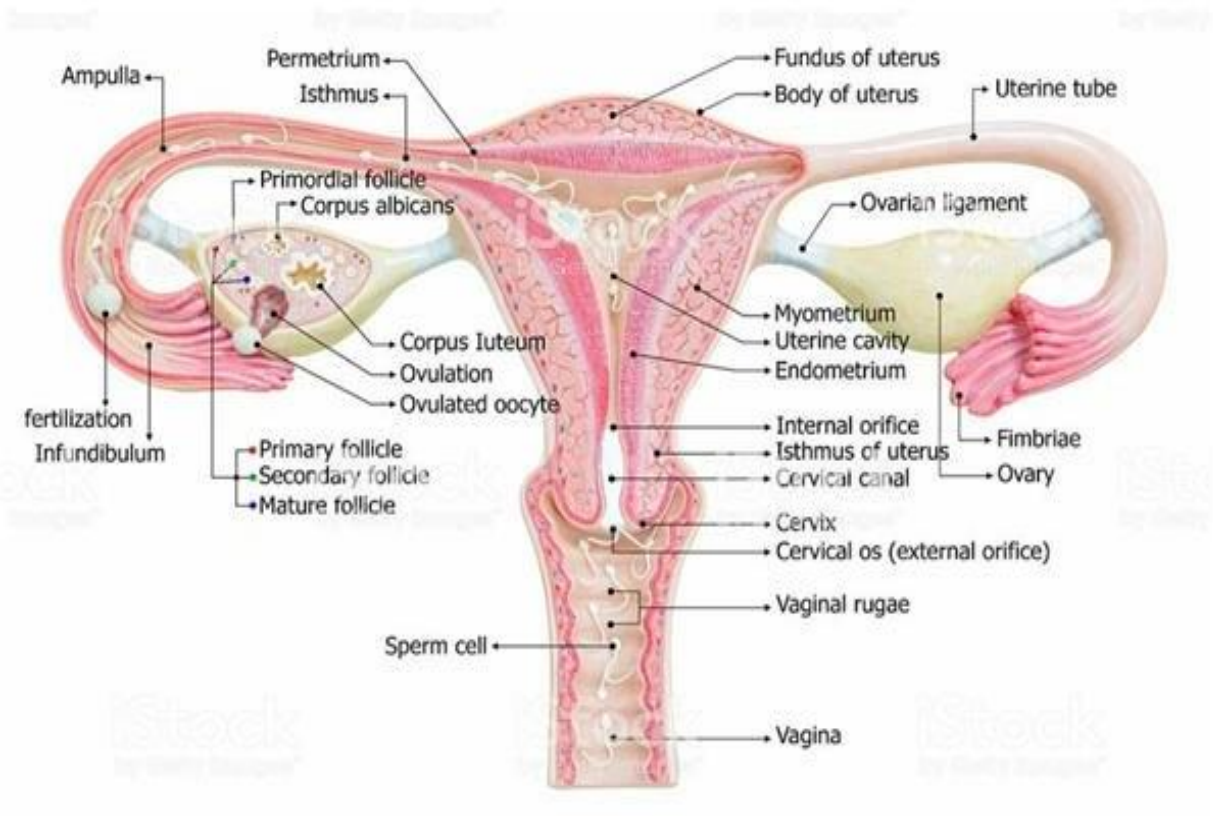


Figure 2.4. Parts of female reproductive organs and fertilization (Zuccotti *et al.*, 1995; Nelson, 2012)

2.1.5. Ovulation Hormone and Female Cycles

Hormones involved in ovulation:

1. Gonadotropin-releasing hormone (GnRH)

Is a tropic peptide hormone made and secreted by the hypothalamus. It is a releasing hormone that stimulates the release of FSH and LH from the anterior pituitary gland through variations in GnRH pulse frequency. Low-frequency GnRH pulses are responsible forFSH secretion whereas high frequency pulses are responsible for LH secretion. During the Follicular phase of the Uterine cycle, estrogen secretion causes the Granulosa cells to autonomously increase its own production of estrogen contributing to elevation in estrogen serum levels. This elevation is communicated to the hypothalamus and contributes to the increase in GnRH pulse frequency eventually stimulating the LH surge that eventually induces the follicular rupture and release from the corpus luteum and luteinization of the granulosa cells enabling the synthesis ot progesterone in place of estrogen. Finally, the low levels of LH following the surge restarts the FSH production by the slow pulsation frequency of GnRH release (Tsutsumi and Webster, 2009).

2. Gonadotropin hormones are heterodimeric glycoproteins with alpha/beta subunits. The alpha subunit is common to all glycoproteins including thyroid-stimulating hormone (TSH) and human chorionic gonadotropin hormone (HCG). The relationship between FSH and LH hormones is responsible for the process that induces follicular development, rupture, release, and endometrial reception or sheddingDisruption in the hormonal communication between the

gonadotropin- releasing hormones, gonadotropic hormones, and their receptors can lead to anovulation or amenorrhea leading to various pathologic consequences(Kara *et al.*, 2019).

3. Follicle-Stimulating-Hormone(FSH) is a gonadotropin synthesized and secreted from the anterior pituitary gland in response to slow frequency pulsatile GnRH. FSH stimulates the growth and maturation of immature oocytes into mature Graafian follicles before ovulation. FSH receptors are G-protein coupled receptors and are found in the granulosa cells that surround developing ovarian follicles.the granulosa cells initially produce the estrogen needed to maturate the developing dominant follicle. After 2 days of sustained elevation of estrogen levels,and the LH surge causes luteinization of the granulosa cells into LH receptive cells. this transition enables granulosa cells to respond to LH levels and produce progesterone (Tsutsumi and Webster, 2009).

4. Estrogen is a steroid hormone that is responsible for the growth and regulation of the female reproductive system and secondary sex characteristics. Estrogen is produced by the granulosa cells of the developing follicle and exerts negative feedback on LH production in the early part of the menstrual cycle. However, once estrogen levels reach a critical level as oocytes mature within the ovary in preparation for ovulation, estrogen begins to exert positive feedback on LH production, leading to the LH surge through its effects on GnRH pulse frequency. Estrogen also has many other effects that are important for bone health and cardiovascular health in premenopausal patients, (Tsutsumi and Webster, 2009).

5. Luteinizing Hormone (L H) is a gonadotropin synthesized and secreted by the anterior pituitary gland in response to high GnRH release. LH is responsible for inducing ovulation, preparation for fertilized oocyte uterine implantation, and the ovarian production of progesterone through stimulation of theca cells and luteinized granulosa cells. prior to the LH surge, LH interacts with theca cells that are adjacent to granulosa cells in the ovary. These cells produce androgens which diffuse into the granulosa cells and convert to estrogen for follicular development. The LH surge creates the environment for follicular eruption by

increasing the activity of the proteolytic enzymes that weaken the ovarian wall allowing for passage of the oocyte. After the oocyte is released, the follicular remnants are theca and luteinized granulosa cells. Their function is now to produce Progesterone, which is the hormone responsible for maintaining the uterine environment that can accept a fertilized embryo (Kumar and Sait, 2011; Laven, 2019).

6.Progesterone is a steroid hormone this responsible for preparing the endometrium for the uterine implantation of the fertilized egg and maintenance of pregnancy. if a fertilized egg implants, the corpus luteum secretes progesterone in early pregnancy until the placenta develops and takes over progesterone production for the remainder of the pregnancy. Progesterone, which is the hormone responsible for maintaining the uterine environment that can accept a fertilized embryo. ovulation occurs around day 14 of a typical 28-day cycle. Estrogen levels rise as a result of increased estrogen production by hormonally active granulosa cells within the follicle. One of the estrogen levels reach a critical point and remain at the level for 2 days, estrogen transitions from a negative feedback modulator of GnRH to a positive feedback modulator on the hypothalamus. This transition point leads to an increased frequency of GnRH secretion onto the anterior pituitary, leading to an LH surge. The LH surge increases intra follicular proteolytic enzymes, (Tsutsumi and Webster, 2009).

The surge also cause the luteinization of thecal and granulosa cells forming the Corpus Luteum, which is responsible for progesterone synthesis levels.the formation of the corpus luteum and ends in pregnancy or luteolysis (destruction of the corpus luteum) (Kumar and Sait, 2011; Laven, 2019) .

FSH and LH stimulate what remains of the mature follicle after ovulation to become the corpus luteum. if fertilization does not occur, progesterone/estrogen levels fall, and the corpus luteum dies forming the corpus albicans. These falling hormone levels stimulate FSH to begin recruiting follicles for the next cycle. If fertilization does occur, human chorionic gonadotropin (hCG) produce by the early placenta preserves the corpus luteum, maintaining progesterone levels until the placenta is able to make sufficient progesterone to support the pregnancy (Reed and Carr, 2008).

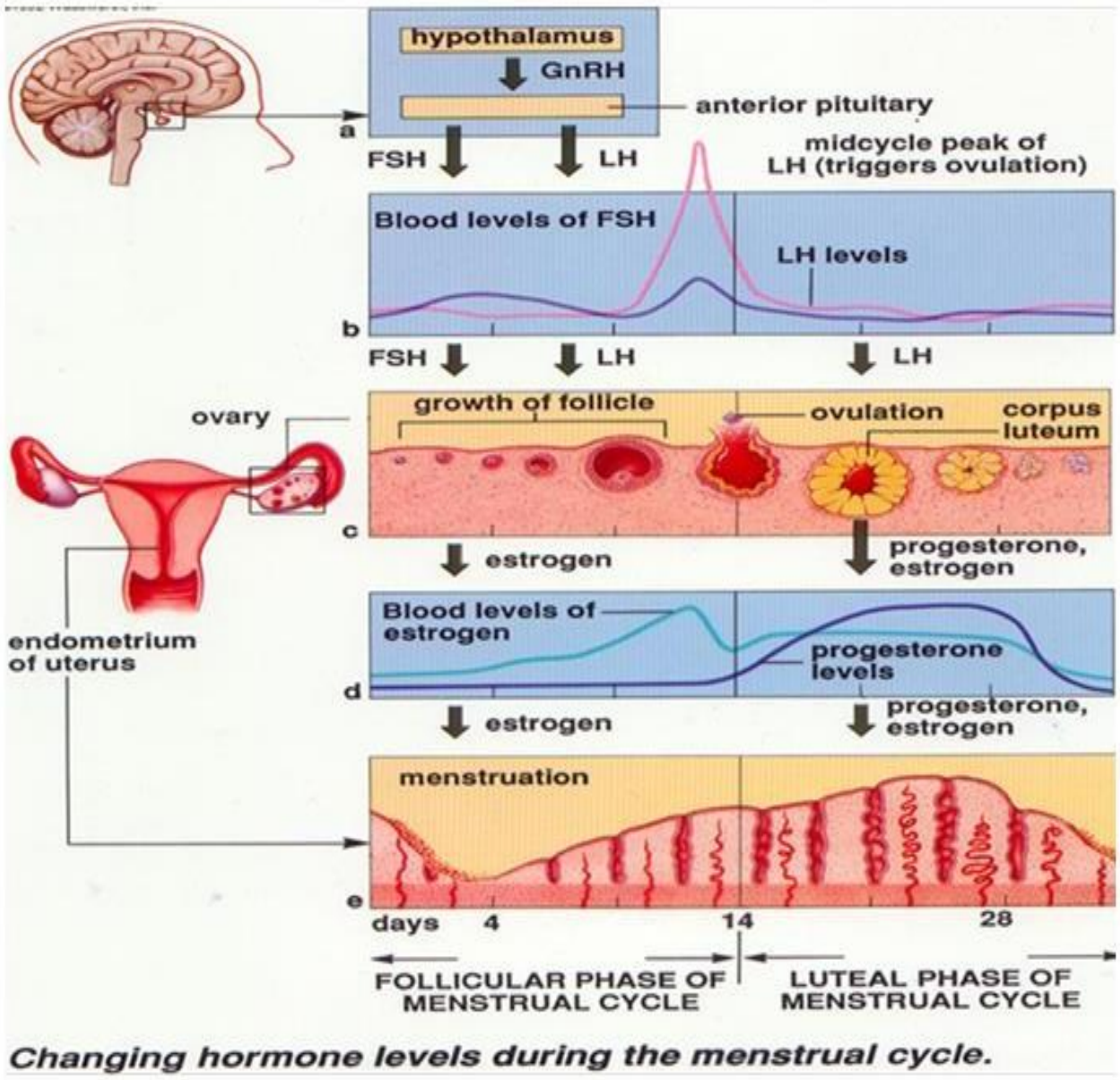


Figure 2.5.Hormonal changes and the female reproductive cycles (Balusik, 2003)

2.2. Infertility:

World Health Organization (WHO defines infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular sexual intercourse and without use any contraceptive so the term " infertility"is sutible for couple complete inability to conceive after a certain period ,while most of the time ,it is degree of subfertility So the term subfertility is more applicabale (Makar and Toth, 2002; Basten, 2009) .

2.2.1. Type of infertility

There are 2 type of infertility:

1. **Primary infertility** refers to couples who have not become pregnant after at least 1 year having sex without using birth control methods (Larsen, 2000; Eraky and EM, 2016).

2. **Secondary infertility** refers to couples who have been able to get pregnant, and previous conception at least once, but now are unable to conceive (Larsen, 2000; Eraky and EM, 2016).

2.2.2. Prevalence of infertility:

The prevalence of infertility had increased significantly in recent years The global prevalence of infertility is reported to be 15% (Mirzaei *et al.*, 2018). The rates of male infertility in North America, Australia, and Europe were reported to be 4%- 6%, 8%, and 7.5%, respectively (Cong *et al.*, 2016). A meta-analysis of the causes of infertility among the patients who referred to several infertility clinics of Iran showed that 78.4% of the couples suffered from primary and 21.6% from secondary in fertility problems. Totally, 34% of them had male factor, 43.5% had female factor and 17% had both male and female factors and

8.1 % had no specified cause for their infertility (Al-Asadi and Hussein, 2015). Finally, preconception care and counseling are recommended to all those who are planning a pregnancy to avoid failure which can make the couples prefer to remain childless or consider an agreement or non -spousal sperm options (Sethi *et al.*, 2016), and 8.5% of women 18-44 years of age who were married or living common-law were considered infertile (Dulberg and Stephens, 1993; Gunby *et al.*, 2010; Dar *et al.*, 2013).

2.2.3 The main causes of female infertility

The main causes of female infertility can be illustrated in table (2.1) as shown by (Nathan, 2007). In his study, 90% of cases of infertility causes have been uncovered, the other 10% of patient has don't been known the causes, whereas (10-30) % of infertility has more than one cause (Duckitt, 2003). Recently there is a growing evidence of possible role of highly reactive products of oxygen, termed free radicals, in case of infertility (Agarwal, Saleh and Bedaiwy, 2003).

Table 2.1. Causes of female infertility (Nathan, 2007).

