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The role of ubiquinone-10 and kisspeptin-10 on reproductive changes of male rat during prepuberty

A thesis

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By

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بسم الله الرحمن الرحيم

﴿وَقُلْ رَبِّ زِدْنِي عِلْمًا﴾

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DEDICATION

I present the fragments of my humble research as a gift to my Master, the Awaited Mahdi, that it may be a flash of light on the path of waiting

To the one who strives to comfort me and make me happy ….my father**.**

To whom that paradise is under her feet …my mother

To my dear … brothers and sisters

To my husband's family who supported and encouraged me with every step

To my role model…present despite the difficulties…. Who was my partner in every step of my research… my husband.

To those who draw hope and smile in my life ……..my lovely son (Kenan) and my daughter (Tasneem)**.**

My second mother …. my dear aunt (Adhra'a)…. may God keep

you and your presence in my life**…**

To the flowers in my life …. my dear friends

I would like to thank all those who supported me to complete successfully my research.

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Summary

Summary

This study is carried out to investigate the effects of pulsatile Kisspeptin administration for12 days on reproductive efficacy in immature male rats, and to assess the protective role of ubiquinone 10. Thirty-six male rats divided into four groups each group have 9 animals were treated as follows; control group receives dimethyl sulfoxide (DMSO) intraperitoneally, twice daily ; kisspeptin group treated with pulsatile kisspeptin (65µg/kg B.W) intraperitoneally, twice daily ; ubiquinone group treated with ubiquinone10 (10 mg/kg B.W) orally via gavage, twice daily and kisspeptin and ubiquinone group treated with pulsatile kisspeptin (65µg/kg B.W) intraperitoneally, Combination with ubiquinone10(10 mg/kg B.W) orally via gavage, twice daily. all treatments were lasted for 12 days. animals were reared till the day 60 of age then Body weight and anogenital distance were measured,

Blood samples were taken via cardiac puncture to assess (Follicle stimulation hormone, luteinizing hormone, Testosterone, and inhibin B), and then animals were sacrificed under anesthesia to dissected & collected (testes and prostate) for measured the testicular measurements, sperm analysis and histological study.

The result revealed a significant decrease ($p \leq 0.05$) in the levels of follicle-stimulating hormone, luteinizing hormone and testosterone in male rats treated with kisspeptin compared to the other groups, while the level of Inhibin B showed a significant increase ($p \leq 0.05$) in male rats treated with kisspeptin compared to animals in other groups. Coadministration of kisspeptin with ubiquinone leads to enhancing these reproductive hormones (follicle-stimulating hormone, luteinizing hormone, Testosterone, Inhibin B).

The result of body weight and testis measurements showed that there was no significant effect ($p \leq 0.05$) on body weight in male rats treated with kisspeptin, while there was a significant decrease in testis weight,

Gonadosomatic index, testis volume and Anogenital distance in male rats treated with kisspeptin compared to animals in control, kisspeptin, ubiquinone and kisspeptin plus ubiquinone groups. Coadministration of kisspeptin with ubiquinone leads to a significant enhancement in testicular measurements (Testis weight, Gonadosomatic index, Testis volume and Anogenital distance).

The result of sperms analysis showed that total spermatid heads, elongated spermatid and daily sperm production have a significant decrease ($p \leq 0.05$) in the kisspeptin group compare with other groups, Coadministration of kisspeptin with ubiquinone leads to a significant improvement in the total sperm heads, elongated spermatid and daily sperm production.

The histological study of the testis of animals treated with kisspeptin in comparison with control animals shows degeneration of seminiferous tubule (sloughing and disruption of germinal epithelium, increased intraepithelial gaps, atrophied epithelial cells and placed irregularly on the basement membrane) and accumulation of pinkish edematous fluid between the seminiferous tubules, vascular engorgement and there are a little number of spermatogonia, while prostate of animals treated with kisspeptin in comparison with control animals shows degeneration of epithelial cells and sloughing of some epithelia, hyperplasia of glandular epithelial cells and of papillary projections toward the alveolar lumen .also, show some prostatic glands appear empty from the prostatic secretion. coadministration of kisspeptin with ubiquinone leads to an improvement in testicular and prostate tissue.

However, when using ubiquinone alone we noted all studied parameters near close to the normal states.

In conclusion, from our result, we conclude that kisspeptin leads to harmful effects on reproductive hormones, testicular measurements, sperm analysis and architecture of testicular and prostate when injected in the

prepubertal stage. Coadministration of ubiquinone with kisspeptin leads to improvements all these studied parameters.

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List of Abbreviations

Abbreviation	Complete words of terms
AGD	Anogenital Distance
ARC	Arcuate nucleus
ATP	Adenosine triphosphate
AVPV	Anteroventral periventricular nucleus
B.w	Body wight
CoQ10	Coenzyme Co10
DMSO	Dimethyl sulfoxide
Eds	Endocrine disrupters
ELISA	Enzyme linked immune sorbent assay
ETC	Electron transport chain
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin –releasing hormone
GPR54	G protein coupled receptor
GSI	Gonadosomatic index
H&E	Hematoxylin and eosin
HCG	Human chorionic gonadotropin
HH	Hypogonadotropic hypogonadism
HPG axis	Hypothalamus pituitary gonad axis
I.P	Intraperitoneal
INH-B	Inhibin B
Kiss1r	Kisspeptin receptor
KP	Kisspeptin
LH	Luteinizing hormone
Mg/kg	Milligram per kilogram
mIU/ml	Microliter international unit per milliliter
M RNA	Messenger RNA
RP3V	Rostral periventricular area of third ventricle
SD	Standard deviation
SEM	Standard error of mean
SPSS	Statistical Program for Social Sciences

Chapter One

Introduction

Introduction

Kisspeptins are a family of neuropeptides encoded by the gene KISS1. Kisspeptins, which were first identified as metastasis suppressors, are now known to serve an important regulatory role in reproduction (Pinilla *et.al*.,2012). Kisspeptin is produced by two main neuron populations in the hypothalamus: the rostral periventricular region of the third ventricle (RP3V) and the arcuate nucleus (ARC). These neurons project and activate neurons of the gonadotropin-releasing hormone (GnRH) (acting in the hypothalamus via the kisspeptin receptor, Kiss1r), resulting in GnRH production. Gonadal sex hormones stimulate the RP3V kisspeptin neurons while inhibiting the ARC kisspeptin neurons, the fundamental mechanism for positive and negative feedback, and it is now widely accepted that the GnRH pulse generator serves as the ARC kisspeptin neurons (Harter *et.al*,.2018).

Windows of vulnerability, often known as critical periods, are periods when the developing system is most sensitive to exposure, are common characteristics of organizational effects (Selevan *et.al*., 2000). Exposures that occur outside of the critical periods have no organizational effects (Ho *et.al*., 2006). Changes in the expression of Kiss1 or kisspeptin during development, as well as variations in expression between sexes, may be a significant driving factor in the maturation of the neuroendocrine reproductive system, particularly during important developmental stages. In fact, alterations in the Kiss1 system are expected to play a role in the timing of puberty threshold, sex variations in LH production, and other aspects of reproductive physiology (Semaan *et.al*.,2013).

According to Thompson *et.al*. (2006;2009), Kisspeptin induces testicular degeneration in adult rats, which can be avoided by pretreatment with cetrorelix, a GnRH antagonist, this characteristic, along with its critical involvement in gonadotropic axis regulation, makes this peptide a viable target for reproductive pharmacological action. Furthermore, it is uncertain if

kisspeptin causes premature puberty in male animals in the same manner as it does in females (Navarro *et.al*., 2004).

Kisspeptin infusion causes gonadotropin release in humans and animals, but prolonged, high-dose kisspeptin administration can cause desensitization (tachyphylaxis), resulting in fast suppression of the hypothalamic-pituitary-gonadal axis and gonadotropin production. this desensitization occurs at the kisspeptin receptor level rather than at the GnRH neurons or pituitary gland level (Ramaswamy *et.al*,.2007 and Jayasena *et.al*,.2009).

Ubiquinone 10 is one of the most important antioxidants and supports reproductive health (Linnane *et.al*., 2007; Sohet *et.al*., 2009 and Prakash *et.al*., 2010). So, the present study attempt here to evaluate its ability to interfere with the endocrine disturbance effect of kisspeptin.

Ubiquinone 10 commonly known as Coenzyme Q10, is an antioxidant molecule that is also a component of the respiratory chain. It is found in human seminal fluid, which contains numerous antioxidants, mitochondrial bioenergetics, and metabolic activities (Ghanbarzadeh *et.al*,.2014). ubiquinne10 deficiency can cause sperm damage, decreased sperm count, and motility. Ubiquinone 10 supplementation has been shown in studies to improve the reproductive process in males who have fertility difficulties (Lewin *et.al*.,1997). Furthermore, ubiquinone 10 concentrations in the seminal fluid are related to sperm motility and count (Balercia *et.al*,.2009). ubiquinone 10's profound participation in male fertility is evidenced by its role in mitochondrial bioenergetics as well as antioxidation processes. There are many mitochondria in spermatozoa, and it is thought that sperm motility necessitates a lot of energy (Fawcett,.1975). Concerning ubiquinone10 supplementation, Mancini *et.al*, (1994) and Nadjarzadeh *et.al*, (2014) reported that following supplementation, ubiquinone 10 levels increased in seminal plasma, reducing oxidative stress and improving semen parameters.

Ubiquinone 10 exists in two forms: reduced (ubiquinol) and oxidized (ubiquinone). There is a significant connection between sperm count, motility, and ubiquinol-10 concentration in seminal fluid (Alleva *et.al*.,1997). Exogenous ubiquinone -10 treatment was found to be helpful in enhancing sperm kinetic characteristics in individuals with idiopathic asthenozoospermia in a Previous study (Balercia *et.al*.,2009).