Ovulatory factors: 30% Central defect: Chronic hyperandrogenemic anovulation. Hyperprolactinemia. Hypothalamic insufficiency. Pituitary insufficiency. Peripheral defect Gonadal dysgenesis. Premature ovarian failure Ovarian resistance
Metabolic diseases: Thyroid disease Liver disease, Renal disease Androgen excess.
Cervical factor: 15% Congenital factor: Mullein duct abnormality, Diethyl stelbosterl (DSE) exposure.
Acquired factor : Surgical treatment , Infection
Pelvic factor: 25% Appendicitis, Pelvic inflammatory disease Uterine adhesion, Endometriosis.
Structural abnormalities: Diethyl stelbosterol (DSE) exposure, Failure of normal fusion of reproductive tract
Additional Factors Behavioral factors: Diet, Exercise, Smoking, Alcohol Drugs
Environmental Factors: Exposure to heavy metals, pesticides and some chemicals

2.2.4. Diagnosis of female infertility:

An accurate history of couple's personal and medical details together with a simple light microscopy seminal analysis will identify the majority of infertility problems related to ovulatory disorders and male subfertility (Smith, Pfeifer and Collins, 2003; Aketayeva *et al.*, 2018). Figure 2.6 shows the diagnostic steps of infertility:

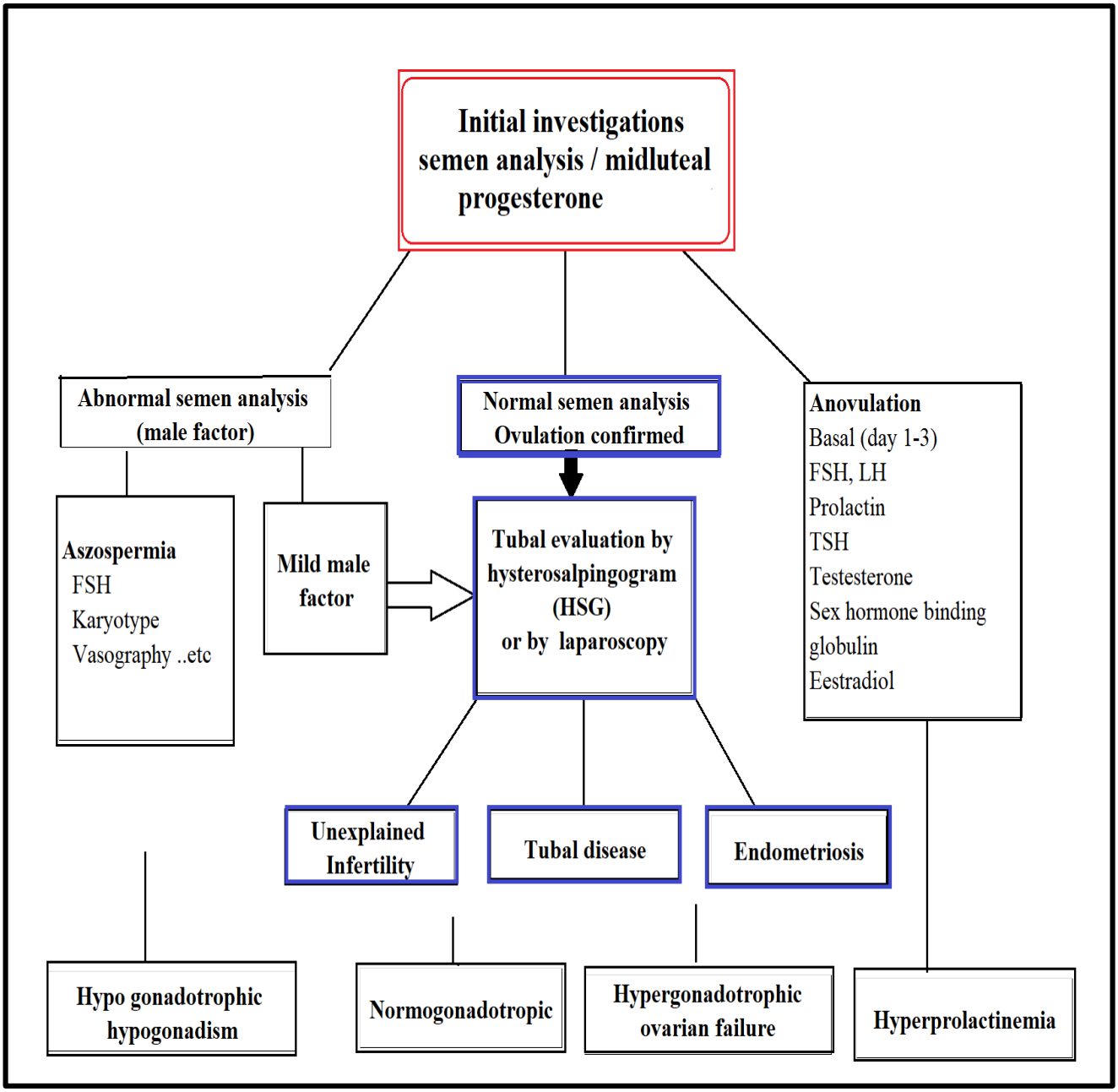


Figure 2.6. Investigations of infertility (Edmonds, 2018)

***The initial steps for diagnosis of infertility includes:**

- Confirmation of ovulation by History and laboratory tests.
- An assessment of the fallopian tubes and the uterus by the use of X-ray called Hysterosalpingogram (HSG).
- an assessment of a seminal fluid analysis (SFA)(Edmonds, 2018).

***Physical examination should document the following:**

- Weight. body mass index (BMI), blood pressure, and pulse
- Thyroid enlargement and presence of any nodules or tenderness. Breast secretions and their character
- Signs of androgen excess
- Vaginal or cervical abnormality, secretions or discharge
- Pelvic or abdominal tenderness, organ enlargement, or masses
- Uterine size, shape, position, and mobility
- Adnexal masses or tenderness
- Cul-de-sac masses, tenderness, or nodularity (Edmonds, 2018)

2.2.5. Treatment of infertility:

The treatment falls into 3 main types as shown in (Table 2.2):

1. Medical treatments.
2. Surgical treatments.
3. Assisted Reproductive Technology

(Nathan, 2007; Kuivasaari-Pirinen *et al.*, 2012; Stevenson, 2016; Aketayeva *et al.*, 2018)

Table 2.2. Infertility treatment (NICE,2013).

Medicine to improve fertility	Anti-oestrogens
	Gonadotrophins
	Pulsatile gonadotrophin-releasing hormone
	Gonadotrophin-releasing hormone analogs
	Dopamine agonists
	Aromatase inhibitors (experimental)
Surgical treatments	Ovarian drilling
	Fallopian tube surgery
	Uterine surgery
	Surgery for endometriosis
Assisted reproductive technology	IUI (Intrauterine Insemination)
	IVF (In Vitro Fertilisation)
	GIFT (Gamete Intrafallopian Transfer) & ZIFT (Zygote Intrafallopian Transfer)
	ICSI (Intracytoplasmic Sperm Injection)
	Donor insemination (eggs or sperm donation)
	PGD (Pre implantation Genetic Diagnosis)
	IVM (In Vitro Maturation)

and the Preconception medical care and counseling is advisable for all those planning a pregnancy failure of which the couple may choose to remain childless or consider adoption, or non-spousal sperm options.

Treatment modalities for infertility include:

- Weight reducing drugs in obese an ovulatory infertile woman, loss of 5-10% of body weight had been discovered to be enough to restore reproductive functions in 55- 100% of women within 6 months (Duval *et al.*, 2015; Balen *et al.*, 2016)
- Induction of ovulation using gonadatrophins, Human Menopausal Gonadotrophin (HMG) (Lunenfeld *et al.*, 2019).
- Bromocriptine in hyperprolactinemic females (Awan *et al.*, 2020).

- Clomiphene citrate-human menopausal gonadotrophin (CC-HMG) combination (Verma *et al.*, 2019).
- Hormone therapy (e.g., Perganol) (Olooto, Amballi and Banjo, 2012).
- Surgical intervention (Park *et al.*, 2019).
- Artificial Insemination (AI): may be achieved by intracervical or intra uterine insemination (IUI) It is performed ill an ovulating woman with patent tubes (Morrell, 2011).

2.2.6 Assisted Reproductive techniques (ART):

The assisted reproduction methods have made a major progress and have become an integral part of the reproductive medicine. Today, the assisted reproduction techniques represent the most effective approach to the treatment of infertility they indicated for specific conditions that cause infertility suchas tubal infertility,male factor infertility or endometriosis(Gupta *et al*., 2010; Kuivasaari-Pirinen *et al.*, 2012). ART involves the handling of both sperm and oocyte outside the body, to start a pregnancy ART are new technologies ; therefore, predictions about the success rate are less certain than other medical procedures Embryos transfer are done mostly on day 2 or 3 after fertilization in order to select the most appropriate ones for transfer

Clinical situations in which ART may be useful include cases of male infertility, cases where problems with number and/or function; in addition, cases of surgically retrieval of sperm such as by sperm aspiration, in which IVF and ICSI may be the best approach to care (Kuivasaari-Pirinen, *et al.*, 2012).

***In Vitro Fertilization (IVF):** is a process by which an egg is fertilized by sperm outside the body: in vitro. The process involves monitoring and stimulating a woman's ovulatory process, removing ovum or ova from the woman's ovaries and letting sperm fertilize them in a fluid medium in a laboratory,

(Elizur *et al*., 2007) and the procedures include gamete intrafallopian Transfer (GIFT), zygote intlafrllopian transfer (ZIFT), (Allen *et al*., 2006).

***Intracytoplasmic sperm injection (ICSI):**

Intracytoplasmic sperm injection (ICSI) (figure 2.7); involves individual sperm cell injection into oocytes cytoplasm ICSI is a useful technique for couples that have low or absent fertilization during IVF, or where the sperm quality and count is too low for normal IVF to be successful (Gremeau *et al*., 2012; Stevenson, 2016)

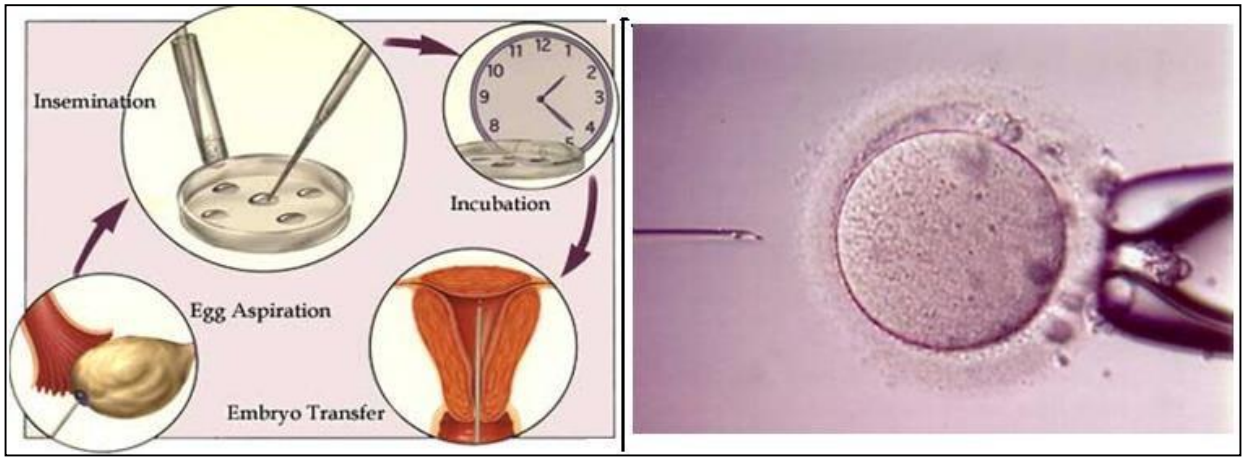


Figure (2.7): Intracytoplasmic sperm injection (Stevenson,2016)

2.3.Leptin hormone

Leptin (from Greek leptos, "thin") is a hormone predominantly made by adipose cells and enterocytes in the small intestine that helps to regulate energy balance by inhibiting hunger, which in turn diminishes fat storage in adipocytes . Leptin acts on cell receptors in the arcuate and ventromedial nuclei, as well as other parts of the hypothalamus and dopaminergic neurons of the ventral tegmental area, consequently mediating feeding(Brennan and Mantzoros, 2006; Bouret, Levin and Ozanne, 2015) . Leptin is a 167-amino-acid peptide that is mainly expressed in white adipose tissue (WAT), but is also found in a variety of tissues including placenta, mammary gland, ovary, skeletal muscle, stomach, pituitary gland, and lymphoid tissue (Moon *et al.*, 2013). Circulating leptin levels are directly in proportion to the amount of body fat, thereby reflecting the status of long-term energy stores. In addition, leptin levels fluctuate according to changes in calorie intake with a marked decrease during starvation (Moon *et al.*, 2013).Leptin is secreted in a pulsatile manner, displaying a circadian rhythm with lowest levels at mid-afternoon and highest levels at midnight. The pulsatile pattern of leptin secretion is similar in obese and lean subjects, but the pulse amplitude is higher in obese subjects (Moon *et al.*, 2013). Leptin levels exhibit sexual dimorphism. Although leptin levels decline significantly after the menopause, women tend to have higher levels than men even after controlling for body fat mass, suggesting a role of sex steroids (Rosenbaum and Leibel, 1999a). Subcutaneous fat produces more leptin than visceral fat, and this may, in part, contribute to higher leptin levels in women compared to men (Rosenbaum and Leibel, 1999a). Besides sex steroids, leptin levels are also regulated by other factors including insulin, glucocorticoids, catecholamine, and cytokines (Ahima and Osei, 2004).

2.3.1.Structure of leptin

Leptin which contains 167 amino acids, was discovered in 1994. It is a hormone secreted by adipocytes and has been found to regulate the intake of food (Grinspoon *et al.*, 1996; Minokoshi *et al.*, 2002) Leptin aids in the regulation of eating behavior through central neuroendocrine mechanisms. It is structurally similar to cytokines and contains an intra-chain disulphide bond which has functional significance. Leptin is produced primarily by white adipose tissues and released as a 16 kilo Dalton (kDa) protein . This circulating leptin correlates positively with leptin mRNA and protein levels in adipose tissue (Grinspoon *et al.*, 1996; Minokoshi *et al.*, 2002), (Figure 2.8).