The present study attempt to understanding the potential effect of kisspeptin on maturing and reproductive efficacy of male rats as well as, clarify the ability of ubiquinone 10 to interfering with the possible effects of kisspeptin

Aims of Study :

The present study conducted to perform the following aims:

1-The present study investigated the effects of 12 days of pulsatile kisspeptin administration on gonadotropins and testosterone release and maturation of immature male gonads.

2-Evaluation the probable ameliorating effects of ubiquinone 10 against the probable changes in the reproductive system of male rat that caused by kisspeptin 10 during maturing period.

Chapter Two

Literature Review

2.Literature Review

2.1 Kisspeptin (Kp)

2.1.1 Kisspeptin Structure

Kisspeptins are synthesized via differential proteolytic processing of a single precursor. Kisspeptin precursor in humans comprises 145 amino acids, including a potential 19-amino acid signal sequence, two dibasic cleavage sites (at amino acids 57 and 67), and one terminal cleavage and amidation site (at amino acids 121–124) (Kotani *et.al*., 2001 and Ohtaki *et al*., 2001), which generates biologically active kisspeptins .Indeed, proteolysis of Pre-pro kisspeptin produces a 54-amino acid peptide (kisspeptin-54), originally dubbed metastin due to its ability to prevent tumor metastasis, and which has been regarded as the main product of the KISS1 gene (Ohtaki *et al*.,2001).

In addition, kisspeptin-14, kisspeptin-13, and kisspeptin-10, have been identified as peptide fragments of the kisspeptin precursor that share the COOH-terminal region of the kisspeptin-54 molecule and comprise an Arg-Phe-NH2 motif characteristic of the RF-amide peptide family.it was later discovered that these fragments bind and activate GPR-54 with similar potency (Kotani *et al*, 2001 and Bilban *et al*., 2004).

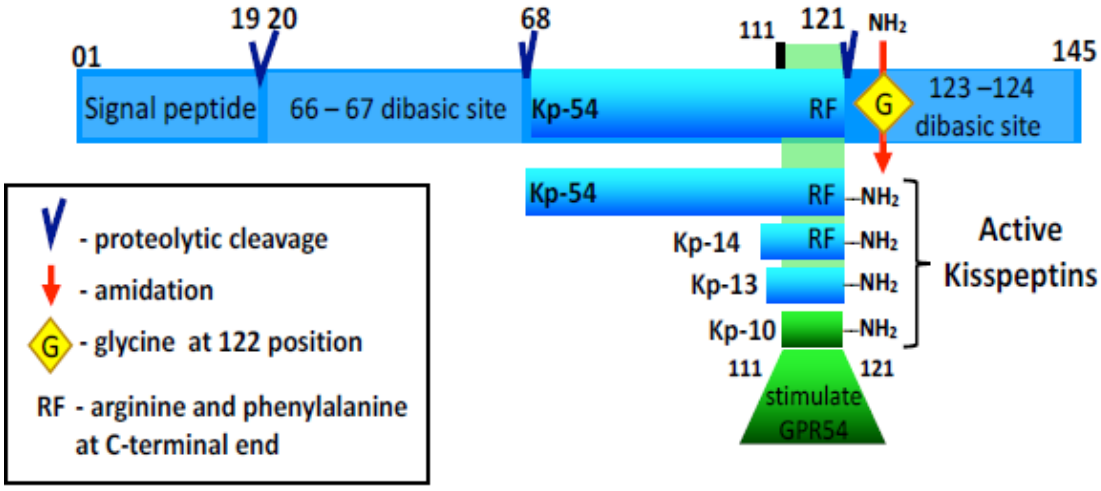

Figure (2-1) Kisspeptin protein structure. Pre-pro kisspeptin and path of proteolytic cleavage, resulting in active kisspeptins (kp-54, kp-14, kp13 and kp-10), (Trevisan *et al.*, 2018).

Kiss1r is the new name for GPR54 and kisspeptin (Oakley *et al*., 2009). Kisspeptin and GPR54 were found in the hypothalamus, brain stem, spinal cord, pituitary, ovary, uterus, liver, pancreas, gut, aorta, coronary artery, umbilical vein, and placenta (Lee *et al*., 1996; Ohtaki *et al*., 2001; Mead *et al*., 2007 and Richard *et al*., 2008). Kiss1 messenger RNA (mRNA) levels in the hypothalamus of mice and monkeys are low before sexual maturation but skyrocket during sexual development (Han *et al*., 2005 and Shahab *et al*., 2005).

2.1.2 History of kisspeptin

KISS1, the gene that encodes kisspeptins, was first identified as a Metastasis suppressor in human malignant melanoma in 1996 (Lee *et al*.,1996). Because the gene was discovered in Hershey (PA, USA), it was called after the famed 'Kisses' chocolate produced in the area. In Kiss, the SS identifies itself as a succession of suppressors. The KISS1 gene is located on chromosome 1q32 and has four exons, the first two of which are not encoded (West *et al*., 1998), The gene encodes 145 amino acid peptide precursors, which are cleaved to a protein of 54 amino acids (West *et al*., 1998).

GPR54 is a G protein-coupled receptor identified as an orphan receptor in rats in 1999 (Lee *et al*.,1999), Galanin does not appear to bind directly to this receptor, despite the fact that GPR54 shares considerable sequence homology with the known galanin receptors (Lee *et al*.,1999). three study teams in 2001, Kotani *et al*., (2001); Muir *et al*., (2001) and Ohtaki *et al*., (2001), soon discovered that the natural ligand for GPR54 is a 54-amino acid gene product known as the Kiss1 gene. Subsequent mutant studies revealed that inactivating mutations in the genes KISS1 and GPR54 cause puberty failure in men, leading to hypogonadotropic hypogonadism (HH) (Lee *et al*., 1996 and Kotani *et al*., 2001). Kisspeptin-GPR54 signaling was therefore found to be essential for initiating gonadotropin (LH/FSH) production throughout puberty (Gottsch *et al*., 2004). Kisspeptin fragments KP-54, KP-13, and KP-10 were identified from the human placenta in 2001 as physiologically active cleavage peptides of the kisspeptin gene (KiSS-1gene) (Kotani *et al*., 2001).

According to two studies published in 2003, mutations in GPR54 (encoding the kisspeptin receptor, also known as KISS1R) induce hypogonadotropic hypogonadism in both humans and animals. Exogenous GnRH injection will fix this by (Gonadotrophin releasing hormone). this result was supported by studies of mice with targeted GPR54 deletions, which revealed that reproductive failure was the sole major mutation-related phenotypic abnormality (Funes *et al*., 2003 and Seminara *et al*.,2003). Kisspeptin-GPR54 signaling is consequently required to begin gonadotropin secretion at puberty and support reproductive function throughout human life.

2.1.3 Characterization of Kisspeptin neuron

Clarkson and Herbison studied the neuroanatomical organization and development of kisspeptin neurons in male and female mice. Kisspeptin neuronal cell bodies were discovered to be mostly present in the ARC and

RP3V. The number of kisspeptin neurons present in RP3V was also shown to be sexually dimorphic, with females having somewhat more than men (Clarkson and Herbison.,2006).

Dual immunofluorescence revealed near appositions between kisspeptin fibers and GnRH cell bodies, which first emerged in pre-pubertal mice (postnatal day 25) and grew in frequency during pubertal development (Clarkson & Herbison., 2006). Kisspeptin neuron projections also extend to the median preoptic nucleus from the midline/periventricular hypothalamic areas (Clarkson *et al*., 2009).

The neuroanatomical organization of kisspeptin neurons and their influence on GnRH production have been studied further. In sheep, we found that kisspeptin treatment significantly increased GnRH production into the pituitary portal system (Smith *et al*., 2011). it travels directly to the anterior pituitary, where it regulates the release of gonadotropins. Furthermore, GnRH neuron terminals are joined to kisspeptin neuron projections in the median eminence (Smith *et al*., 2011), resulting in a new 'axo-axonal' system of kisspeptin-GnRH regulation in which kisspeptin promotes GnRH release. (d'Anglemont de Tassigny *et al*., 2008; Smith *et al*., 2011 and Uenoyama *et al*.,2011).

2.1.4 Kisspeptin and its physiological role as a GnRH modulator

GnRH secretion is essential for the hypothalamic-pituitary-gonadal axis (Colledge *et al*., 2010), with enhanced pulsatile promoting pubertal growth (Plant.,2015). Researchers have long attempted to categorize the variables that generate this (the "GnRH pulse generator"), Kisspeptin is an excellent in *vitro* and in *vivo* GnRH release stimulator, and its role as a regulator has been

an exciting step in establishing reproductive health control (Pinilla *et al*., 2012).

Kisspeptin administration to rats increases circulating gonadotropins, with this effect being notably missing in Kiss1r mice (Messager *et al*., 2005), indicating that kisspeptin activates the hypothalamic-pituitary axis. Kisspeptin antagonist treatment suppresses GnRH secretion (Irwig *et al*., 2004; Roseweir *et al*., 2009), indicating that it functions upstream of GnRH. Such effects are seen in both animals and humans (Seminara *et al*.,2003; Gottsch *et al*., 2004; Dhillo *et al*., 2005 and Messager *et al*., 2005). However, recent research has discovered that additional coregulatory peptides impact the interaction between kisspeptin and GnRH.

2.1.5 Uses of Kisspeptin

Kisspeptin was named metastin when it was shown to be a metastasis suppressor in 1996 (Lee *et al*.,1996 and Kauffman *et al*.,2003). Over the last two decades, increasing evidence has confirmed its unique role. Kisspeptins prevent metastasis by limiting the development of the secondary tumor (Prabhu *et al*.,2013). Kisspeptin binding to KISS1R/Kiss1r increases intracellular calcium and Mitogen-Activated Protein Kinase (MAPK) activation while decreasing cell motility and proliferation (Babwah *et al*.,2012). Kisspeptins have been studied as potential treatment targets for melanoma (Shirasaki *et al*.,2001), thyroid cancer (Ringel *et al*.,2002), bladder cancer (Sanchez-Carbayo *et al*.,2003), esophageal squamous cell carcinoma (Ikeguchi *et al*.,2004), hepatocellular carcinoma (Lee *et al*.,1996; Olbrich *et al*.,2010) and gastric cancer (Dhar *et al*.,2004).

2.1.6 Potential Clinical Applications of kisspeptin

Kisspeptin agonists and antagonists might be used for diagnostic and therapeutic purposes. Kisspeptin agonists can be used to identify lesions in

HPG axis dysfunction and to assess the gonadotrophic potential of infertile patients. They can also be used to treat infertile women and lead to stimulating LH, which causes ovulation. Kisspeptin antagonists can disclose kisspeptin's function in various physiological and pathological states of the HPG axis (Rance *et al*.,2010).

Kisspeptin antagonists, which reduce LH pulse frequency and amplitude without affecting basal LH secretion, may be useful as contraceptives in women or in the treatment of sexually steroid-dependent diseases such as prostate and breast cancer, endometriosis, and uterine fibroids (Roseweir *et al*., 2009; Pineda *et al*., 2010). Furthermore, they can inhibit early luteinization during in vitro fertilization (Durnerin *et al*.,2008; Drakakis *et al*.,2009). Finally, kisspeptin has the potential to be used in the treatment of metastatic malignancies.

2.1.7 Effects of Kisspeptin on Male Reproductive Tract

Kisspeptin and its receptor have been suggested to be involved in the regulation of human sperm motility and male fertility. It has been evidenced by detection of kisspeptin and its receptor in human sperm, which could be activated by kisspeptin treatment while sperm activity was blocked by kisspeptin antagonists (Hoffman *et al*., 2011). Similarly, Kiss1 and Kiss1r have been detected in the testes of mice and have been suggested to regulate sperm function, although kisspeptins failed to release testosterone form seminiferous tubule explants (Homma *et al*., 2009; Horikoshi *et al*., 2003).

2.1.7.1 Kisspeptin and puberty

The hypothalamus-pituitary-gonad (HPG) axis and associated hormones regulate all phases of reproduction throughout a person's life. For example, puberty is marked by the (re)activation of the HPG axis (Peper.,2010). in a nutshell, gonadotrophin-releasing hormone (GnRH) neurons produce GnRH

into the hypophyseal portal circulation, which goes to the anterior pituitary. GnRH then attaches to and activates the hormone-releasing gonadotrophin receptor, which stimulates the production and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Marques *et al*.,2018).

LH and FSH play distinct roles in the reproductive system, such as stimulating gonadal steroid production and increasing gonad development. Meanwhile, gonadal steroids such as estrogen and testosterone serve as a feedback signal to GnRH neurons. Natural sexual growth loss and aberrant puberty are caused by HPG axis defects (Clarke & Dhillo.,2016).

2.1.7.2 Kisspeptin and Onset of Puberty

The time puberty starts is influenced by genetic and environmental variables, as well as gene-environment interactions, and effectively distinguish between males and females. Puberty has been demonstrated not to exist in the absence of appropriate interaction with Kisspeptins and their associated receptors, for example, inactivating GPR54 gene mutations in hypogonadotropic hypogonadism patients (Funes *et al*., 2003; de Roux *et al*., 2003; Seminara *et al*., 2003).

The Hypothalamic Kiss1 mechanism regulates the onset of puberty and is thought to include at least four related major components:

1. An increase in endogenous kisspeptin tone, which appears to be sufficient in and of itself to activate the GnRH/gonadotropin axis.
2. An elevation insensitivity to the stimulatory effects of kisspeptin in terms of GnRH/LH responses.
3. An enhancement in GPR54 signaling efficiency, which appears to be connected to a state of resistance to kisspeptin stimulation and desensitization.

4. An elevated number of kisspeptin neurons, depending on the species, in the AVPV and/or ARC, and in their projections of GnRH neurons, primarily arising from the AVPV in rodents; (Pinilla *et al.*,2012).

2.1.8 Intratesticular activity of kisspeptins

2.1.8.1 Kisspeptins, Leydig cell physiology and steroidogenesis

Androgens are steroid hormones released by Leydig cells in male testes (primarily testosterone). To present, there is no conclusive evidence that peripheral kisspeptin influences the production of androgens in Leydig cells. First, disruption of Kiss1 expression is linked to lower testosterone levels in rats (Ayturk *et al*.,2017). Second, while Kiss1r is expressed by the immortalized Leydig cell line MA-10, it does not respond to KP-10 stimulation (Hua *et al*.,2013). Furthermore, Sertoli cells respond to kisspeptin and stimulate androgen-binding protein (ABP) production, suggesting a possible role of kisspeptin in the production of ABP (Irfan *et al*.,2016).

The Kisspeptin mechanism is the major central regulator of reproduction and puberty initiation in both animal and human models (de Roux *et al*.,2003; Seminara *et al*., 2003; Pinilla *et al*., 2012). Kisspeptin peripheral administration accelerates spermatogenesis by stimulating hypothalamic GnRH; repeatedly obtaining functional mutations in KISS1/KISS1R may cause central precocious puberty in humans (Chianese *et al*., 2016).

Inactivating KISS1/KISS1R mutations, on the other hand, contribute to hypogonadotropic hypogonadism in mice and humans (Seminara *et al*., 2003; de Roux *et al*., 2003; Chianese *et al*., 2016), with a drastic decrease in testosterone biosynthesis that is not reversed by repeated brief and sustained human chorionic gonadotropin (hCG) stimulation experiments during infancy, puberty, adolescence, and early adulthood) (Nimri *et al*., 2011),This

implies that the kisspeptin system may be directly involved in the production of steroids that enhance the development of spermatogenesis and sperm maturation. The collapse of the HPG axis following conditional Kiss1R reactivation in GnRH secreting neurons in Kiss1R-/-mice highlights the importance of intratesticular activity in ensuring effective spermatogenesis (León *et al*., 2016).

2.1.8.2 Kisspeptins and spermatogenesis progression

Subcutaneous injection of synthetic Kiss1 Penta decapeptide accelerates spermatogenesis in male chub mackerel before puberty (Anjum *et al*.,2012). The commencement of Kiss1/Kiss1r expression in the mouse testis coincides with the production of spermatozoa, according to gene expression profiling in animals (Hua *et al*.,2013), indicating a relationship between spermatogenesis and the kisspeptin/Kiss1r pathway in animals. Kisspeptin also has anti-metastatic effects by reducing cell chemotaxis and migration, which is critical in the early stages of spermatogenesis (Harms *et al*.,2003). In addition, KP-13 will cause an improvement in sperm intracellular Calcium concentration ([Ca2+]i) in the late stages of spermatogenesis, which will improve human sperm motility and hyperactivation (Pinto *et al*.,2012).

Kisspeptin production in seminal plasma and sperm consistency has a positive association, indicating that the kisspeptin pathway is important in spermatogenesis (Zou *et al*.,2019). addition, Continuous or repeated injection of Kp-54 and Kp-10 induces testicular degeneration (Thompson *et al*., 2006, 2009; Ramzan and Qureshi, 2011). Adult rats' germ cell degeneration is caused by a decrease in plasmatic inhibin B, a biomarker typically linked with Sertoli cell activity (Thompson *et al*., 2006,2009), Kiss1R has been found in Sertoli cells of frogs (Chianese *et al*., 2013), goats (Han *et al*., 2020), and monkeys (Irfan *et al*., 2016), suggesting a possible paracrine interaction mediated by kisspeptin between Leydig cells (ligand synthesis) and Sertoli

cells (receptor possession). Chronic Kp-10 treatment has a negative influence on the physiology of Sertoli cells in prepubertal male rats, reducing type A spermatogonia, spermatocytes, elongation spermatids and normal sperm production (Ramzan and Qureshi.,2011).

The study found that chronic intraperitoneal injection of Kp-10 (50 nmol) for 13 days resulted in high germ cell death rates as well as enhanced proliferation rates, a state that persisted to a limited extent 7 days following chronic treatment. On the contrary, a single intraperitoneal injection (acute treatment) raised testosterone levels and proliferation rates, possibly as a result of testosterone-mediated actions, but had no impact on apoptosis in the germinal epithelium (Aytürk *et al*., 2017). Kisspeptin has previously been shown to have impacts on testosterone and testicular tissue histology in prepubertal and pubertal rats (Thompson *et al*.,2009; Ramzan and Qureshi, 2011).

2.1.8.3 Effect of Kisspeptin on Sex Steroids

Kisspeptin directly activates GnRH secretion from the anterior pituitary, hence LH pulse remains the surrogate marker of GnRH pulse since each GnRH pulse is connected with LH and FSH pulse, and LH is more closely related to GnRH production than FSH (George *et al*.,2013). In adult male rats, central and peripheral administration of kisspeptin-10 leads to elevated plasma LH, FSH, and total testosterone (Thompson *et al*., 2004). According to Whitlock *et al*., (2008), reproductive steroids boost somatotropic axis exposure to physiologically adequate Kp10 dosages and make Kp10 more likely to be an integrator of luteinizing hormone and GH release.