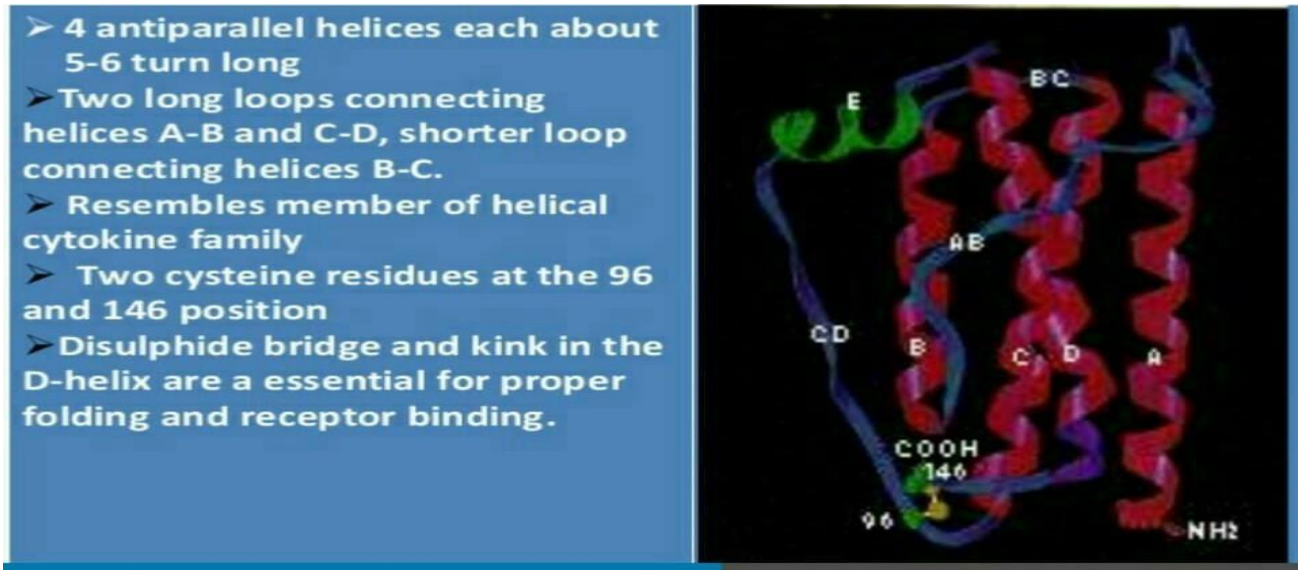


Figure 2.8. Structure of leptin (Minokoshi *et al.*, 2002)

The structure of leptin shows that Glu 100 lies on the surface of the molecule, with its side chain pointing toward the solvent. Therefore, substitution of the exposed Trp with Glu at this position apparently reduces intermolecular hydrophobic interactions and improves the solubility of the protein

Hydrophobic residue side chains are in white. with the exception of Trp100, other hydrophobic residues like Phe 92, Leu 142, Trp138, and Phe 41 are also located

on the surface maintain high potency but eliminate deficiencies of the native hormone. (Zhang *etal*.,1997), (Figure 2.9).

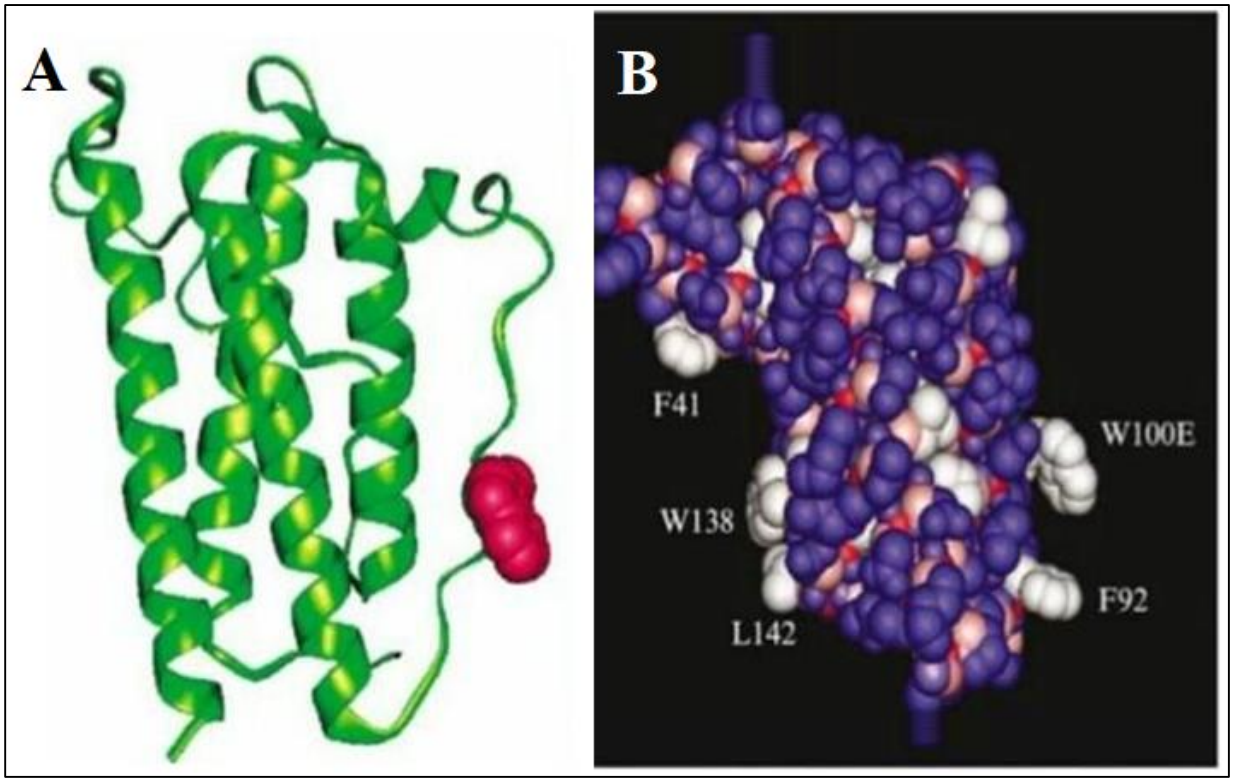


Figure 2.9. Surface structure molecule of leptin (Zhang etal.,1997) (**a**) Ribbon diagram of the four-helical conformation. Four helixes take an up-up-down- down arrangement, with a small helix E in the CD loop. The 100 Trp position was shown as a red CPK model on the surface of the molecule. (**b**) The surface structure of leptin. Blue is for N, red for O, and white for C atoms.

2.3.2.Physiological role of leptin (Rosenbaum and Leibel, 1999b)

1. Regulation of food intake, immune function, energy expenditure and body weight.
2. Thermogenesis.
3. Reproductive function.
4. Suppressed bone formation.
5. Directly act on the cells of liver and muscles.
6. Related to inflammatory response.
7. Contribute to early haemotopoiesis.

The hypothalamus senses the nutritional state of the body through signaling provided by the leptin hormone. Leptin decreases the intake of food through the upregulation of neuropeptides such as a melanocyte-stimulating hormone, which known to be anorexigenic. It concurrently down regulates orexigenic factors such as neuropeptide Y. Obesity can be caused by genetic defects in anorexigenic signaling, such as mutations in the melanocortin-4 or leptin receptors . The administration of leptin in humans and mice induces the reduction of excessive eating and obesity(Campfield, Smith and Burn, 1996; Cunningham, Clifton and Steiner, 1999; Obradovic *et al.*, 2021).

Obesity, however is associated with high leptin levels which can indicate leptin resistance in obese individuals. Leptin works in contrast to ghrelin, a peptide primarily produced by the stomach which stimulates the appetite (Castracane *et al.*, 1998). An increase in hunger is directly proportional to an increase in the ratio of ghrelin to leptin. Clinical studies show that leptin levels increase with sleep. Research also found that ghrelin levels are inversely related to sleep duration (Spiegel *et al.*, 2004; Taheri *et al.*, 2004).

2.3.3.The effect of some factors on leptin levels

The physiological factors that influence leptin secretion include gender, age, adiposity, physical exercise, feeding, and caloric restriction. Several hormones, including insulin, glucocorticoids,growthhormone, estradiol,testosterone,insulin-like growth factor-I, and somatostatin also modulate leptin (Dalamaga *et al.*, 2013; Moon *et al.*, 2013)

2.3.4. Factors regulating circulating Leptin levels:

Table 2.3. Factors regulating circulating leptin level (Moon *et al.*, 2013)

Factors increasing Leptin	**Factors reducing Leptin**
Excess energy stored as fat (obesity)	Low energy states with decreased fat stores (Leanne's)
Overfeeding	Fasting
Glucose	Cold exposure, and adrenergic agonists
Insulin	Thyroid hormone
Estrogen	Testosterone
Pro inflammatory cytokines (TNF-α , IL-6)	
TNF, (tumor necrosis factor), IL(interleukin).	

2.4.Leptin and reproductive processes in women

Administration of leptin induces an increase both LH concentrations and in ovarian and uterine weights. leptin could play a physiological role during the normal menstrual cycle. The fact that serum leptin values are lower in men than in women and lower in postmenopausal than in premenopausal women indicates that leptin production may be affected by gonadal steroids (Agarwal *et al.*, 1999; Kendall *et al.*, 2004). It is possible that gender differences are related to testosterone which in men shows an inverse relationship with leptin values (Osuna C *et al.*, 2006). however, leptin as a signal of nutritional status linked to the reproductive process The amount of body fat stored is known to influence fertility, indicating a link between adipose tissue and the reproductive system (Mathew, Castracane and Mantzoros, 2018), an interesting hypothesis is that leptin is a peripheral signal indicating the adequacy of nutritional status for reproductive function. Neuropeptide Y synthesis is decreased, diminishing the appetite and increasing whole-body energy expenditure and weight loss. Therefore, leptin communicates the size of the adipose reserve to the hypothalamus. Leptin is also synthesized in the reproductive tissues and it is related to the hypothalamus-pituitary-ovary axis function. Gonadotropin-releasing hormone (GnRH) and LH pulses can be related to leptin actions. Leptin may directly regulate the function of the reproductive organs and, via paracrine effects, may regulate estradiol synthesis. In addition, estradiol concentrations could also influence leptin synthesis. Leptin may be a signal of metabolic status to the reproductive system (Odle *et al.*, 2018; Iwasa *et al.*, 2021).Leptin could be a regulator of hypothalamic –pituitary-ovarian function (Figure 2.9) It has been suggested that leptin is a metabolic signal to the reproductive axis in primates, where leptin increases the plasma concentrations of LH and follicle stimulating hormone (FSH) (Malendowicz *et al.*, 2007), and LH pulse frequency and amplitude (Licinio *et al.*, 1997). Different degrees of

subnormal gonadotrophin secretion with lower LH compared with FSH secretion have been found in patients suffering from diverse degrees of severity of weight loss (Conway and Jacobs, 1997). Severely food-restricted animals have reduced circulating concentrations of leptin, which are associated with markedly reduced secretion of LH and FSH (Conway and Jacobs, 1997). Reducing the amount of nutrition during adulthood can lead to infertility, primarily through reduction of gonadotrophin-releasing hormone (GnRH) secretion(Brannian, Zhao and McElroy, 1999; Odle *et al.*, 2018). Furthermore, patients with hypothalamic amenorrhea are characterized by lower leptin concentrations than in eumenorrhoeic controls (Andrico *et al.*, 2002). FSH administration induces a parallel increase in serum oestrogen and leptin concentrations (Licinio *et al.*, 1997).

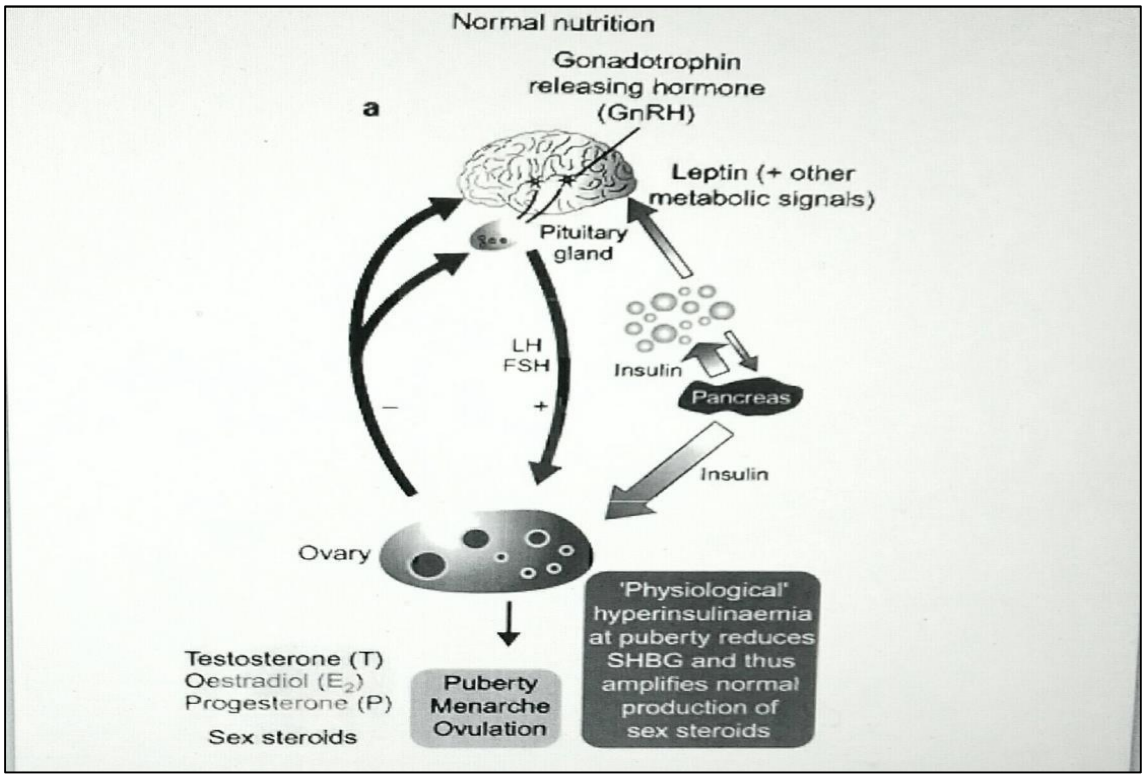


Figure 2.10. Hypothetical model of relationships between leptin and the pituitary-ovary axis (Metwallyetal.,2007)

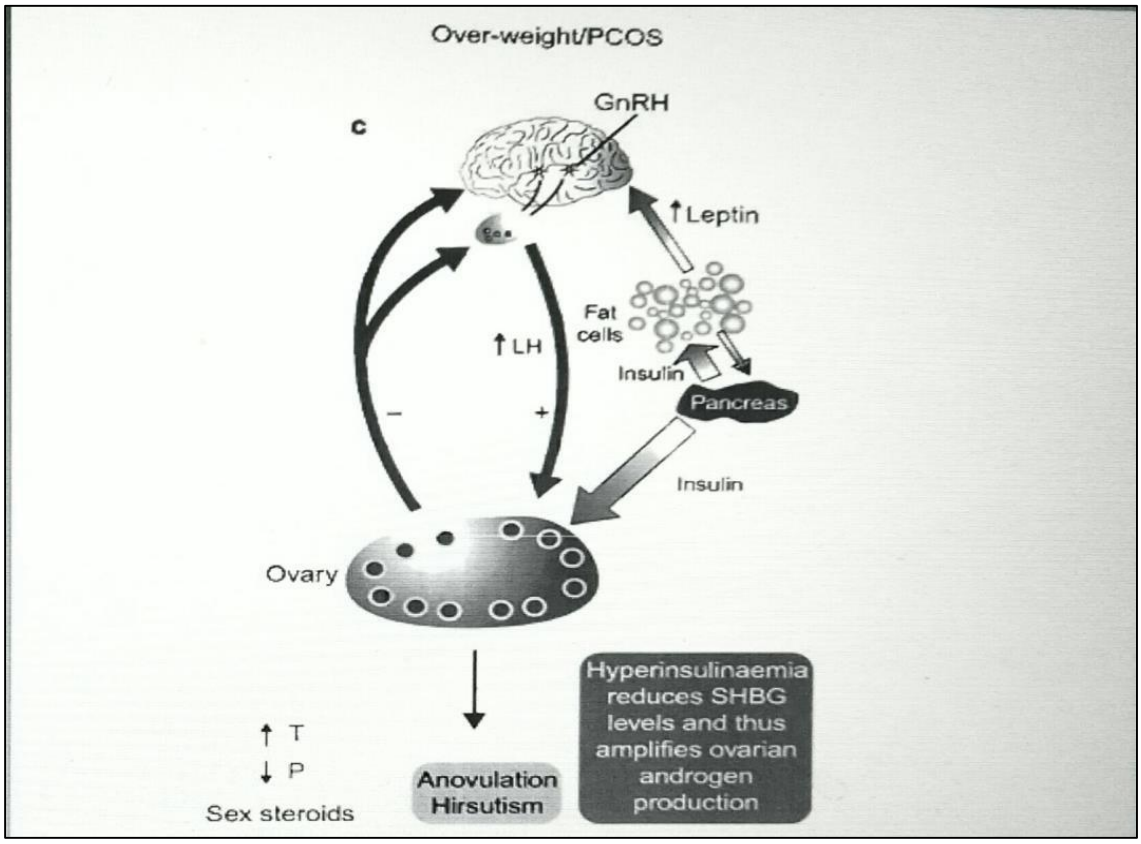
Leptin is a molecular signal from adipose tissue that regulates the food intake,

presumably through neuropeptide Y actions. Recent data support the idea that leptin binds to the short form of its receptor to transport itself into the arcuate hypothalamic region (Zhou and Rui, 2013)**.**