We previously demonstrated that intraperitoneal kisspeptin-10 treatment causes dose-dependent degenerative alterations in rat testicular tissue in the prepubescent stage (Ramzan and Qureshi.,2011). the discharges of GnRH

neurons in mice right increased dramatically from 25% (juvenile) to 50% (pre-pubertal) and >90% (pubertal) during the onset of puberty, showing increasing sensitivity to kisspeptin as they grew older (Navarrao *et al*.,2009). Kiss1 and kiss1r mRNA were shown to be significantly increased in the hypothalamus during puberty (Navarrao *et al*.,2009).

2.1.9 The relationship between kisspeptin and endocrine disruption

Endocrine disruptors (EDs) are either naturally occurring compounds that interfere with the endocrine system's function or man-made substances (Knez 2013; Tripathy & Nagar., 2018). Eds can be any estrogen-like and antiandrogenic chemicals, environmental factors such as dioxin and some pesticides, biological stressors such as oxidative stress, or pharmacological agents like radiation and medicines. These chemicals elicit their behavior by imitating, inhibiting, and stimulating hormone actions and altering the whole neuro-endocrine balance.

EDs may be due to functional anomalies, including decreased semen content, decreased sperm counts, or behavioral abnormalities, including altered sexual activity, decreased libido, and infertility (Tripathy & Nagar., 2018). EDs accumulated primarily in the neuroendocrine and HPG (hypothalamic-pituitary-gonadal) axis and deregulated natural physiological functions and actions, directly influencing the human reproductive system, triggering pathologies such as cancers and urogenital malformations, altering specific gender hormone networks, and occasionally resulting in transgenerational reproductive effects (Koppe *et al*.,2006).

EDs may have multifaceted and pleiotropic impacts on animal reproductive function. Given the complexity of the endocrine system, the mechanisms of action of EDs are challenging to decipher. Most EDs, which

can have either synergistic or antagonistic effects, are predicted to work via a variety of pathways. Eds inhibit hormone production at the place of origin or further along the transport route by interfering with single or multiple hormone pathways or even forming single ED-receptor or EDs-multi receptor complexes. It is far more difficult and unpleasant to achieve the expected consequences (Poongothai *et al*.,2007; Diamanti-Kandarakis *et al*.,2009).

During the prenatal period, testicular development is important for human reproductive potential and is highly hormonally controlled. The development of fetal androgen is required for the differentiation of the male reproductive system. Testicular dysgenesis can be caused by disruption of androgen production, especially during the time of virilization (weeks 8-14) of fetal development (Welsh *et al*,.2008). Endocrine disruptors (such as BPA) have been linked to urogenital tract developmental abnormalities, reduced epididymis size, prostate enlargement, decreased ejaculate volume, and sperm concentration in males (Chiba *et al*.,2012). BPA also binds to the LH receptor, influencing testosterone synthesis in Leydig cells (Wetherill *et al*,.2007).

Endocrine disruptors were systemic different combination classes that might negatively impact stable humans, animals, and fisheries or progeny through interaction with endocrine systems (Porte *et al*.,2006). disorders in all endocrine systems that need both hyperactive and underactive hormone production eventually lead to illness, the symptoms of which can extend to several organs and functions and can be life-threatening or disabling. When regarded in this broad context, the threat presented by environmental substances having endocrine action (either agonist or antagonist) is potentially significant (Gore *et al*.,2006). Exogenous compounds that interfere with the metabolism or function of hormone production are known

as endocrine-disrupting chemicals (EDs). Furthermore, because the growing body is so reliant on sex hormones for maturation, the fetus and kid are extremely sensitive to any hormonal environment change (Bromer *et al*., 2010).

Kiss1 is a major regulator of gonadotropin secretion, and its activity is critical for puberty onset, steroid-mediated sexual input regulation, and adult fertility management. Kisspeptin, a functional product of the Kiss1 gene, has been shown to be inhibited by EDs such as bisphenol A, dichlorodiphenyltrichloroethane (DDT), vinclozolin, and certain polychlorinated biphenyls (PCBs), as well as complex ED combinations. Kisspeptins are neuropeptides that are produced in separate neuronal communities within the hypothalamus and play significant roles in brain sex differentiation, puberty initiation, and reproduction via the GPR54 receptor. Sex steroid hormones, particularly estradiol, play an important role in the sex-specific structure and function of the kisspeptin system (Patisaul.,2013).

Kiss1 neurons, another neuropeptide with important reproductive roles, have been demonstrated to co-express neurokinin B (NKB). Kisspeptins also stimulate the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). While GnRH neurons are an important component of the reproductive axis, kisspeptin (KP) peptides have recently been identified as critical upstream regulators that combine central and peripheral signals with GnRH release, thereby playing a key role in reproductive control (D'Anglemont de Tassigny and Colledge,.2010).

As a result, these compounds (EDs) can disrupt sexually dimorphic ontogeny and kisspeptin signaling pathway function, as well as have negative effects on neuroendocrine physiology. EDs disrupt steroid hormone signaling (Tena-Sempere.,2010). Toxic compounds may impact critical periods in the creation of the reproductive system, such as primordial germ

cell determination, gonadal differentiation, pubertal initiation time, estrous cycles, gametogenesis, genitalia, or signaling activities that govern human sexual behavior. EDs affect male reproductive function by interfering with testicular development and function (i.e., steroidogenesis and spermatogenesis). Reproductive success is determined by the development of the female reproductive system throughout fetal life (Gregoraszczuk and Ptak., 2013).

Sex steroids are also important as puberty progresses for increased production of hypothalamic kisspeptins. Synthetic estrogen treatment decreased hypothalamic Kiss1 mRNA expression as well as GnRH activation and gonadotropin secretion throughout critical stages of rodent development. Kisspeptin treatment reverses the inhibition of pubertal activity caused by estrogen exposure, indicating the potential of EDCs to alter the kisspeptin pathway.

EDCs action is also gendered dimorphic, with estrogenic chemicals eliciting opposing effects in males and females. Some substances provide disparate results, with the amount and timing of treatment during sexual development are especially important. Prenatal genistein exposure, for example, causes a postnatal delay in female rats, whereas postnatal exposure causes early puberty (Rasier *et al*.,2006; Dickerson & Gore.,2007). Because various ED has been linked to changes in sexual behavior, puberty, and fertility, the kisspeptin system became an ideal target for investigation (Tena-Sempere.,2010). In rats, neonatal exposure to estrogenic ED, such as BPA and genistein, resulted in different degrees of kisspeptin suppression, most likely via decreased amounts of hypothalamic kisspeptin mRNA (Bateman & Patisaul.,2008; Navarro *et al*.,2009).

The relative importance and potential interplay of endocrine malfunction in each of these brain networks that control reproduction (GnRH and

kisspeptin neurons) remain unknown. Various chemical signals impact the development of the central nervous system. Sex steroids play important roles in the development of numerous dimorphic brain circuits and behaviors, in addition to the creation of primary and later secondary sexual characteristics. Proteins such as SHBG and AFP protect the developing brain from excessive exposure to maternal sex hormones (De Mees *et al*.,2006) When BPA and another ED bypass this regulatory system, they are able to enter the immature blood-brain barrier (Rubin., 2011).and, as a result, impair the development of sexually dimorphic regions (such as the dopaminergic, nitrergic, and kisspeptin systems), reversing the neurochemical phenotype, which has a detrimental effect on adult fertility and sexually differentiated behaviors (Panzica *et al*.,2011).

2.2 Ubiquinone 10 (CoQ10)

Ubiquinone, also known as CoQ10, is a fat-soluble, vitamin-like benzoquinone that is endogenously synthesized in the human body from tyrosine (Nelson & Cox., 2017). It has a quinone group and a side chain of 10 isoprenoid units (PubChem.,2019). Ubiquinol the completely reduced form of ubiquinone 10 is a powerful lipophilic antioxidant capable of neutralizing free radicals and regenerating vitamin E's reduced form (Kagan, Fabisiak, and Quinn, 2000; Ouchi, Nagaoka, and Mukai 2010).

It is a 1,4-benzoquinone, where Q indicates the quinone molecular group and 10 denotes the number of chemical subunits of isoprenyl in its tail. For natural ubiquinone, the number can range from 6 to 10. This class of fat-soluble compounds resembling vitamins is found in all respiratory eukaryotic cells, particularly the mitochondria. It is a component of the electron transport chain and is involved in aerobic cellular respiration, which generates energy in the form of ATP. As a result, 95% of the energy in the human body is produced (Ernster and Dallner., 1995; Dutton *et al*., 2000).

Ubiquinone 10 is considered an antioxidant and free radical eliminator and is considered as a super vitamin (vitamin Q) that helps in the protection of DNA, cells membrane lipids, and proteins from the hazards of oxidative damage assists in regenerating vitamin E and reliefs healthy energy levels (Bentinger *et al*., 2007; Linnane *et al*., 2007).

The addition of ubiquinone 10 aids to improve human and animal health in the case of many diseases such as obesity, diabetes, heart disease, muscle atrophy, aging, cancer, Alzheimer's disease, etc. (Roffe *et al*., 2004; Adarsh *et al*., 2008; Sohet *et al*., 2009; Prakash *et al*., 2010; Varela-López *et al*., 2016). in addition, in vitro data show that it has anti-inflammatory properties (Schmelzer *et al*., 2009).

Ubiquinone 10 inhibit lipid peroxidation in biological membranes and protects mitochondrial proteins and DNA from oxidative damage. In fact, it is the only lipophilic antioxidant that can be de novo synthesized by cells and that has enzymatic mechanisms to regenerate its reduced form (Brayfield, 2017). There has been an interest in identifying reversible causes of male infertility recently, and multiple studies have been carried out to investigate whether seminal parameters can be improved by supplementing infertile males with antioxidants (Showell *et al*.,2011). ubiquinone 10 (as a part of the mitochondrial respiratory chain) seems to play an important role in energy metabolism among the various antioxidants tested, as well as acting as a liposoluble chain-breaking antioxidant for cell membranes and lipoproteins (Showell *et al*.,2011).