2.5. Leptin and Female obesity and Infertility

Obesity is not only influenced by lack of leptin but also leptin resistance . Leptin has been power to increase with increasing adiposity in humans and rodents (Myers Jr *et al.*, 2010). Given that the presence of leptin reduces food intake and body weight, elevated levels of leptin in obese persons is viewed as leptin resistance. (Metwally, Li and Ledger, 2007; Obradovic *et al.*, 2021). In these cases, humans lack the responsiveness to the appetite reducing effects of leptin. The effects of leptin resistance are however reversible. If the fat content of obese mice is reduced, the mice will recover leptin sensitivity and glycemic control (Scarpace and Zhang, 2009; D'souza *et al.*, 2017; Izquierdo *et al.*, 2019). believed that decreased leptin sensing in the melanocortin circuits influences the pathology of leptin resistance (Enriori *et al.*, 2007; Myers, Cowley and Münzberg, 2008). In fact leptin acting through the receptors on the theca and granulosa cells inhibits production of sex steroids in the ovary (Brannian and Hansen, 2002; Moschos, Chan and Mantzoros, 2002) The inverse correlation Of adiponectin to insulin ratio (Gil-Campos and Ramon, 2004) causes elevation of androgen levels (Metwally, Li and Ledger, 2007). In addition, insulin acting via insulin like growth factor1(IGF1) enhances luteinizing hormore-mediated increases in ovarian androgens (Bergh *et al.*, 1993). On the other hand, peripheral conversion of androgens to estrogen in adipose tissue inhibit gonadotroph in secretion (Corbould, 2008). Increased androgens in turn contribute to apple and not pear shaped obesity (Corbould, 2008); thus, a vicious circk evolves where abdominal fat accumulation increases insulin resistance and

androgen production, with hyperandrorogenemia promoting hyperinsulinemia and so forth(Garg, 2004; Knudsen *et al.*, 2010).



Figure(2.11) Leptin and female overweight and hormones (Metwallyetal.,2007)

The strongest consequence of obesity on fertility is anovulation. Polycystic Ovarian Syndrome (PCOS) related to obesity, being a well- known cause of ovulatory dysfunction. is furthermore exacerbated by increase insulin resistance and hyperinsulinemia (Brannian and Hansen, 2002; Pasquali, Patton and Gambineri, 2007) in 65%of patients with PCOS Obesity shares one common denomitutor to anovulation (Pasquali *et al.*, 2003). Most of the studies have shown that BMI is the regulator for all the correlations seen between leptin and other parameters in women with PCOS (Jalilian, Haghnazari and Rasolinia, 2016; Daghestani *et al.*, 2018)

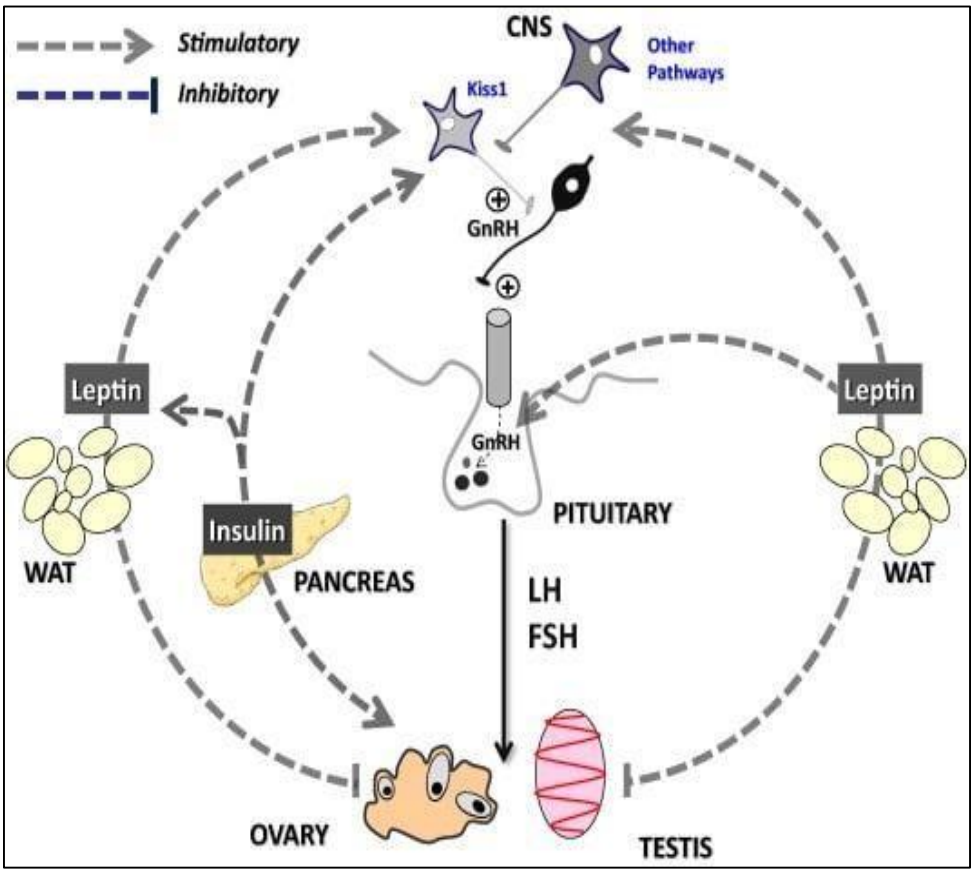


Figure 2.12. Leptin level Regulation and hormone (Cunningham, Clifton and Steiner, 1999)

Chapter Three

Materials and Methods

3.1.Subjects and Study design:

This study was designed a case control study conducted during the period from December 2020 to July 2021 at Kamal Alsamurai Hospital (center for fertility and treatment infertility & IVF) and in the privet clinic in Baghdad Iraq**.**

The study protocol approved by the research Local and Ethical Committe in College of Medicine in University of Babylon under the reference number. BMS/0256/016 a verbal and signed consent obtained from all participants prior to their Enrollment in the study, all individuals participating in the study gives , a verbal agreement.

3.2. Patients' groups:

The study included 200 participants attending to Kamal Alsamurai Hospital center for (fertility and treatment infertility& IVF) and in the privet clinics at reproductive age (15-45) years.

As cases group, 100 women with confirmed primary subfertility were included. Additionally, 100 healthy fertile women almost matched to cases regarding their demographic characteristics were included as control group.

3.3. Inclusion criteria

1.Women aged 15-45 years

2.Women consented to participate who have confirmed primary subfertility due to Female factors, unexplained or combined male and female factor.

3.4.Exclusion criteria

Women with one or more of the following were excluded from the study:

1.History of diabetes mellitus.

2.Currently have thyroid disease.

3.non- smoker**s**

3.5. Data evaluation:

Data evaluated using data evaluated sheet including**: -**

***Demographic variables**; age, address, level of education, occupation (job), family history, medical and surgical history, smoking habit, menstruation history, previous IVF, ICSI, and other.

***Anthropometric measurements;**

Thigh circumference, arm circumference, body weight, body height and then body mass index (BMI) which was calculated according to the standard equation (Fryar *et al.*, 2018) as :

$$\text{BMI} = \frac{\text{Body weight (kg)}}{(\text{Body height in meter})^2}$$

Body mass index categorized according to the World Health Organization classification of BMI for adults as followed (Fryar *et al.*, 2018; Weir and Jan, 2021):

BMI (kg/m^2)	Category
< 18.5	Underweight
18.5 – 24.9	Normal
25 – 29.9	Overweight
≥ 30	Obese

***Biochemical tests**: were performed in the second day of menstrual cycle including FSH, LH, AMH, Prolactin, Estradiol, testosterone and Leptin were performed for all study participants.

***Ultrasonography findings**: Trans-vaginal ultrasonography performed for all participant by experienced professional radiologist to assess endometrial thickness and antral follicle count (AFC). Vaginal ultrasound scan is performed using real time ultrasound devise (Philips11, E), using vaginal probe (5-7MHZ). The scan is used to check the uterus, ovaries and surrounded structures,at day 2 or 3 of cycle all these data were gathered through, detailed history taking, thorough physical examination, laboratory tests and ultrasonography examination.

3.6.Instruments and Equipment used in the study :

Table (3.1) The apparatus used in this study.

Instrumental	Supplied Company
ELISA System	Italy reader: PPC246 washer:PPC245
Eppendorf's	Germany
Microliter wells No.96	Germany
Micro Pipette	USA
Centrifuge	German
Microliter Plate for wells	Italy
Digital camera	Canon IXUS 11015, Japan
Tape measurement for length	China
Distillator	England
Air drier	USA
Balance for measurement weight	USA
Syringe with needle	USA
Digital timer	Germany
Absorbent paper	USA
Pipette Tips	USA
Cylinder & beaker	Germany
Laminar air flow hood	Germany
Transvaginal ultrasound	Germany
Autoclave	Germany
test tube	USA
BMI calculator	USA
Refrigerator deep frizzing for keep the samples	Germany
Container to keep samples	USA

3.7.Chemicals used in the study

Table(3.2) The Chemicals used in this study.

Chemicals	Origin
Serum LH Kit	Germany
Serum FSH Kit	Germany
Serum AMH Kit	Germany
Serum Prolactin Kit	Germany
Serum Testosterone Kit	Germany
Serum Estradiol (E2) Kit	Germany
Serum Leptin Kit	Germany
Reagents	Germany
Standard solution from 0-5 (S0,S1,S2,S3,S4,S5)	Germany
Control low-lyoph solution	Germany
Control high-lyoph solution	Germany
Assay Buffer solution	Germany
Anti-serum solution	Germany
Enzyme complex solution	Germany
Wash solution	Germany
Substrate solution	Germany
Stop solution	Germany

3.8. Methods

3.8.1 Specimen collection and preparation:

(1). Collection of samples

A sample of 10 ml venous blood was collected, 6 ml for the analysis of estradiol, testosterone, prolactin, LH, FSH and AMH levels was conducted in the biochemistry laboratory by ELISA. The remained 4 ml blood samples were centrifuged at 5000 rpm for 5 minutes (1792 g), by placing them into Eppendorf tubes, the samples collected were preserved at-8 °C before assessment.

(2). Fertility Hormones reference ranges

Table (3.3) Normal value of fertility hormones for women

Fertility Hormones			
Test name		**Reference range**	**Unit**
LH	Follicular phase	2.4 – 12.6	mIU/ml
	Mid-cycle	14 – 96	
	Luteal	1.0 – 11.4	
	Postmenopausal	7.7 – 59	
FSH	Follicular phase	3.5 – 12.6	mIU/ml
	Mid-cycle	4.7 – 21.5	
	Luteal	1.7 – 7.7	
	Postmenopausal	25.8 – 134.8	
AMH	Optimal fertility	4 – 6.8	ng/mL
	Satisfactory fertility	2.2 – 4.0	
	Low fertility	0.3 – 2.2	
	Very low undetected	0.0 – 0.3	
	High level	(>6.8)	
Prolactin		N: 6.0 – 29.9	ng/mL
Testosterone (total)		0.06 – 0.82	ng/mL
Estradiol (E2)	Follicular phase	12.5 – 166	Pg/mL
	Luteal	43.8 – 211	
	Postmenopausal	5.0 – 45.7	
	Ovulatory phase	85.5 – 498	

3.9. Leptin Kit procedure

All procedures and methods were performed according to the manufacturer's instructions guide provided with the Leptin-Sandwich ELIZA kit, DRG diagnostics, Instruments GmbH, Germany, (Chow and Phoon, 2003; DRG Instruments GmbH, 2021)

3.9.1.Reagent Preparation

1. **Standard**

   Reconstitute the lyophilized contents of each vial with 0.5 mL deionized water and let stand for at least 10 minutes at room temperature. Mix several times before use. The reconstituted standards are stable for 6 weeks at 2 - 8 °C for longer storage freeze at -20 °C

2. **Controls:**

   Reconstitute the lyophilized content each vial with 0.5 mL deionized water and let stand for at least 10 minutes at room temperature. Mix the control several times before use. The reconstituted controls are stable for 6 weeks at 2 - 8 °C for longer storage freeze at -20

3. **Wash Solution**

   Add deionized water to the concentrated Wash Solution.

   Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 me The Diluted Wash Solution is stable for 2 weeks at room temperature.

4. Test Procedure:

Each run must include a standard curve.

1. Secure the desired number of microliter wells in the frame holder.
2. Dispense 15 mL of each Standard, Control and samples with disposable tips into appropriate wells.
3. Dispense 100 Assay Buffer into each well. Thoroughly mix for 10seconds, it is important to have a complete mixing in this step.
4. Incubate for 120 minutes at room temperature.
5. Briskly shake out the contents of the Wells.
6. Rinse the wells 3 times with 300 mL diluted Wash Solution per well. Strike the wells sharply on absorbent paper to remove residual droplets. Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 100 mL Antiserum to each well.
8. Incubate for 30 minutes at room temperature.
9. Briskly shake out the contents of the wells.
10. Rinse the wells 3 times with 300 ml- diluted Wash Solution per well. Strike the wells sharply on absorbent paper to remove residual droplets.
11. Dispense 100 ml- Enzyme Complex into each well.
12. Incubate for 30 minutes at room temperature.
13. Briskly shake out the contents of the wells. Rinse the wells 3timeswith300 ml- diluted Wash Solution per well. Strike the wells sharply on absorbent paper to remove residual droplets.

14.Add 100 mL of Substrate Solution to each well.

15.Incubate for 15 minutes at room temperature.

16.Stop the enzymatic reaction by adding 50 NL of Stop Solution to each well.

17. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microliter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

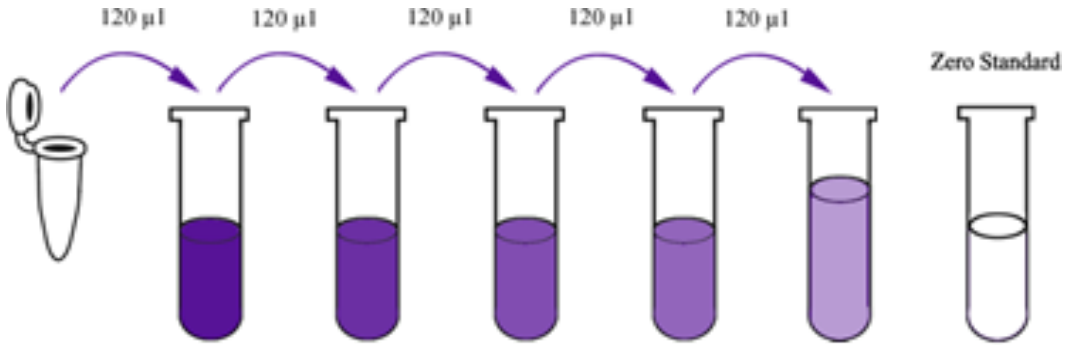


Figure 3.Steps of test procedures

(DRG Instruments GmbH, 2021)

3.9.2. Principle of the test

The DRG Leptin Sandwich ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Leptin in serum.

The DRG Leptin Sandwich ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microliter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule. An aliquot of patient sample containing endogenous Leptin is incubated in the coated well with a specific biotinylated monoclonal anti-Leptin antibody. A sandwich complex is formed. After incubation the unbound material is washed off and a Streptavidin Peroxidase Enzyme Complex is added for detection of the bound Leptin. Having added the substrate solution, the intensity of color developed is proportional to the concentration of Leptin in the patient sample.

Figure 3.1. measurement of leptin serum sample in microplate by ELISA system

3.9.3.ELISA Analysis (DRG Instruments GmbH, 2021)

Two Human Sandwich leptin ELISA kits EIA-2395 Lot 45K070 (company name DRG instrument Gmbh.),were used to evaluate leptin in blood samples, and two ELISA kits were used to evaluate leptin by Chromate, Microplate Reader P4300 (Awareness Technology, USA). The values of coefficient variance (CV) was tagged as <10 by the kit by the company that manufactured it. The kit measurement gap was 15.6-1000 pg./ml leptin, changed to ng/ml according to current leptin research studies in this field. Our findings could therefore be easily contrasted with other studies that examined leptin in different diseased conditions.

Figure 3.2. ELISA system at measurement of serum leptin

3.10.Calculation of Results

a) Calculate the average absorbance values for each set of standards, controls and patient samples.Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each Standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

b) Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

c) Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rod bard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.

d) The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Table3.4.Relation between concentration of controls &standard with optical density (OD)

Controls	Acceptance Range ng/mL	Observed concentration ng/mL	Standards (Calibrators) ng/mL name		OD at 450 nm 620 nm Ref	Acceptance Range Max. OD
45KL040	3.86 - 10.2	7.49	S0	0	0.02	≥ 1.20
45KH040	27.2 -71.6	50.9	S1	2	0.18	
			S2	5	0.41	
			S3	25	1.26	
			S4	50	1.72	
			S5	100	2.14	

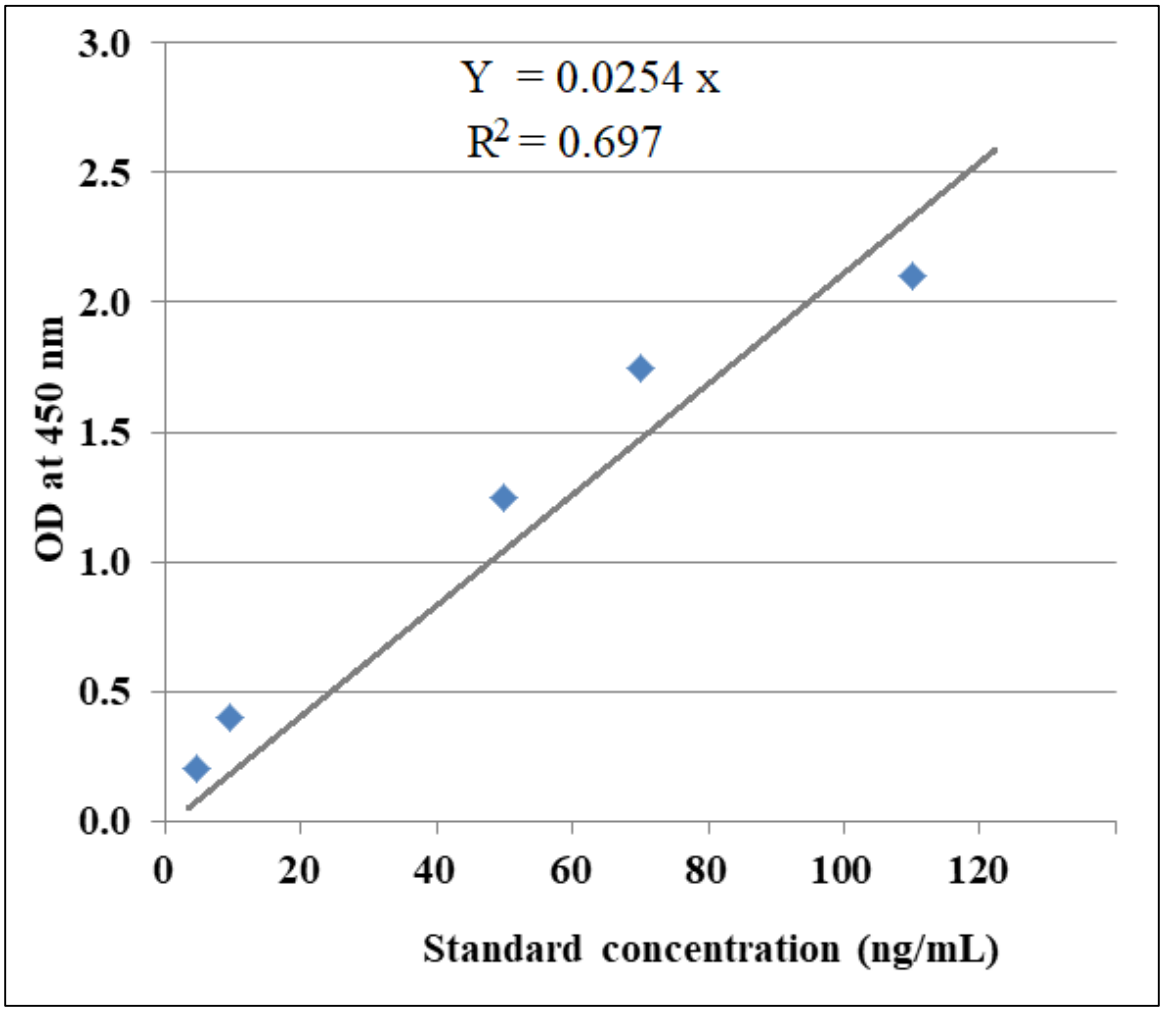


Figure 3.3. Standard curve

3.11.Expected Normal Values

laboratory should determine its own normal and abnormal values, in a study conducted with apparently healthy adults. using the DRG Leptin sandwich ELISA, the following data were Observed (DRG Instruments GmbH, 2021).

Table 3.5.Normal value about leptin hormone both in females &males

Population	**ng /mL**
Males	3.84 ± 1.79
Females	7. 36 ± 3.73

The results alone should the only reason for any therapeutic consequences the results should be correlated to other clinical observations and diagnostic tests.

3.12.Quality Control

Controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. validity of results. Use controls at both normal and pathological levels.

3.13.Performance characteristics

Assay Dynamic Range : Between 0.7 - 100 ng/mL

3.14. Statistical analysis:

Data of cases and controls were checked for any error or inconsistency , then transformed into computerized database using Microsoft Excel Software, 2019.

The entered data then exported to the Statistical package for Social Sciences (SPSS) Software, IBM, Chicago, US, version 26. All continuous variables like age, anthropometric measurement, hormones, endometrial thickness and antral follicle count were assessed for normal statistical distribution.

Continuous variables presented as mean (± Standard deviation), median and range, accordingly. Categorical variables , like age groups, education level, hob, family history and surgical history expressed as frequencies (number of subjects) and percentages.

Continuous variables compared between cases and controls using the Student's t test for two independent groups, categorical variables compared using chi-square test or Fisher's exact test when chi square was inapplicable. Binary Regression analysis was performed to assess the association between subfertility and other variables as predictors, Odds Ratio (OR) was calculated which is an indicator of the strength of association. Moreover, to assess the validity of leptin as predictor of subfertility, Receiver Operating Characteristics (ROC) curve analysis was performed at different cutoff point to get the optimal cutoff point and the higher area under the curve (AUC) associated with that cutoff point that produce the highest validity parameters (sensitivity, specificity, accuracy, positive and negative predictive values). It is worth mentioned, that the higher AUC close to one indicates the good validity of a test while AUC of 0.600 or lower indicates

failure of a test to predict the outcome. All statistical tests and procedures performed under the assumption that power of the study of 0.80 or more and a level of significance, P. value, of 0.05 or less to be significant difference or association. Finally, all findings presented in tables and or figures with an explanatory paragraph for each using Microsoft Word version 2019.

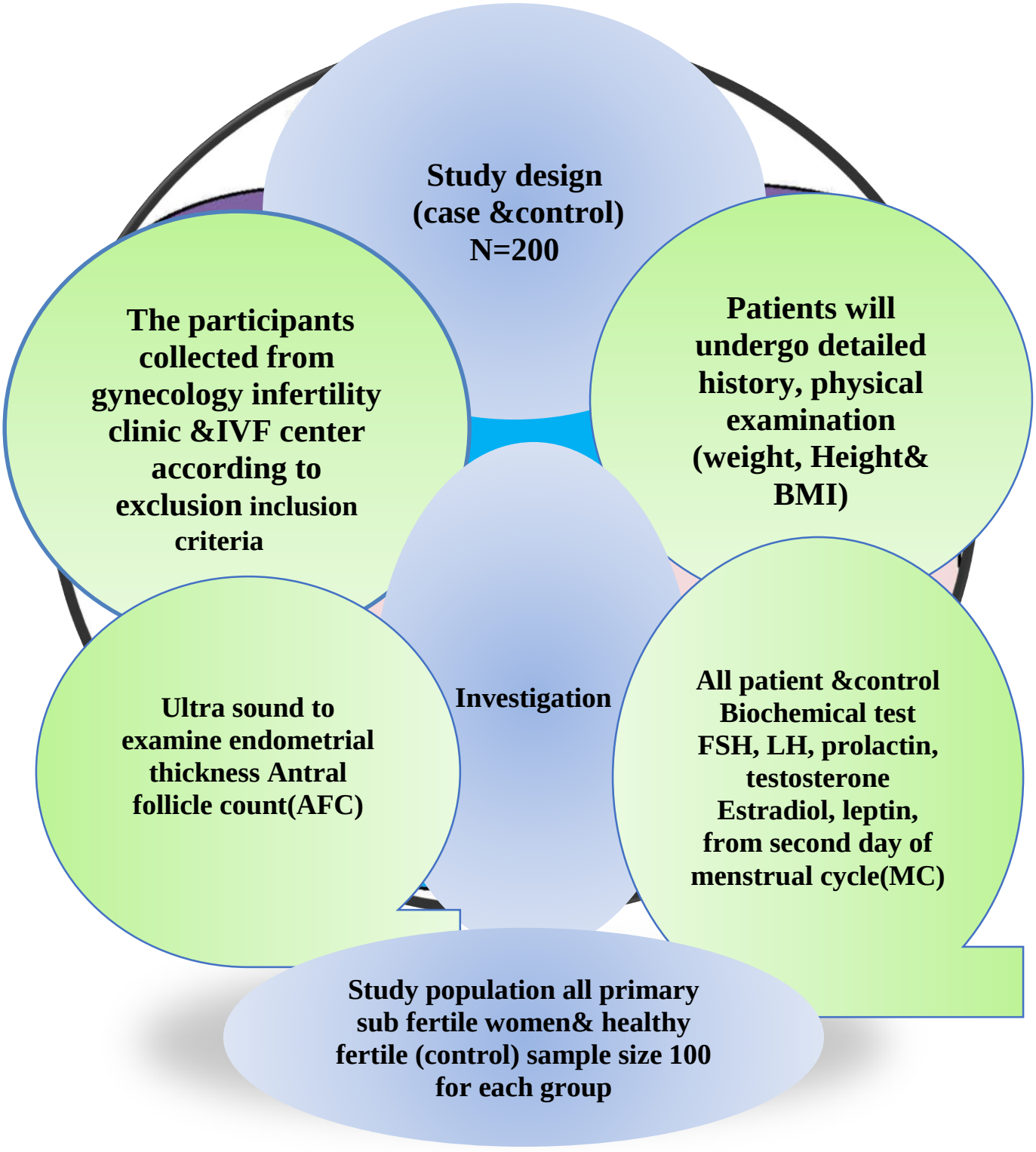


Figure 3.4. Schematic diagram of study design

Chapter Four

Results

4.1. Demographic Characteristics of the studied groups

One hundered infertile women (cases) and 100 fertile women (control) enrolled in this study, with a mean age for cases and control of 29.7 ± 6.5 years and 29.1 ± 8.2 years, respectively. The demographic characteristics are shown in (Table 4.1) where both groups show no significant difference in age, level of education, job, family history of infertility or surgical history, in all comparisons ($P > 0.05$).