2.2.1 Chemical form of ubiquinone 10

Ubiquinone 10 is produced by the mevalonate cycle of acetyl-CoA, which produces cholesterol, dolichol, and ubiquinone 10 (Molyneux *et al*.,2008). ubiquinone 10 is also known as CoQ10 in its oxidized form and ubiquinol in

its reduced form. In humans, ubiquinone (2,3-dimethoxy-5 methyl-6-decaprenyl-benzoquinone) has a chain of isoprene units and is derived from the combination of a benzoquinone ring with a hydrophobic isoprenoid chain, all of which have a double bond and a trans orientation (Litarru and Tiano.,2010).

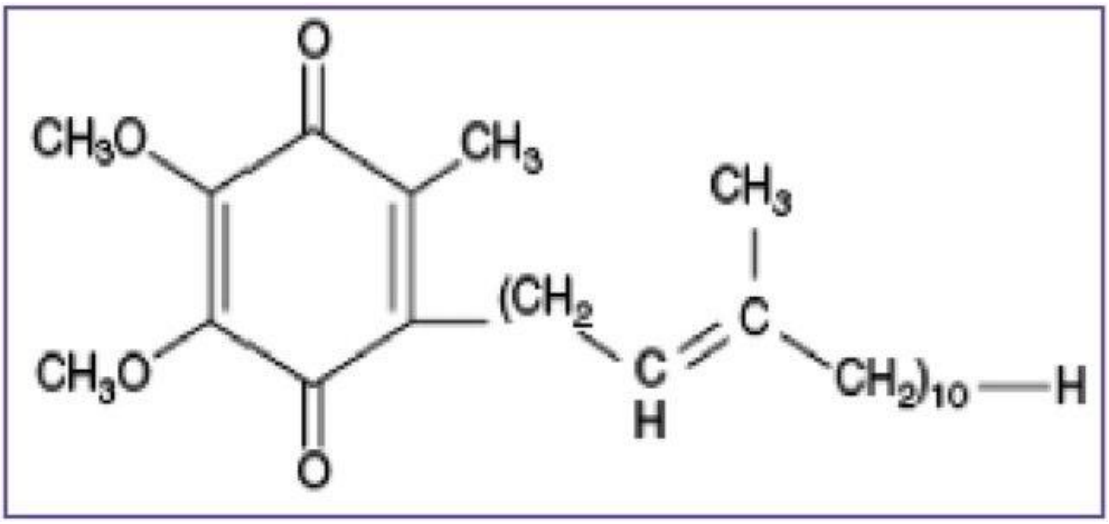

Figure (2-2) Chemical structure of ubiquinone 10 (reproduced from Prakash *et al.*, 2010).

2.2.2 Sources of Ubiquinone 10

Ubiquinone 10 is found in plant and animal cells and is the most prevalent type in humans and most mammals. ubiquinone 9 is the most common type in rats and mice. Ubiquinone 6, ubiquinone 7, and ubiquinone 8 are rich in yeast and bacteria. Ubiquinone is found in all tissues, but it is abundant in the heart and skeletal muscles, the liver and kidneys, and the lungs. Except in the brain and lung tissues, the reduced form of ubiquinone is the most effective source (Kumar *et al*., 2016).

The concentration of ubiquinone in human plasma ranges between 0.75 and 1.0 Ug/ml, with 75 percent in the reduced form. The total amount of

ubiquinone in the body has been calculated to be 1.0-1.5 g, with the majority of it found in muscle cells. Aging can be associated with the reduction of ubiquinone levels. Ubiquinone levels can be improved through biosynthesis, food, and supplementation. Exercise also stimulates the biosynthesis of ubiquinone in the body. Pork core, fish, vegetables, especially cauliflower, soya bean, and sweet potato are good sources of ubiquinone (Singh *et al*.,2003).

2.2.3 Functions of Ubiquinone 10

Ubiquinone 10 has a variety of vital cellular functions, particularly within mitochondria, but also within the cell elsewhere (Crane.,2001). Within mitochondria, during oxidative phosphorylation, ubiquinone 10 plays a key role as an electron carrier (from complexes I and II to complex III) in the mitochondrial electron transport chain (METC). It is also involved in the metabolism of pyrimidines, fatty acids, and mitochondrial uncoupling proteins (as a cofactor of the enzyme dihydrate dehydrogenase) and in the regulation of mitochondrial transfer pore permeability (Crane.,2001).

also, ubiquinone 10 is an essential lipid-soluble antioxidant that protects both mitochondrial and extra-mitochondrial cell membranes (Golgi apparatus, lysosomes, endoplasmic reticulum, peroxisomes) from free oxidative stress (OS) caused by free radicals (Hargreaves.,2003). In addition to functioning specifically as an antioxidant, ubiquinone 10 is also active in the regeneration of vitamin C and vitamin E antioxidants (Rodick *et al*.,2018), respectively.

Ubiquinone 10 also has a role as an inflammatory mediator, a role in the metabolism of cholesterol (Schmelzer *et al*.,2008), and a role in the maintenance of lysosomal pH (Heaton *et al*.,2020).

A function in the metabolism of sulfides as a sulfide quinone oxidoreductase cofactor (Quinzii *et al*.,2017), and a role in the metabolism of amino acids (as a choline dehydrogenase and proline dehydrogenase cofactor in the synthesis of glycine and proline/arginine) (Salvi and Gadda.,2013; Hancock *et al*.,2016). It has been shown that ubiquinone 10 specifically influences the expression of a variety of genes (Gutierrez-Mariscal *et al*,.2019).

2.2.4 Formulation

Ubiquinone 10 is a fine yellow to orange crystalline powder that, when exposed to light, decomposes and darkens. With a slight odor, it is tasteless, virtually insoluble in water, mildly soluble in ethanol and soluble in acetone and ether, and with a melting point of around 48 ◦C (Ubidecarenone, 2019). It has poor oral bioavailability in humans because of its elevated molecular weight (863.34 g/mol) and high hydrophobicity (PubChem.,2019). (Miles, 2007).

In addition, ubiquinone 10 is brittle and sensitive to fire, light, and oxygen, which also limits its therapeutic and practical food formulation applications (Fir *et al*., 2009). Its stability is around 24 months when stored in the original bottle, shielded from light, in a dry position at a low temperature (below 25◦C). Due to its physicochemical properties, high dose and stable ubiquinone 10 formulations are difficult to achieve with regard to its formulation in food supplements (Li & Chen., 2017).

As a fine powder with poor rheology and low melting point, ubiquinone 10 is difficult to correct dosage and press into tablets, especially when the temperature rises beyond its melting point, resulting in stickiness and adherence to machinery surfaces (Abdel-Hamid & Betz, 2012; Nakamura, Otsuka, Yoshino, Sakamoto, & Yuasa, 2016).

Furthermore, as ubiquinone 10 is influenced by light, heat, and oxidation, it should be kept in a cool, dark position, ideally in an airtight container (Ubidecarenone, 2019). For this cause, and taking into account the growing interest in ubiquinone 10 over the years, research has concentrated on overcoming the problems that restrict its formulation in food supplements and medicinal products. Study activities have also been performed to increase its solubility, oral bioavailability, and stability (Beg *et al*., 2010; Kumar *et al*., 2016).

2.2.5 Biochemical and physiological effects

Three major steps are involved in the biosynthesis of ubiquinone 10 (Olson& Rudney, 1983) Synthesis of the ring structure from tyrosine or phenylalanine, formation of the isoprenoid side chain from acetyl-CoA residues via the mevalonate pathway, and finally condensation of these structures via the enzyme polyprenyl transferase, most likely in the Golgi apparatus. The coenzyme hydroxy methyl glutaryl (HMG) A reductase reaction appears to be an important step in regulating synthesis, as it is with cholesterol synthesis, but other steps may also be regulated. As previously stated, ubiquinone 10 is an important electron and proton transporter in the mitochondria. Situations that alter the amount of ubiquinone 10 in the inner mitochondrial membrane alter the electron transport rate, thereby influencing the efficacy of ATP production.

Ubiquinone 10 exists in both oxidized and reduced forms, ubiquinone and ubiquinol. As a result, ubiquinone 10 also serves as a lipid-soluble antioxidant. Several studies have found that ubiquinone 10 supplementation reduces low density lipoprotein (LDL) oxidation in vitro and in vivo (Alleva *et al*, 1995; Kaikkonen *et al*, 1997). It has been shown to have a vitamin E sparing effect as well as a direct anti-oxidative effect (Stocker *et al*,1999).

2.2.6 The mevalonate pathway of ubiquinone 10

The mevalonate pathway is made up of reactions that begin with acetyl-coenzyme A (acetyl-CoA) and end with farnesyl pyrophosphate (FPP), which is the substrate for the biosynthesis of ubiquinone 10, cholesterol, dolichol, and isoprenylated proteins (Fig. 2-3) (Paredes-Fuentes *et al*.,2020). As a result, the conversion of acetyl-CoA to FPP is shared by all end products. This organization is unusual because the biosynthetic sequence for several lipids is identical, and one would expect that the production of one lipid has a large influence on the synthesis of the other lipids.

However, the mevalonate pathway lipids are synthesized at widely disparate rates and amounts, implying that, in addition to central regulation, a terminal regulation is involved. The regulatory enzymes are most likely the branch-point enzymes that use FPP. Because of this, the mevalonate pathway in animal cells is extremely complex.

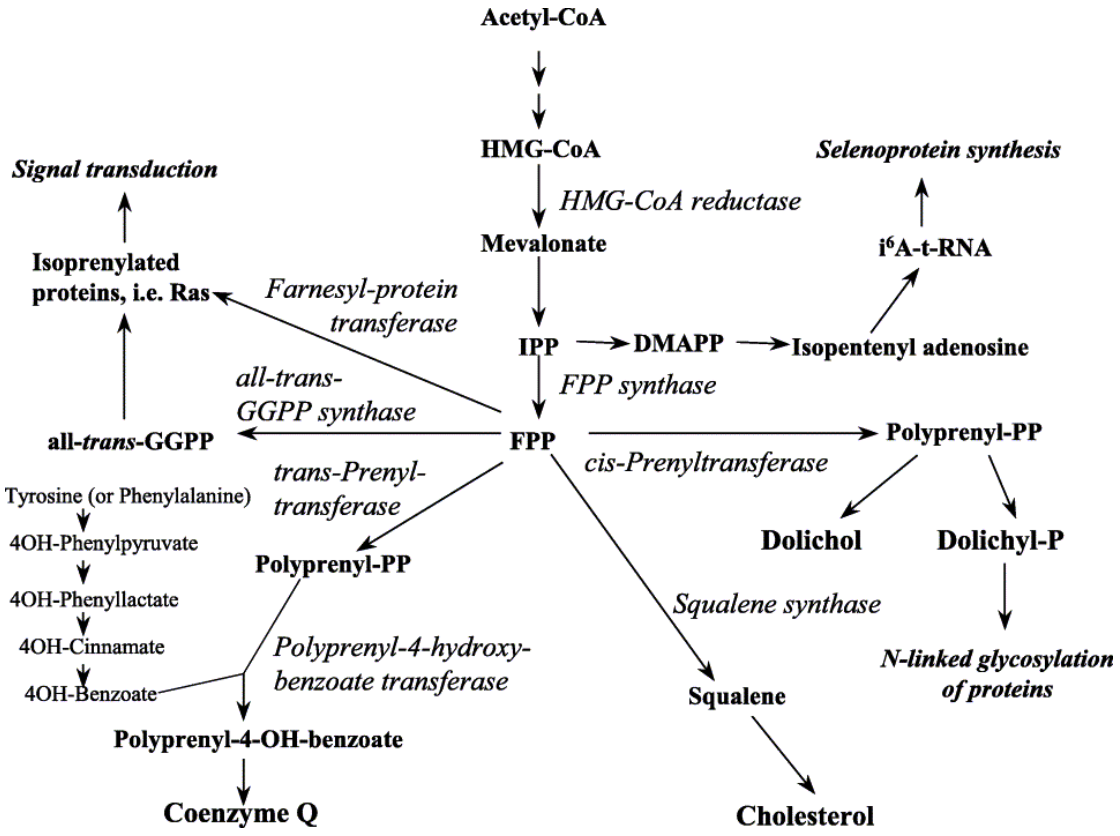

Figure (2-3) The enzymatic conversion and condensation of acetate to farnesyl-PP and subsequent biosynthesis of ubiquinone 10, cholesterol, and dolichol. Abbreviations: CoA, coenzyme A; DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; HMG, 3-hydroxy-3-methyl glutaryl; 4OH, 4-hydroxy; IPP, isopentenyl pyrophosphate. Key enzymes are indicated in italic, (Paredes-Fuentes *et al*.,2020).

2.2.7 Ubiquinone 10 and male fertility

Ubiquinone 10 is essential for energy production as well as the prevention of oxidative damage. In sperm motility and function, energy production and low levels of oxidation are critical. A recent study found a strong correlation between ubiquinone 10 levels and sperm counts in seminal plasma, as well as a negative correlation with hydroperoxide levels (Lewin &Laron, 1997). Furthermore, ubiquinone 10 appears to improve sperm motility in sperm samples that were poor motile (Alleva *et al*, 1997 and Balercia *et al*., 2009).

2.2.8 Absorption and metabolism of ubiquinone 10

Ubiquinone 10 is a crystalline powder that is water-insoluble. Absorption is comparable to that of lipids; the method of absorption appears to be similar to that of vitamin E, another lipid-soluble nutrient. This process in the human body involves the release of pancreatic enzymes and bile into the small intestine, which promotes the emulsification and micelle creation necessary for the absorption of lipophilic substances (Bhagavan & Chopra.,2006).

Food consumption (particularly the presence of lipids) accelerates the biliary excretion of bile acids and substantially increases ubiquinone absorption. Exogenous ubiquinone 10 is absorbed from the small intestine and is most effectively absorbed when given with a meal. In fed conditions, the serum content of ubiquinone10 is greater than in fasting ones (Ochiai *et al*.,2007). Because of its insolubility in water, limited solubility in lipids, and relatively large molecular weight, given ubiquinone 10 orally has a low absorption efficiency. According to one rat research, only around 2–3 percent of orally given ubiquinone10 was absorbed (Zhang *et al*.,1995).

In general, the larger the ingested dosage, the lower the percentage dose absorbed. In the case of supplementary ubiquinone10 in final dosage forms, absorption is also affected by factors like as nature of the formulation, and solubilized formulations of ubiquinone 10 have been found to have improved bioavailability (Chopra *et al*.,1998; Zaghloul *et al*.,2002).

Data on the metabolism of ubiquinone10 in animals and humans are limited. (Zmitek *et al*., 2008). A study with 14C-labeled ubiquinone10 in rats showed most of the radioactivity in the liver two hours after oral administration when the peak plasma radioactivity was observed, but ubiquinone 9 (with only 9 isoprenyl units) is the predominant form of ubiquinone in rats (Kishi *et al*.,1964). Ubiquinone10 appears to be

metabolized in all tissue, with biliary and fecal excretion being the primary routes of elimination. Regardless of the formulation employed, levels revert to normal within a few days of discontinuing ubiquinone 10 treatments (Ozawa *et al*.,1986).

2.2.9 Effect of ubiquinone 10 in sperm

Ubiquinone 10 is an antioxidant molecule that contributes to the respiratory chain. The ability of antioxidants to reduce male infertility is investigated, as well as whether antioxidant supplementation to infertile men can improve seminal indices. Ubiquinone 10 was one of several compounds studied because of its role in energy metabolism and antioxidant status via its function as a liposoluble chain-breaking agent for lipoproteins and cell membranes (Showell *et al*., 2014).

A significant amount of Ubiquinone 10 is found in seminal plasma and spermatozoa, which helps to reduce oxidative stress and protect sperm viability (Mancini & Balercia., 2011). The concentration of Ubiquinone 10 in seminal plasma is significantly related to sperm number and motility (Alleva *et al*., 1997). Ubiquinone 10 is normally found in seminal fluid, where it improves testicular and sperm function (Balercia *et al*., 2009).

Ubiquinone 10, as an antioxidant, may be useful in balancing out high levels of ROS (Mancini *et al*., 2005). In fact, enzymatic and non-enzymatic antioxidants, such as Ubiquinone 10, are naturally present in sperm cells and seminal plasma. It is found in sperm mitochondria at particularly high concentrations, where it plays a role in generating energy. It is considered to be a pro-motility for this purpose, an antioxidant molecule capable of inhibiting the formation of super oxides (Majzoub., 2018).

Ubiquinone 10 can also be quantified in seminal fluid, where there is a link between its abundance and peroxide loss, as well as sperm count and

motility increase (Mancini & Balercia., 2011). Furthermore, lower seminal Ubiquinone 10 concentrations were linked to defective sperm parameters. As a result, it was critical to investigate whether exogenous Ubiquinone 10 administration could improve the consistency of sperm and pregnancy rates. Mancini *et al*., (1994) and Nadjarzadeh *et al*., (2014) reported that Ubiquinone 10 supplementation increased CoQ10 levels in seminal plasma, which reduced oxidative stress and improved semen parameters.

2.2.10 Pharmacokinetics and bioavailability of the dietary supplement ubiquinone 10

Ubiquinone 10's molecular composition consists of a group of quinones and a side chain of 10 units of isoprenoids. It has a high molecular weight and is highly hydrophobic (863.34 g/mol) (PubChem, 2019). It is also strongly insoluble in the aqueous system (O'Neil.,2013; Brayfield., 2017) and is consumed from the small intestine steadily and incompletely, resulting in poor oral bioavailability in humans (Miles., 2007).

Ubiquinone10 absorption follows zero-order kinetics and is thought to follow the same pathway as other lipophilic agents. It is a dynamic process that takes place in the small intestine by a mixture of passive and active transport pathways (Miles., 2007), with duodenum, colon, ileum, and jejunum regions from greater to lower permeability to ubiquinone 10 according to murine models (Palamakula, Soliman, & Khan., 2005).

Studies of absorption and bioavailability indicate that the person's responsibility to ubiquinone 10 supplementation is highly variable as a high-complexity mechanism and can be influenced by various factors such as age, gender, diet, microbiota, and intestinal fat absorption capability, among others (Martucci *et al*., 2019). It should be remembered that although its sluggish absorption and low bioavailability are related to its high molecular

weight, high lipophilicity, and weak aqueous solubility, as with other water-insoluble compounds, the absorption of ubiquinone 10 is improved by the presence of a lipid medium, so it is advised that it be taken with fat-containing meals or encapsulated inside an acceptable delivery method (Xia, Xu, S & Zhang.,2006).

Chapter Three

Materials and Methods

Chapter Three----------Materials and Methods

3.Materials and Methods:

3.1 Materials:

3.1.1 Experimental animals:

The experiment began in October, Prepubertal male rats used in this experiment were obtained from the laboratory animal unit of the College of Veterinary Medicine, University of Baghdad, Iraq. the age was (35 days) old with an average body weight (100-135gm). kept under hygienic conditions and air-conditioned room. the light system was 14/10 hrs light/dark cycle;23 ±2 c° with a relative humidity of 50±5%. the animals were housed in plastic cages, food and water were offered daily, tap water was provided via glassy bottles, Rats were fed as pellets as shown in appendix I.