Table 4.1. Demographic characteristics of the studied groups

Variable		Cases (n=100)		Controls (n=100)		P. value
		No.	%	No.	%	
Age (year)	≤ 20	6	6.0	7	7.0	0.832^{ns}
	21 – 30	56	56.0	54	54.0	
	31 – 40	33	33.0	31	31.0	
	41 – 50	5	5.0	8	8.0	
Mean age ±SD		29.7 ± 6.5		29.1 ± 8.2		0.567 ^{ns}
Education	Primary	10	10.0	8	8.0	0.751^{ns}
	Secondary	35	35.0	32	32.0	
	College or higher	55	55.0	60	60.0	
Job	Housewife	69	69.0	65	65.0	0.547^{ns}
	Employed	31	31.0	35	35.0	
Family history of infertility	Yes	4	4.0	3	3.0	0.700^{ns}
	No	96	96.0	97	97.0	
Surgical history	Gynecological ad obstetrical	7	7.0	8	8.0	0.580^{ns}
	General surgeries	6	6.0	3	3.0	
	None	87	87.0	89	89.0	

ns: not significant

4.2. Anthropometric measurement of the studied groups

No significant differences had been reported between both groups regarding their anthropometric measurement including, thigh circumference, arm circumference , body weight , body height and body mass index (BMI), in all comparisons, P value > 0.05, nonetheless, infertile women seemed to have relatively higher BMI, where 33 infertile women were obese compared to 29 women in control group, but the difference did not reach the statistical significance, (P>0.05), (Table 4.2 and Figure 4.1)

Table 4.2. Comparison of anthropometric measurements of the studied groups

Variable*	Cases (n=100)	Controls (n=100)	P. value
Thigh circumference (cm)	50.6 ± 6.3	50.4 ± 5.7	0.759^{ns}
Arm circumference (cm)	32.1± 4.9	31.6 ± 6.1	0.514^{ns}
Body weight (kg)	72.8 ± 11.8	71.6 ± 11.6	0.469^{ns}
Body height (cm)	161.4 ± 6.0	161.8 ± 4.9	0.604^{ns}
BMI (kg/m^2)	28.9 ± 4.7	27.4 ± 4.3	0.233^{ns}

All variables presented as Mean ± standard deviation

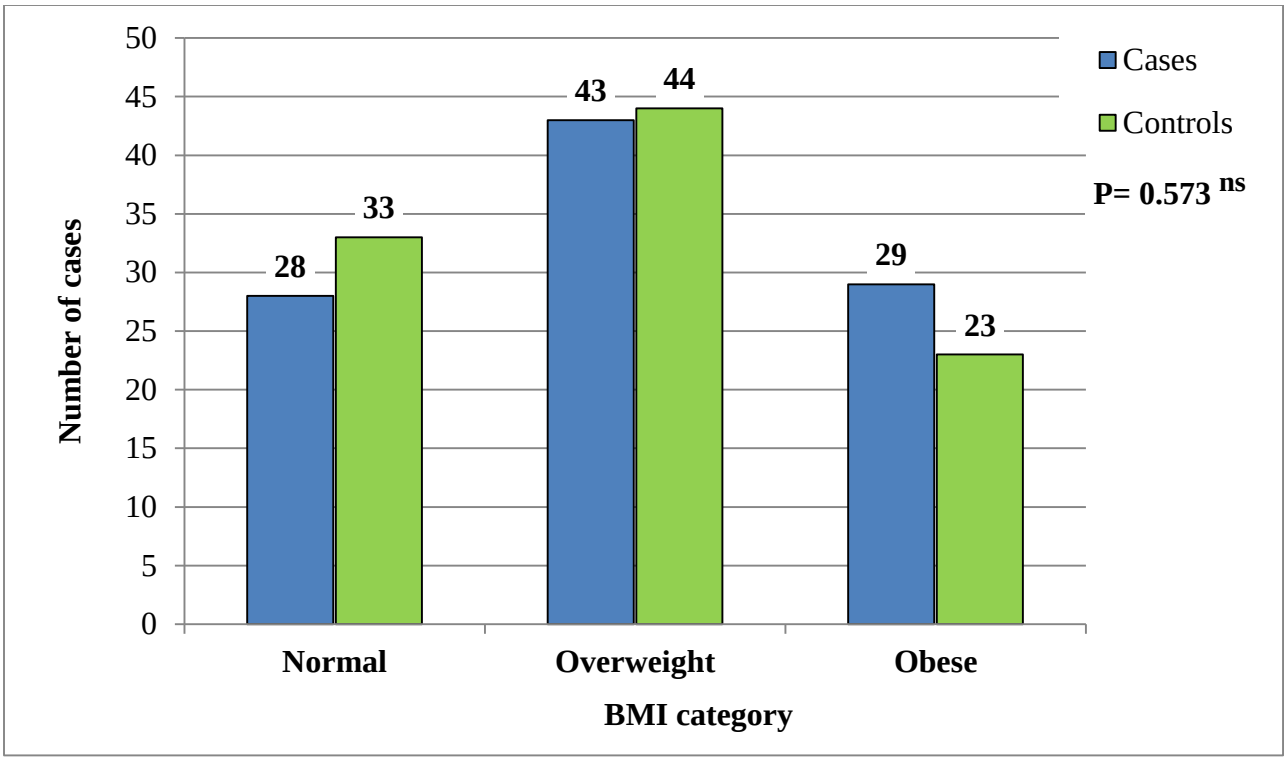


Figure 4.1. Distribution of the studied groups according to their body mass index (BMI) categories.

4.3. Hormonal indices, endometrium thickness and antral follicle count

Regarding hormonal indices, follicle stimulating hormone (FSH) , luteinizing hormone (LH), testosterone and prolactin were significantly higher in infertile women than controls, (P. value <0.001). Leptin level in infertile women was much higher than controls with highly significant difference, the mean level was 26.8 ± 15.2 ng/mL vs. 6.4 ± 2.3 ng/mL in cases and controls, respectively, (P. value < 0.001). On the other hand, anti-Müllerian hormone (AMH) and antral follicle count (AFC) were significantly lower in cases than controls, (P<0.001). Additionally, neither estradiol (E2) nor the endometrial thickness showed significant differences between cases and controls (P>0.05), (Table 4.3)

Table 4.3. Comparison of hormonal indices , endometrium thickness and Antral follicle count of the studied groups

Parameter**	Cases (n=100)	Controls (n=100)	P. value
FSH (mIU/mL)	8.2 ± 2.9	6.3 ± 1.7	< 0.001*
LH (IU/L)	7.8 ± 2.1	5.9 ± 1.6	< 0.001*
Testosterone (ng/mL)	0.85 ± 0.17	0.53 ± 0.11	< 0.001*
Prolactin (ng/mL)	19.4 ± 9.0	13.9 ± 6.1	< 0.001*
Leptin (ng/mL)	26.8 ± 15.2	6.4 ± 2.3	< 0.001*
AMH (ng/mL)	1.7 ± 1.2	3.0 ± 1.8	< 0.001*
AFC (per ovary) median (range)	10 (2 – 32)	13 (7 – 50)	0.015*
E2	35.7 ± 14.5	37.6 ± 20.7	0.451^{ns}
Endometrial thickness	4.3 ± 1.7	4.6 ± 2.5	0.436^{ns}

FSH: Follicle stimulating hormone , LH: luteinizing hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count .
*significant difference , ns: not significant
**all levels of parameters presented as mean ± standard deviation (SD) except AFC as median and (range)

4.4. Causes and duration of infertility among infertile group

The main causes of infertility among cases were female factors which are reported in 49 cases (49%) followed by Unexplained infertility in 39% of cases then combined male and female factors in 12% of cases. Regarding the duration of infertility it ranged between 1-18 years, however, majority of cases, (77%), had duration of 3 years or more, (Table 4.4)

Table 4.4. Causes and duration of infertility among infertile (cases) group (N = 100)

Variable		No.	%
Causes	Female factor	49	49.0
	Unexplained factor	39	39.0
	Male and female factor	12	12.0
Duration (year)	1 - < 3	23	23.0
	3 - < 6	43	43.0
	6 - < 10	23	23.0
	> 10	11	11.0

4.5. Serum Leptin levels according to causes of infertility

As shown in (Table 4.5),when infertile women categorized into subgroups according to the cause of infertility and their leptin concentration compared to that of controls, all these subgroups had significantly higher serum leptin levels than controls, (P<0.001). From other point of view, women with female factors had lower serum leptin levels compared to other subgroups, however, the difference between these subgroups in serum leptin levels was statistically insignificant, (P>0.05).

Table 4.5. Multiple comparison of serum Leptin levels among cases subgroups with different causes of subfertility against control group

Group		Number of subjects	Serum Leptin level (ng/mL)	P. value vs. control
			Mean ±SD	
Cases' subgroup	Female factor	49	26.6 ± 16.9	<0.001*
	Unexplained factor	39	26.7 ± 13.6	<0.001*
	Male and female factors	12	28.4 ± 18.4	<0.001*
Control		100	6.4 ± 3.9	-
P. value between subgroups of subfertile women			0.935^{ns}	

*significant, ns: not significant

4.6.Serum Leptin levels according to BMI

Comparison of serum leptin levels according to BMI in both studied groups, revealed that 28 infertile women who had normal BMI had the lower serum leptin level, (mean ± SD: 19.4 ± 5.6 ng/mL) compared to the mean level in 43 overweight subfertile women (26.3 ± 6.7 ng/mL) and obese women had the higher serum leptin level (mean ± SD: 34.7 ± 8.9 ng/mL), with highly significant difference, (P<0.001), indicated that serum leptin level much increased with heavier BMI. In control group, similar trend where obese women had relatively higher leptin level than overweight and normal BMI subgroup, but the difference in leptin level across BMI categories did not reach the statistical significance, (P>0.05). On the other hand, subfertile women in all subgroups of BMI (normal, overweight and obese) had much higher leptin levels compared to controls, in all comparisons against controls, (P. value < 0.001), (Table 4.6)

Table 4.6. Comparison of serum Leptin levels among cases and controls according to BMI

BMI category	Cases (n = 100)		Control (n = 100)		P. value between groups
	N	Serum Leptin (ng/mL) Mean ± SD	N	Serum Leptin (ng/mL) Mean ± SD	
Normal	28	19.4 ± 5.6	33	6.3 ± 2.4	< 0.001*
Overweight	43	26.3 ± 6.7	44	6.4 ± 1.9	< 0.001*
Obese	29	34.7 ± 8.9	23	6.7 ± 1.8	< 0.001*
P. value within group		<0.001*		0.769 ns	
*significant difference, ns: not significant					

4.7.Serum Leptin levels according to age

According to age, no significant difference was found in serum leptin levels when compare across the age groups in cases and control group, (P> 0.05). But subfertile women in all age groups had significantly higher serum leptin levels than their corresponding controls, (P<0.001), (Table 4.7)

Table 4.7. Comparison of serum Leptin levels among cases and controls according to age

Age (year)	Cases (n = 100)		Control (n = 100)		P. value between groups
	N	Serum Leptin (ng/mL) Mean ± SD	N	Serum Leptin (ng/mL) Mean	
≤ 20	6	26.7 ± 9.3	7	6.0 ± 2.9	< 0.001*
21 – 30	56	25.6 ± 9.8	54	6.5 ± 2.0	< 0.001*
31 – 40	33	29.0 ± 14.9	3	6.4 ± 2.1	< 0.001*
41 – 50	5	25.9 ± 11.2	8	6.1 ± 2.1	< 0.001*
P. value within group		0.746 ns		0.912 ns	
*significant difference, ns: not significant					

4.8. Correlation of serum Leptin with age , duration of infertility and anthropometric variables

Bivariate correlation analysis was performed to assess the significance of correlation between serum Leptin levels from one side with age, duration of infertility and anthropometric variables from the other side. Among cases, results of this analysis revealed no significant correlation between serum Leptin level and these variables except body weight and BMI , where a significant positive correlation was reported with body weight, (R = 0.356, P. value = 0.001), and BMI (R = 0.707, P. value < 0.001), (Table 4.8) Among controls, none of these variables showed significant correlation with serum Leptin levels, in all comparisons , P value > 0.05, (Table 4.8).

Table 4.8. Correlation of serum Leptin with patient age and anthropometric measurements in infertile women

Variable	Correlation coefficient (R)	P. value
Age	0.052	0.605 ns
Duration of infertility	0.042	0.678 ns
Thigh circumference (cm)	0.070	0.489 ns
Arm circumference (cm)	0.007	0.944 ns
Body weight (kg)	**0.356**	**0.001 ***
Body height (cm)	0.068	0.503 ns
BMI	**0.707**	**<0.001***
*significant, ns: not significant		

Table 4.9. Correlation of serum Leptin with age and anthropometric measurements in control fertile women

Variable	Correlation coefficient (R)	P. value
Age	0.133	0.188ns
Duration of infertility	0.187	0.722 ns
Thigh circumference (cm)	0.010	0.924 ns
Arm circumference (cm)	0.060	0.552 ns
Body weight (kg)	0.119	0.240 ns
Body height (cm)	0.046	0.649 ns
BMI	0.071	0.481 ns
ns: not significant		

4.9. Correlation of serum Leptin with hormonal indices and endometrial thickness

Bivariate correlation analysis for the correlation between serum Leptin with other hormonal indices and endometrial thickness in both studied groups revealed no significant correlation among these parameters with Leptin in both infertile women and control groups , (P. value > 0.05), (Tables 4.10 and 4.11)

Table 4.10. Correlation of serum Leptin with other hormonal indices and endometrial thickness in infertile women group

Parameter	Correlation coefficient (R)	P. value
FSH	0.120	0.234^{ns}
LH	0.102	0.144 ^{ns}
Testosterone	0.011	0.917 ^{ns}
AMH	0.001	0.994 ^{ns}
E2	0.063	0.532 ^{ns}
Prolactin	0.092	0.361 ^{ns}
AFC	0.038	0.709 ^{ns}
Endometrial thickness	0.013	0.899 ^{ns}
ns: not significant		

Table 4.11. Correlation of serum Leptin with other hormonal indices and endometrial thickness in fertile women control group

Parameter	Correlation coefficient (R)	P. value
FSH	0.137	0.176 ns
LH	0.136	0.141 ns
Testosterone	0.086	0.394 ns
AMH	0.120	0.233 ns
E2	0.160	0.111 ns
Prolactin	0.087	0.390 ns
AFC	0.044	0.664 ns
Endometrial thickness	0.058	0.568 ns
ns: not significant		

4.10.Association between the subfertility and other variables(Predictors) using binary logistic regression analysis

Further analysis with binary regression testing was performed to assess the predictors effect of different variables as predictors of subfertility , for this purpose, the parameters that showed significant differences between subfertile and fertile women such as hormonal levels and BMI used as covariates (independent variables) in the equation while subjects' group used as dependent variable, results of this analysis are shown in (Table 4.12) where 6 parameters appeared to be significant predictors of subfertility, however, the higher odds ratio (OR) reported with leptin (OR= 2.793) followed by testosterone (OR = 2.468), LH (OR = 1.421), AMH (OR = 1.360) , prolactin (OR = 1.182). BMI was also appeared as predictor of subfertility, (OR= 1.391, P = 0.011). Comparison of odds ratio of these parameters, indicated that leptin level was the stronger predictor of subfertility after adjustment for other variables where women with higher serum leptin levels about 2.8-fold more likely to be

subfertile. Other parameters; FSH, AFC and E2 appeared to be not significant predictors after adjustment for other parameters.

Table 4.12. Results of Binary regression analysis for the predictors of subfertility

Variables in the Equation	Odds ratio (OR)	95% C.I. for OR		P. value
		Lower	Upper	
FSH	1.381	0.9	2.118	0.139
LH	1.421	1.24	1.63	**0.018**
Testosterone	2.468	1.379	4.418	**0.002**
Prolactin	1.182	1.049	1.333	**0.006**
Leptin	2.793	1.189	6.561	**0.001**
AMH	1.36	1.108	2.642	**0.024**
AFC	1.067	0.926	1.229	0.369
E2	1.026	0.955	1.101	0.487
BMI	1.391	1.06	2.602	**0.011**

Highlighted cells refer to significant predictors of subfertility
Control group was the reference group

4.11. Validity of serum Leptin in prediction of Infertility

To assess the validity of serum Leptin as predictor of infertility, Receiver Operating Characteristics (ROC) curve analysis was performed (Figure 4.2), ROC is a plot of true positive rate against false positive rate for a test, giving an area under the curve (AUC) ranged between 0 and 1 , the higher AUC value close to one indicates the good prediction rate, in this analysis serum leptin level showed an AUC of 0.956 at a cutoff point of Leptin = 11 ng/mL, giving a high sensitivity specificity and accuracy of 96%, 98% and 96.9%, respectively with a positive predictive value of 98% and negative predictive value of 96.1%, which

indicate that serumLeptin level is an excellent predictor of infertility independent of other parameters., (Table 4.13).

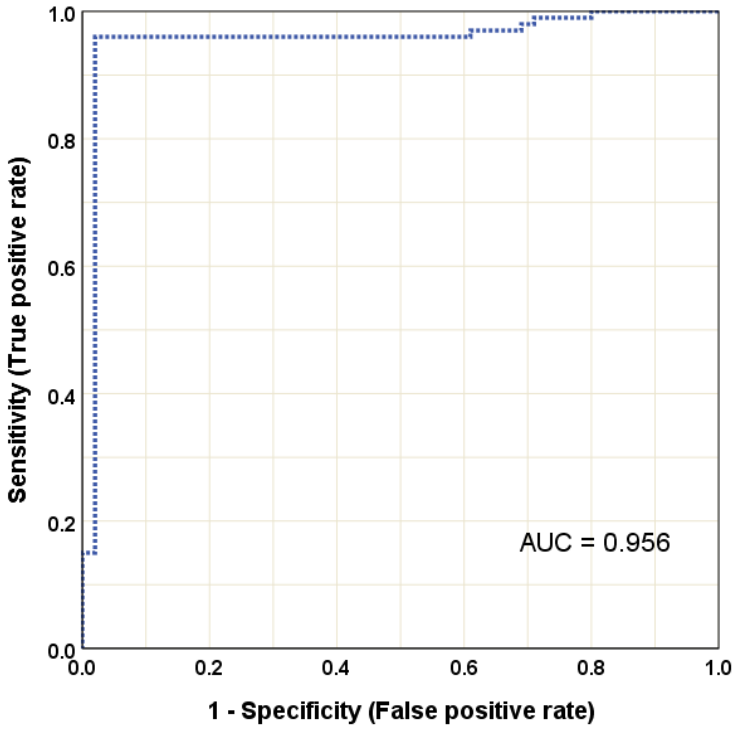


Figure 4.2. Diagram of Receiver Operating Characteristics (ROC) curve for the validity of serum Leptin level in prediction of infertility

Table 4.13. Validity parameter of serum Leptin level in prediction of infertility

Validity parameter	Value
AUC	0.956
Cutoff point of Leptin (ng/mL)	11.0
Sensitivity	96.0%
Specificity	98.0%
Accuracy	96.9%
Positive predictive value	98.0%
Negative predictive value	96.1%

Chapter Five

Discussion

Subfertility represents one of the significant health problem worldwide, and most of its burden on women. Previous studies are conflicting and there still a debate about the role of leptin in the process of fertility (Gambineri *et al.*, 2019; Al-Jawadi, 2020; Kargasheh *et al.*, 2021; Maharlouei *et al.*, 2021).

5.1. Demographic characteristics of the studied groups

The current case control study included 100 women suffering from primary subfertility for different causes and duration in addition to 100 fertile women as control group. The studied groups were almost matched for their demographic characteristics. Family history of infertility and surgical history were almost similar in both groups, this reflect the well homogenous sampling and selection of participants in both groups. It is well known that matching and homogenous population sample are important factors to control confounders and possible bias that affect case-control study designs, therefore, this matching strengthen our study and increased its power (De Graaf *et al.*, 2011; Ananth and Schisterman, 2017; Mansournia, Jewell and Greenland, 2018).

5.2. Anthropometric measurement of the studied groups

No significant differences were found in anthropometric measurement of women between cases and control groups. Nonetheless, we noticed that BMI of subfertile women was relatively higher than control groups, this could be attributed to the larger number of women with female causes of subfertility and had PCOS so they seemed to be heavier and more likely to be obese, where 29 subfertile women were obese compared to 23 women in control group. Previous studies documented a significant association between obesity and subfertility

particularly when PCOS was the cause of subfertility in addition to the relationship between obesity itself with PCOS (Al-Shattawi, Al-Jumili and Al-Azzam, 2018; Baheerati and Devi, 2018; Moridi *et al.*, 2019).

5.3. Hormonal indices, endometrium thickness and antral follicle count

Regarding hormonal studies, the current study found that FSH, LH, testosterone and prolactin were significantly higher in infertile women compared to controls, (P. value <0.001), these findings agreed that reported in previous studies; in a study conducted by Odiba et al. from Nigeria , the level of FSH and LH were significantly higher in primary infertile women compared to controls and also to those with secondary infertility (Odiba *et al.*, 2014). Similarly, Prasad et al. found that the mean levels of FSH and LH were significantly higher in infertile compared to fertile women (Prasad, Parmar and Sharma, 2015). From other point of view, a study conducted by Hashemi et al. from Iran found that FSH and LH were significantly higher in infertile women in general and in those with PCOS than controls (Hashemi, Mozdarani and Naghavi, 2016).

Conversely, Mohan K and Sultana M. reported lower levels of FSH and LH in women with primary infertility (Mohan and Sultana, 2010), inconsistent with our study, a recent Iraqi study conducted by Zena Al-Jawadi in 2020, found that the mean FSH level was significantly lower in infertile than fertile women, also she found lower LH level but not significant between both groups (Al-Jawadi, 2020).

However, it had been suggested that FSH and LH levels above the normal values indicate that the infertility may be due to a defect in the negative regulatory mechanisms in the hypothalamus by estrogen and progesterone, where a problem in the negative feedback regulation, while lower levels of FSH and LH than controls indicate that defects in pituitary gland , GnRH or hypothalamus could be the associated factors lead to infertility (Odiba *et al.*, 2014).

In our study we found that testosterone and prolactin were significantly higher in infertile women than controls, (P. value <0.001).

Zena Al-Jawadi from Iraq (Al-Jawadi, 2020), reported in 2020 a mean prolactin level of 30.7 ± 11.5 ng/ml in infertile women compared to 15.68± 7.1 ng/ml in control group.

Mohan and Sultana also reported higher level of prolactin in infertile women compared to controls(Mohan and Sultana, 2010). In an Iranian study conducted in 2015 by Kamyabi and Gholamalizade , authors found no significant difference in testosterone, FSH and LH levels between women with infertility (explained or unexplained) compared to fertile women (Kamyabi and Gholamalizade, 2015).

Isong et al. found in their study significant higher prolactin level in infertile women compared to normal control group (Isong *et al.*, 2016). So as the study of Prasad et al.(Prasad, Parmar and Sharma, 2015) documented significant higher prolactin levels in infertile women than controls , where they found a mean prolactin level in infertile women of 18.59 ± 7.5 compared to 13.44 ± 5.82 ng/ml in fertile women which close to our reported levels in both groups. It is worth mentioning that elevated levels of prolactin in infertile women was not unexpected, the high levels of prolactin with their effect on gonadotropins releasing hormone (GnRH) and ovulation inhibition are documented to result in women infertility (Henderson, Townsend and Tortonese, 2008). However, despite the fact that hyperprolactinemia causes infertility, but the exact mechanism for its effect is not well defined (Grattan *et al.*, 2007; McCann *et al.*, 2020).

Regarding serumLeptin levels in our study , we found much higher levels of serum leptin in infertile compared to fertile women, the mean level of srumleptine was 26.8 ± 15.2 ng/mLl vs. 6.4 ± 2.3 ng/ml , respectively, (P. value < 0.001), these findings agreed that reported in other studies; Zena Al-Jawadi found

a significantly higher mean serum leptin level of 39.2 ± 1.2 in infertile women and almost 35.4 ± 0.9 ng/ml in fertile control group (Al-Jawadi, 2020). Moreover, our finding consistent with that reported by Kumari et al. (Pratibha Kumari *et al.*, 2017), who found higher leptin levels in infertile than fertile women, however, the mean serum leptin level reported in Kumari et al. study was lower than ours, (Pratibha Kumari *et al.*, 2017) this may be due to difference in the included infertile women in their study where they recruited only women with unexplained infertility.

In contrary to our finding, a study conducted by Biag et al. (Baig *et al.*, 2019b) studied the association of serum leptin with other hormones, in women with unexplained infertility compared to fertile women and found no significant difference in serum leptin levels between fertile and unexplained infertile women. Also, an Iranian study performed by Tafvizi and Masomi documented a lower leptin level, (24.89 ± 2.93 ng/ml) in infertile than fertile women (31.2 ± 2.85 ng/ml) but the difference was statistically insignificant (Tafvizi and Masomi, 2016).

In the present study we found that AMH and AFC were significantly lower in infertile women than controls, this findings agreed that reported by EO et al. who found that AMH was significantly lower in infertile women and significantly associated with leptin level (Eo *et al.*, 2020). From other point of view, Kalaiselvi et al. showed that AMH is a novel marker for assessment of ovarian reserves (Kalaiselvi, Saikumar and Prabhu, 2012). Other studies found inverse correlation between leptin and AMH and other studies found stronger correlation in fertile than infertile women, therefore findings are conflicting regarding the correlation between AMH and leptin from one side and the role of AMH in infertility from the other side (Eo *et al.*, 2020). However, lower level of AMH in infertile women could be attributed to the reduced number of remaining primordial ovarian

follicles left (Al-Taee *et al*.,2012; Eo *et al.*, 2020).

In the present study AFC was significantly lower in infertile than fertile women (P<0.001). Similarly,Rosen et al. stated that lower AFC associated with infertility and the lower AFC suggest that factors affecting the size of the remaining follicle pool also affect oocyte quality and the likelihood of conception (Rosen *et al.*, 2011). Similar findings reported in India where Nayak et al. documented lower AFC in infertile than fertile women and that AFC was a good marker of ovarian reserve (Nayak, Mukherjee and Mitra, 2018). From other point of view, in cases with unexplained infertility, Greenwood et al. found no significant difference in AFC between cases with unexplained infertility and controls (Greenwood *et al.*, 2017).

5.4. Causes and duration of infertility among infertile group

In the present study, female factors were the more frequent causes of infertility, followed by unexplained infertility and the least frequent were the combined male and female factor which reported in 49%, 39% and 12%, respectively, these findings were not unexpected, it is widely postulated that, female factors and unexplained infertility are the main causes of infertility, however, there some variation in the rate of these factors in different studies and populations; in Iran , Kazemijaliseh et al reported that more than 55% of infertility was due to female factors among Iranian women, while unexplained infertility in 14.4% and male factors in 29.1%. (Kazemijaliseh *et al.*, 2015). The Iranian study attributed the high rate of female factors to ovulatory disorder among the Iranian women which may be due to older age at marriage and tendency to delayed childbearing among couples. Aging also blamed as one of the reasons of infertility, however, the risk factors and causes of infertility and their relationship with age are out of scope of our aim for the present study. Later study in 2019 from India conducted by Deshpande and Gupta found that only female factors represented 46.7% of all

causes of infertility, unexplained infertility in 33.3%, only male factor in 9.16% and combined causes in 10.3% (Deshpande and Gupta, 2019).

The duration of infertility in our study ranged between 1-18 years, and majority of cases, (77%), had a duration of 3 years or more, this relatively longer duration of infertility could be attributed to the delay in seeking medical care among infertile women or social cultural factors in our population that lead to longer duration before taking treatment, additionally, men could not accept the idea about the male factors of infertility that make the couples to be more delayed. An earlier Iraqi study documented a duration of primary infertility for a mean of 6.5 years in 2013 (Taha and Rashid, 2013) , However, this is not only in our society and population; Deshpande and Gupta from India documented that majority of couples take a mean of 6.7 ± 2 years before taking treatment for their infertility (Deshpande and Gupta, 2019). Other factors associated with longer duration before taking medical care and treatment could be due to taking complementary and alternative medicine (Bardaweel *et al.*, 2013; Özkan, Karaca and Sarak, 2018; Belhachemi *et al.*, 2020)

5.5. Serum Leptin levels according to causes of infertility

The present study did not find a significant differences in the levels of leptin across the causes of infertility, but in all subgroups (Female factors, unexplained, combined female and male factors), serum leptin levels were significantly higher than controls, which agreed the results of previous studies compared leptin levels in different causes of infertility (Chou and Mantzoros, 2014; Kamyabi and Gholamalizade, 2015; Baig *et al.*, 2019b).

Several earlier studies investigated the association between leptin and infertility, but the results are conflicting; Chou et al. (Chou and Mantzoros, 2014) documented that leptin concentration has no important role in the hypothalamic pituitary gonadal axis dysfunction or hyperandrogenemia and no significant role

in women infertility and that leptin did not have a direct role in the regulation of ovarian functionality , but its effect was through its direct correlation with BMI where obesity was the cause of infertility. Interestingly, Wartel et al. (Wertel *et al.*, 2005) studied the levels of leptin in three groups of infertile women according to their cause of infertility; and concluded that leptin was not involved in the pathophysiology of infertility. On the other hand, there is an evidence that leptin may have an inhibition effect on the ovarian sterodogenesis and inhibitory effect on development of ovarian follicles (Childs *et al.*, 2021; Nikanfar *et al.*, 2021).

5.6.Serum Leptin levels according to BMI

In the present study we found that serum leptin level much increased with heavier BMI in infertile women, while no such correlation in control group. The correlation between serum leptin and body mass index attributed to the fact that leptin secreted by white adipose tissue and its level reflects the volume of fat in the body (Childs *et al.*, 2021; Nikanfar *et al.*, 2021).

5.7. Serum Leptin levels according to age

According to age, no significant difference was found in serum leptin levels when compared across the age groups in both cases and controls, but subfertile women in all age groups had significantly higher serum leptin levels than their corresponding controls, despite aging is one of the significant risk factors of infertility because older women more likely to have ovulatory dysfunction, but in the current study 95% of infertile women aged at 40 or younger, so the correlation with age appeared to be not significant, additionally, the leptin level proved to be declined with aging, so that the higher level of serum leptin in infertile group could mask the effect of age on level of leptin in this group(Isidori *et al.*, 2000).Previous study, reported that after adjustment for BMI no significant association still between leptin and age of Chinese women(Zhong *et al.*, 2005)

5.8.Correlation of serum Leptin with age, duration of infertility and anthropometric variables

The current study did not find a significant correlation between serum leptin and each of age, duration of infertility and anthropometric variables in both studied groups, except body weight and BMI, where a significant positive correlation was reported with body weight and BMI in infertile women, but not among controls . Similar to our finding, Al-Jawadi et al. found no significant correlation between leptin level and adiposity indices except BMI. In contrast (Al-Jawadi, 2020), our findings inconsistent with that reported by Senghor and Vinodhini who found moderate positive correlation between leptin and adiposity indices including hip circumference and waist circumference, however, we included other indices which are thigh and arm circumference which may not reflect the real status of adiposity(Senghor, Shivashekar and Vinodhini, 2018). Moreover, a highly significant correlation was found between BMI and serum leptin among infertile Indian women while no such correlation among fertile women (P Kumari *et al.*, 2017)

5.9. Correlation of serum Leptin with hormonal indices and endometrial thickness

In the current study we did not find a significant correlation between leptin level and each of hormonal indices in both studied groups, ($P>0.05$). The correlation between serum leptin and other reproduction hormones is widely studied but the results of different studies are not all consistent and there still a need for further investigations. Baig et al. studied the relationship between leptin level and reproduction hormones at two phases , pre-ovulatory and luteal phase and found no significant correlation between leptin and each of FSH and LH but a significant correlation with E2 in both infertile and fertile women (Baig *et al.*, 2019b). In our study, despite the non-significant difference in E2 levels between

infertile and fertile groups, the infertile women seemed to have relatively lower E2 level compared to fertile women, Al-Jawadi et al. found significantly lower level of E2 in infertile group than controls , also our results agreed that of Isong et al. (Isong *et al.*, 2016)

In contrary, Farooq *et al.* found a strong significant negative correlation between leptin level and each of FSH , LH and testosterone in fertile obese women and health normal males and females (Farooq, Ullah and Ishaq, 2013).