3.1.2 Instruments:

The instruments that were used in this study with their suppliers and sources are summarized in Table (3-1).

Table (3-1): instruments that were used in this study.

NO.	**Instruments**	**Company**	**Country**
1.	Analytical sensitive balance	Sartorius	Germany
2.	Balance for animals	Shimadu company	Japan
3.	Centrifuge	Hettich Roto fix11	Japan
4.	Digital camera	Toup Cam	China
5.	Digital vernier calipers	INGCO	China
6.	Disposable Syringes	Medical ject	S.A.R.
7.	Dissecting tools	S.I.E.	Pakistan

8.	ELIZA reader	Bio Kit	USA
9.	ELIZA washer	Bio Kit	USA
10.	Eppendorf tube	Fisons	England
11.	Freezer	Hitachi	Japan
12.	Incubator	BINDER	Germany
13.	Insulin Syringe	Medical ject	S.A.R.
14.	Jell tube	AFMA-Dispo	Japan
15.	Latex gloves	Great glove	Malaysia
16.	Light microscope	Lieca	China
17.	Liquid nitrogen	Rockefeller	USA
18.	Micropipette 100-1000 µl	CYAN	Germany
19.	Micropipette 1-100 µl	CYAN	Germany

3.1.3 Chemicals:

All laboratory chemicals and sources that used in this study are listed in Table (3-2)

Table (3-2): The chemicals were used in this study and their sources:

NO.	Chemicals	Company	Country
1.	Chloroform	Noorbrok	England
2.	CoQ10(Coenzyme Q10)	AMS (America medic and science)	USA
3.	DMSO	LOBA	Chemie
4.	Eosin-Hematoxilin Stain	Merck	Germany
5.	Ethanol	Merck	Germany

6.	Ethanol 80%	Labort	India
7.	Follicle Stimulating Hormone (FSH) ELSA Kit	Monobind Inc	USA
8.	Formalin 10 %	TEDIA Company	USA
9.	Luteinizing Hormone (LH)ELISA Kit	Monobind Inc	USA
10.	Negrosin	Merck	Germany
11.	Normal saline	Labort	India
12.	Paraffin Wax	Merck	Germany
13.	Rat inhibin B(INH-B) Elisa kit	Elisys Uno Human	Japan
14.	Rat kisspeptin powder	Elabscience	USA
15.	Sodium Citrate	Fluka	
16.	Testosterone ELISA kit	Human	Germany
17.	Xylene	Scharlau	Spain

3.2 Methods:

3.2.1 Experimental Design:

Thirty-six prepubertal male rats aged 35 days, were randomly divided into Four groups comprising nine animals for each group as the following:

1.First group:(control group): Animals in this group were given DMSO intraperitoneally twice daily for 12 days.

2.Second group:(Kisspeptin group): rats have administrated kisspeptin at a dose (65μg/kg B.W) dissolved in DMSO, Intraperitoneally, twice daily for 12 days, according to (Hameed *et al*., 2013).

3.Third group (ubiquinone group): Animals in this group were intubated orally via gavage ubiquinone 10 only (10 mg /kg B.W) dissolved in DMSO, twice daily for 12 days, according to (Mazen and Elnegris., 2013)

4.Fourth group: (kisspeptin plus Ubiquinone group): rats received ubiquinone 10 (10 mg /kg B.W) orally via gavage and kisspeptin 10 (65µg/kg B.W) intraperitoneally, twice daily for 12 days, according to (Hameed *et al*., 2013 and Mazen and Elnegris., 2013).

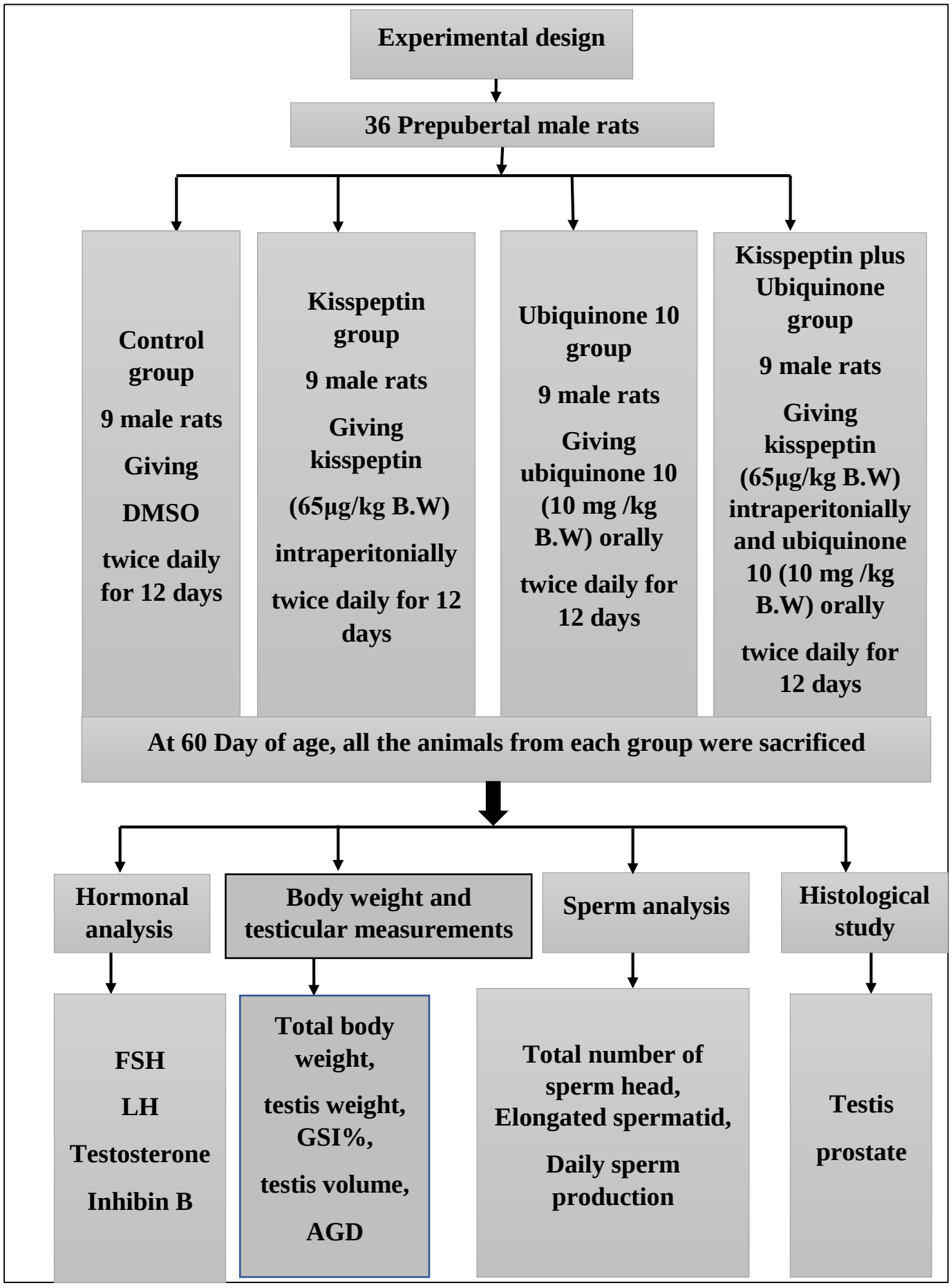

Figure (3-1): experimental design.

3.2.2 Animal Sacrifice and Sample Collection:

Treatments were done for 12 days, animals were reared till day 60 of age then body weight and anogenital distance were measured, then anaesthetized by placing them in a closed jar containing cotton sucked with chloroform anaesthesia, rats of each group were sacrificed.

Blood samples were taken by heart puncture. Then the blood sample was dropped directly from the heart by using a 5 ml disposable syringe, blood was collected in a gel tube to be centrifuged (3000 rpm for 15 minutes) to obtain the serum which is then transferred to Eppendorf tubes, for assessment of reproductive hormones concentrations in serum (FSH, LH, testosterone and Inhibin B). All samples were collected in the morning (8.30 AM-10.30 AM) in order to minimize the diurnal variation of hormone levels.

3.2.3 Organs Collection for Histological Section

Animals (prepubertal male rats) were sacrificed using chloroform anesthesia, and the animals were dissected to remove samples (testes and prostate), all were weighted with an electronic analytical and precision balance. The two testes of each male rat, two of prostate each male rat, which was isolated separately, and one of them were stored in formalin at a concentration of 10% in sterile plastic containers for histological examination and the other one was stored in liquid nitrogen to perform testicular spermatid head.

3.3 Studied parameters

3.3.1 Hormones Assays *(Enzyme-Linked Immunosorbent Assay "ELISA")*

The basic principle of an Enzyme linked immunosorbent is to use an enzyme to detect the binding of antigen (Ag) antibody (Ab). The enzyme converts a colorless substrate to a colored product, indicating the presence of Ag: Ab binding (Ma *et al.*, 2006).

3.3.1.1 Estimation of Follicle Stimulating Hormone (FSH) Concentration (µlU/ml):

Measurement of serum follicular stimulating hormone concentration was generally regarded as valuable tool in the diagnosis of homeostasis of fertility regulation via the hypothalamic –pituitary–gonad axis; kit was used (Monobind Inc. lake forest CA 92630, USA).

Principle of the Test:

The Monobind (FSH) ELISA is based on the principle of competitive enzyme immunoassay; the essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. as show appendix II.