Chou *et al.* reported that leptin level was not correlated with LH, FSH, testosterone or E2 levels (Chou and Mantzoros, 2014)

However, it is suggested that increased fat mass in infertile women may play a significant role in this correlation and act as confounder for such correlation(Childs *et al.*, 2021) . It has been suggested that leptin can have dual role in regulation of reproduction; it has negative effect on the neuroendocrine regulation when its level reduced below the normal level. When its level elevated above normal limit it will negatively affecting the normal ovarian function and even development and viability of embryo in fertile cases (Ashrafi Mahabadi and Tafvizi, 2020).

Endometrial thickness was not significantly different between fertile and infertile women in the current study and in both groups no significant correlation was found between leptin and endometrium thickness. In subfertile women, the endometrium seems to be thinner than fertile women,

Lindhard et al. found no significant difference in endometrium thickness among women with different cases of infertility and fertile women (Lindhard *et al.*, 2006). It has been suggested that leptin involved in angiogenesis and stimulation of endothelial cells enhances the angiogenesis, however, in some ovulating subfertile cases the endometrium become non receptive for implantation of embryo due to lack of leptin receptors (Alfer *et al.*, 2020). A study conducted by

Chakrabarti et al. (Chakrabarti *et al.*, 2012) found a negative (inverse) correlation between the level of serum leptin and endometrial thickness where patients with serum leptin level above 30 ng/ml had the thinner endometrium compared to those with lower level of leptin (Chakrabarti *et al.*, 2012), which inconsistent with our finding where no significant correlation was found in our study. However, endometrium thickness thought to be an indirect marker endometrium potential to maintain pregnancy and has been suggested that endometrium can be respond to leptin when there is expression of mRNA in secretory endometrium (Chakrabarti *et al.*, 2012)

5.10.Association between the subfertility and other variables(Predictors) using binary logistic regression analysis

In the present study, Binary regression analysis was performed and revealed 6 significant predictors of subfertility, these are leptin (OR=2.793), testosterone (OR = 2.468), LH (OR = 1.421), AMH (OR = 1.360) , prolactin (OR = 1.182). BMI was also appeared as predictor of subfertility, (OR= 1.391, P = 0.011). Comparison of these odds ratio revealed that Leptin was the stronger predictor after adjustment for other variables and women with higher leptin levels of 11 ng/ml or higher, about 2.8-fold more likely to be subfertile

Other parameters; FSH, AFC and E2 appeared to be not significant predictors after adjustment for other parameters.

A recent study conducted by Plenkin and Dashilinko from Ukraine (Plenkin and Dashilinko, 2021), assessed the value of Leptin level as predictor of Infertility in women who failed to conceive after treatment for infertility, they included 82 infertile women with primary infertility after failure of treatment for 5 years compared them to matched 164 fertile women as control, who were conceived normally. Authors found that failure of treatment could be attributed to higher levels of leptin after adjustment for different possible risk factors and hormonal

levels and concluded that women with raised serum level of leptin of more than 15 ng/ml were significantly at high risk to fail to response to treatment independent of other factors such as obesity, old age, dietary habit, alcohol use with an odds ratio of 3.18 (1.89 – 6.27) after controlling for other confounder. Kumari et al. stated that leptin is a predictive marker for unexplained infertility (P Kumari *et al.*, 2017)

Regarding other parameters that appeared to be significantly associated with infertility, previous studies concerned with these parameters in different aspect attributed the correlation of these parameters with infertility to intercorrelation between these parameters, for instance, Lin et al. found that serum testosterone level between 0.20 – 0.27 associated with higher risk of subfertility (OR = 3.12) when AMH level was < 1.2 but the risk increased significantly in women with AMH level higher than 5 (OR = 6.54) (Lin, Li and Tsui, 2021).

Conversely, Tafvizi and Masomeh from Iran found no statistically significant difference in serum leptin levels between fertile and infertile women and also no significant correlation between leptin levels and other risk factors of infertility including hormone profile and AFC (Tafvizi and Masomi, 2016)

Hernáez et al. found in multiple regression analysis that greater BMI of ≥ 30 kg/m^2 , i.e. obese women had 18% greater odds of subfertility, also they found higher risk in lower BMI , < 20 kg/m^2 and concluded a J-shaped relationship between BMI and subfertility, while BMI between 23-25 kg/m^2 linked to lower risk of subfertility (Hernáez *et al.*, 2021)

From other point of view, among Qatari women, Musa and Osman found that age of > 35 years, steady weight gain since marriage and menstrual cycle irregularity have shown to be significant predictors of primary infertility with an odds ratio of 3.7, 2.4 and 4.2, respectively (Musa and Osman, 2020).

In Iran, Kazemijaliseh et al. used logistic regression in analysis of predictors of

infertility and found that primary infertility was independently related to old age (OR=1.37), higher BMI (OR = 1.95), active smoking (OR=1.38) and higher education level (OR = 2.23).

However, in our study, we controlled the effect of demographic variables at the study design stage by matching to control their confounding effect (De Graaf *et al.*, 2011; Ananth and Schisterman, 2017; Mansournia, Jewell and Greenland, 2018). In Oman, Al Maskari and Alnaqdy ascertain the relationship between Leptin and obesity among a group of Omani women (Al Maskari and Alnaqdy, 2006)

5.11. Validity of Leptin in prediction of Infertility

Moreover we assessed the validity of leptin as a predictor marker for infertility using ROC curve analysis and revealed a significant high validity parameter indicated that leptin can be excellent predictor for infertility when its level exceeded 11 ng/ml giving a sensitivity specificity and accuracy of 96%, 98% and 96.9%, respectively with a positive predictive value of 98% and negative predictive value of 96.1%. and Dashilinko reported relatively higher cutoff point of 15 ng/ ml for leptin above which women has to be at high risk of infertility and authors considered leptin as good predictor of infertility (Plenkin and Dashilinko, 2021). However, to best of our knowledge, no previous studies concerned with validity parameters of leptin in prediction of infertility.

Chapter Six

Conclusions and Recommendations

6.1. Conclusions:

According to the finding of the study it can be concluded that

1. Leptin level was much higher in sub fertile than fertile women in total group and also across the subgroups according the causes of subfertility compared to controls.

2. Women with higher leptin level cutoff 11ng/ml or higher, about 2.8-fold more likely to be subfertile.so higher leptin level was the stronger predictor of subfertility,

3. Leptin was excellent valid predictor of subfertility with a high sensitivity specificity, accuracy, positive predictive value and negative predictive value.

4.Female factors were the more frequent cause of subfertility followed by unexplained subfertility and the reported rates were comparable to that in previous studies and literatures.

5. There were a significant variation in all fertility hormones except estradiol; between subfertile and fertile women. The levels of LH, FSH, Testosterone, and Prolactin were higher in subfertile women. Estradiol was not different between subfertile and fertile women

6. Subfertile women tend to have lower AMH and Antral follicle count than fertile women.

7. Significant predictors of subfertility Higher (LH, FSH, testosterone, prolactin, leptinlevels, BMI) and lower AMH levels while AFC and E2 failed to predict subfertility.

8. Obese women had the highest level of leptin than the overweight and normal subfertile women in a dose dependent association There were a significant correlation between higher leptin level and increased BMI;

9. Leptin level and anthropometric parameters except body weight no significant correlation was found between us.

10. Leptin and each of age and duration of subfertility and hormonal indices or endometrial thickness no significant aassociation was found between us.

11. Subfertility is mainly affected women at age 21-40 years and its frequency increased with level of education.

6.2. Recommendations:

1. In clinical practice presence of good diagnostic and predictor marker is necessary in management of primary subfertility therefore it is recommended to consider leptin as one of the markers of subfertility in addition to the traditional hormonal and songraphic studies and IVF centers , and laboratories as one of the routine tests .
2. Leptin it can play a significant role and has a promising value in clinical practice.
3. Further studies are suggested to assess the factors that affect the leptin levels among Iraqi women at reproductive age.
4. Further studies with larger sample size and multiple centers are highly suggested for further assessment and evaluation.

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الخلاصة

يمثل ضعف الخصوبة إحدى المشكلات الصحية الكبيرة في جميع أنحاء العالم ، حيث يعاني ملايين الأزواج من العقم والذي له تأثير مهم على الأزواج وعائلاتهم ويشير إلى تحدٍ حقيقي لأطباء النسائية والتوليد من حيث العلاج الذي قد يستغرق وقتًا طويلاً. اجريت دراسات عديدة على اللبتين وعلى نطاق واسع في أنواع مختلفة من العقم، ومع ذلك، فإن نتائج الدراسات السابقة متضاربة ودور اللبتين في عملية الخصوبة لا يزال قيد النقاش.

هدف هذه الدراسة هو تقييم تركيز اللبتين في مصل النساء اللواتي لديهن عقم اولي (ابتدائي)، والتحري عن العلاقة بين مستوى اللبتين وبعض الهرمونات التناسلية بالإضافة الى تقييم العلاقة بين مستوى اللبتين وسماكة بطانة الرحم عند النساء اللواتي لديهن عقم ابتدائي.

تصميم الدراسة كان من نوع الحالة والشواهد التحليلية واجريت بعد أن تمت الموافقة عليها من قبل اللجنة المحلية لأخلاقيات البحوث في كلية الطب جامعة بابل. أجريت الدراسة خلال الفترة من كانون الاول ٢٠٢٠ إلى تموز ٢٠٢١ في مستشفى كمال السامرائي للخصوبة وعلاج العقم وأطفال الأنابيب والعيادات الخاصة في بغداد. اشتملت الدراسة على ما مجموعه ١٠٠ امرأة مصابة بضعف الخصوبة الأولي (الحالات)، و ١٠٠ امرأة خصبة كمجموعة ضابطة، واللواتي استوفين معايير الاشتمال ، وكانت كلتا المجموعتين متطابقتين تقريبًا لخصائصهما الديموغرافية. تم الحصول على الموافقة الشفوية من جميع المشاركات في كلا المجموعتين. تم جمع البيانات من خلال التاريخ المرضي الكامل والفحص السريري الشامل. تم جمع عينات الدم من جميع المشاركات وإرسالها إلى المختبر لإجراء الدراسات الهرمونية. اجري الفحص بالموجات فوق الصوتية لتقييم سمك بطانة الرحم وعدد البصيلات الغارية. التحليل الإحصائي كان باستخدام الحزمة الإحصائية للعلوم الاجتماعية الإصدار ٢٦. تم تطبيق الاختبارات الإحصائية المناسبة حسب نوع المتغيرات والمعلمات. تمت مقارنة المتغيرات الديموغرافية والقياسات الجسمانية والمستويات الهرمونية بين المجموعات. علاوة على ذلك، تم إجراء تحليل الانحدار الثنائي لتقييم المتغيرات والمعاملات المرتبطة بالعقم، وتم حساب نسبة الأرجحية ومقارنتها لتقييم أهمية وقوة التنبؤ بالعقم الاولي من خلال هذه المتغيرات والمعلمات. تم إجراء تحليل منحنى خاصية تشغيل المتلقي لتقييم صلاحية اللبتين كمؤشر على ضعف الخصوبة ومعايير صحتها؛ تم حساب الحساسية والنوعية والدقة.

أظهرت نتائج الدراسة عدم وجود فروقات ذات دلالة احصائية بين كلتا المجموعتين في خصائصهما الديموغرافية بما في ذلك العمر والتعليم والوظيفة والتاريخ العائلي للعقم أو التاريخ الجراحي، في جميع مقارنات هذه المتغيرات، قيمة الدلالة الاحصائية (**P**) أكبر من ٠,٠٥. ايضا لم تكن هناك فروقات معنوية في القياسات الأنثروبومترية بين المجموعتين، (قيمة ٠,٠٥ <P.). كانت القيم المتوسطة للهرمون الملوتن ، هرمون محفز الجريبات المبيضية، التستوستيرون، والبرولاكتين أعلى بشكل ملحوظ في النساء العقيمات ، (قيمة الدلالة الاحصائية 0.05> P). كان الهرمون المضاد لمولر وعدد الجريبات الغارية أقل بكثير في النساء العقيمات (حيث قيمة ٠,٠٥> P). لم يكن كل من الاستراديول وسماكة بطانة الرحم مختلفة بشكل معنوي احصائيا بين المجموعتين، (٠,٠٥ <P). كان متوسط مستوى اللبتين لدى النساء العقيمات ٢٦,٨ ± ١٥,٢ نانو غرام / مل وكان أعلى بكثير منه لدى المجموعة الضابطة حيث كان متوسط اللبتين ٦,٤ ± ٢,٣ نانوغرام / مل، (P<0.001). كان السبب الرئيسي للعقم هو العوامل المتعلقة بالمرأة حيث شكلت هذه العوامل ما نسبته ٤٩٪ من جميع المسببات ويليه العقم غير المبرر في ٣٩٪. لم تكن مستويات اللبتين مختلفة بشكل كبير عند مقارنتها حسب سبب العقم، ولكن في جميع المجموعات الفرعية كان متوسط مستوى اللبتين أعلى بكثير منه في المجموعة الضابطة، (قيمة ٠,٠٠١> .P). وجدت الدراسة أن مستوى اللبتين يزداد مع ارتفاع مؤشر كتلة الجسم حيث كان متوسط مستوى اللبتين عند النساء العقيمات البدينات هو ٣٤,٧ ± ٨,٩ كجم / م ² مقارنة بـ ٢٦,٣ ± ٦,٧ كجم / م² في مجموعة الوزن الزائد و١٩,٤ ± ٥,٦ كجم / م² في مجموعة الوزن الطبيعي الفرعية. (٠,٠٠١> P. value). لم يتم العثور على ارتباط مماثل بين اللبتين ومؤشر كتلة الجسم في المجموعة الضابطة، (قيمة ٠,٠٥ <P.). وجدت الدراسة أن ارتفاع مستوى الليبتين هو مؤشر هام على ضعف الخصوبة حيث أن النساء اللواتي لديهن مستويات من الليبتين بمقدار ١١ نانوغرام / مل أو أعلى يصبحن أكثر عرضة لقلة الاخصاب بمقدار ٢,٨ ضعف مقارنة بالنساء الاخريات، (نسبة الأرجحية = ٢,٧٩٣). أظهر مستوى اللبتين حساسية عالية ونوعية ودقة بمعدلات ٩٦٪ و٩٨٪ و٩٦,٩٪ على التوالي كمؤشر على ضعف الاخصاب.

استنتجت الدراسة ان مستوى اللبتين الأعلى هو مؤشر قوي، ممتاز وذو مصداقية عالية للتنبؤ بضعف الاخصاب ويتمتع بمعدلات عالية من الحساسية والنوعية، والدقة والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية. لذلك يوصى باستخدامه كفحص مهم في التقييم السريري للنساء العقيمات بالإضافة إلى الفحوصات المستخدمة الأخرى

وزارة التعليم العالي والبحث العلمي
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دور مستوى اللبتين في مصل النساء اللواتي يعانين من قلة الخصوبة الاولي

رسالة مقدمة الى
مجلس كلية الطب - جامعة بابل
كجزء من متطلبات نيل درجة الماجستير
في علوم / الفسلجة طبية

من قبل

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