3.3.1.2. Estimation of Luteinizing Hormone (LH) concentration (ng/ml)

Measurement of serum gonadotropin (LH) concentration was generally regarded as valuable tool in the diagnosis of homeostasis of fertility regulation via the hypothalamic–pituitary–gonad axis; kit was used (Monobind Inc. lake forest CA 92630, USA).

Principle of the test:

The monobind (LH) ELISA was based on the principle of competitive enzyme immunoassay; the essential reagents required for a solid phase

enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. as show appendix III.

3.3.1.3 Estimation of Testosterone (T) Concentration (ng/ml):

The serum testosterone was estimated by ELISA test for the quantitative determination of total testosterone in serum or plasma kit (Human, Germany).

Principle of the test:

The testosterone ELISA was based on the competitive interaction of testosterone and the hormone-enzyme conjugate for a limited number of immobilised monoclonal anti-testosterone antibodies (mouse). This amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of testosterone in the specimen. as show appendix IV.

3.3.1.4 Estimation of rat inhibin B (INH-B) Concentration (ng/ml) by using the appropriate enzyme-linked immunosorbent assay (ELISA) (Elisys UNO Japan):

The kit allows for the determination of INH-B concentrations in rat serum, plasma, tissue homogenates and other biological fluids.

Principle of the test:

The kit assay rat INH-B level in the sample, use purified rat INH-B antibody to coat microtiter plate Welle, make solid-phase antibody, then add INH-B to the wells, combined antibody which with HRP labelled, become antibody-antigen-enzyme-antibody complex, after washing completely, add TMB substrate solution, TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm .the concentration of INH-B in the samples is then

determined by comparing the O.D. of the samples to the standard curve.as show appendix V.

3.4 Body weight and testicular measurements

3.4.1 final body weight

The body weight of all-male rats and of all groups was recorded at the end of the experiment, body weights were measured using the electronic balance.

3.4.2 Testis weight

It's mean of weight two testes divided on 2.

Testis weight= weight right testis +weight left testis/2

3.4.3 Gonadosomatic index

The rats were weighed before the study and at its termination. Testes were removed and weighed. Gonadosomatic index percent (GSI %) was determined using the following formula (Ramzan and Qureshi.,2011):

$$\text{GSI (\%)} = \frac{\text{testes weight}}{\text{final body weight}} \times 100$$

3.4.4 Testis volume:

The length and width of both testes were determined using the digital vernier calipers. Testis volume was estimated from the equation of an ellipsoid according to (Pochron and Wright., 2002).

$$Testis\ volume\ (mm3) = 3 \times 4 \times \pi \times w^2$$

3.4.5 Anogenital distance (AGD):

Anogenital distance (AGD) is the distance from the anus to the base of the penis**.** The AGD is measured as follows: from the center of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum in males.

AGD was measured using digital calipers according to (Eisenberg and Lipshultz., 2015).

3.5 Sperm analysis

3.5.1 Testicular spermatid head count

For 2–3 minutes, frozen testes were thawed. In the tunica albuginea, a small incision was created. Tunica and blood vessels connected with it were removed from the testes. Testes were homogenized for 1 min at1000×g. Samples were stained with 0.1% w/v trypan blue solution for≥1 min. The modified Neubauer's chamber was used to count spermatid heads. For each animal, two notes were collected, and the mean of both was deemed to be a typical measurement. The total number of spermatid heads in the testis was calculated as described by (Seung *et.al*. 2003) using the formulae:

- **Total number of spermatid heads**

$$= \frac{\text{mean count of spermatid heads}}{0{:}\,00004\ \text{ml}} \times 50{:}\,5\ \text{ml}$$

- **Elongated spermatids per gram of testis weight**

$$= \frac{\text{Total number of spermatid heads}}{\text{testis weight}}$$

- Daily sperm production (DSP) was calculated as

Daily sperm production per gram testis weight per day

$$= \frac{\text{Total number of spermatid heads per grm of testis weight}}{6{:}\,10(\text{time divisor})}$$

3.6 Histological study:

The animal's anatomy in the laboratory and the organs of rats were dissected (testes and prostate) rapidly excised for histological study and rinsed in normal saline then fixed by immersing deeply in a large volume of 10% formalin at least ten times the volume of the tissue as rapidly as was feasible thereby keeping postmortem changes at a minimum. The samples were put in a labelled container contain 10% formalin and shaking of the container gently several times to make certain that the fluid reached all surfaces and that the pieces were not sticking to the bottom or sides (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom) according to (Luna., 1968).

3.7 Histopathological Technique:

The testis and prostate of each animal were quickly removed and rapidly weighed then prepared for histological study according to Luna method (1968) with aid of the light microscope as the following steps:

1.Fixation

The specimen was fixated in the natural buffered formalin 10 % for 24 – 48 hours.

2.Washing and dehydration

After fixation, the specimens were washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later . By dehydration, the water had been completely extracted from fragments by bathing them successively in a graded series of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

3.Clearing

Bathing the dehydrated fragments insolvent (xylene) for 30 – 60 minutes, this step was repeated 3 times. As the tissues clearing, they generally became transparent.

4.Infiltration and embedding

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 – 60 C. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

5.Sectioning

After hold from the oven, the specimen was let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by the microtome, steel blade into sections 5 micrometers thick. The sections were floated on a water bath (50 – 55 o C), then transferred into glass slides coated with Mayers albumin as an adhesive substance and left to dry.

6.Staining

The histological sections of the studied organs were stained with

Hematoxylin - Eosin stain.

3.8 Statistical Analysis:

Statistical analysis of the results was conducted according to SPSS (2016) version 24.00 where one way (ANOVA) was used to assess the significance of changes between the groups' results. The data were expressed as Mean, ± Standard Errors (SE) and P-value ≤ 0.05 were considered as statistically

significant, LSD test was carried out to test the significant levels among means of treatments (Green and Salkind., 2016).

Chapter Four

Results

4. Result

4.1 Effect of kisspeptin and ubiquinone 10 on serum hormones levels of pituitary –Gonad axis of the pre-pubertal male rat:

The statistical analysis of variance revealed the presence of a significant decrease ($P \leq 0.05$) in the level of FSH hormone level in rats treated with the kisspeptin as compared with other groups. On the other hand, Coadministration of ubiquinone with kisspeptin considerably increases its value, (Table 4-1).

Regarding the LH serum level, there was a significant decrease ($p \leq 0.05$) in the group of rats treated with kisspeptin compared to the other groups. in contrast, Kisspeptin administration with ubiquinone concurrently resulted in a substantial increase in its value, (Table 4-1).

Concerning testosterone level, as appear in the Table (4-1), the rats treated with kisspeptin only, show a significant decrease ($p \leq 0.05$) in their value compared with other groups. Kisspeptin coadministration with ubiquinone improved its value as well.

While Inhibin B level, Table (4-1) showed significant increase ($p \leq 0.05$) in male rats treated with kisspeptin when compare with the control group, ubiquinone with kisspeptin group and ubiquinone alone group. In comparison to kisspeptin alone, coadministration of kisspeptin with ubiquinone improves its value, but it was not significantly different in the control group. In comparison to the other groups, ubiquinone alone shows a substantial reduction in Inhibin B.

Table (4-1) Effect of kisspeptin and ubiquinone 10 on serum hormones levels of pituitary –Gonad axis of the pre-pubertal male rat (mean +SE):

Parameters / Groups	FSH (µIU/ml)	LH (µIU/ml)	Testosterone (ng/ml)	Inhibin B (pg/ml)
Control	A 1.91±0.11	A 1.88±0.18	B 2. 13±0.23	B 312.6±54.10
Group 1 (Kisspeptin 65µg/kg B.W.)	B 0.86±0.16	B 0.63±0.12	C 1.08±0.16	A 476.3±43.14
Group 2 (ubiquinone10 10mg/kg B.W.)	A 2.17±0.21	A 2.03±0.21	A 2.83±0.26	B 263.2±31.05
Group 3 (Kisspeptin+ ubiquinone.)	A 2.02±0.11	A 1.65±0.11	B 1.68±0.12	B 295.7±33.11
LSD	0.84	0.53	0.67	83.4

N=6

Different letters represent significant differences at ($p \leq 0.05$).

4.2 Effect of kisspeptin and ubiquinone 10 on body weight and testicular measurements of prepubertal male rat:

Table (4-2) illustrated statistical analysis of variance of body weight in the present study reveals no significant difference between the control group and the treated group.

Testis weight was a significant decrease ($p \leq 0.05$) in rats treated with the kisspeptin group compare to other groups. on the other hand, coadministration of ubiquinone with kisspeptin leads to a significant enhancement in the value of testis weight, (Table 4-2).

Regarding the gonadosomatic index, there was a significant decrease ($p \leq 0.05$) in the group of rats treated with kisspeptin compared to the other groups. On the other hand, administration of kisspeptin with ubiquinone concurrently led to a significant improvement in its value, (Table 4-2).

Also, as appear in the table (4-2) a significant decrease($p \leq 0.05$) in the testis volume in the rats exposed to kisspeptin, Furthermore, concurrent administration of kisspeptin with ubiquinone results in a significant improvement in its value.

Anogenital distance (AGD) is the distance from the anus to the base of the penis, and reduction in this length is an indicator of abnormal male reproductive tract masculinization. AGD, together with penile length, provide accessible end-point markers for male reproductive health.

According to anogenital distance (AGD), the rats treated with kisspeptin only show a significant decrease ($p \leq 0.05$) in their value compared with other treated and control groups. while coadministration of kisspeptin and ubiquinone causes a significant increase ($p \leq 0.05$) in its value in comparison with kisspeptin only but it was not significantly different in the control group. ubiquinone alone reveals a significant increase in AGD in comparison with all groups, (Table 4-2).

Table (4-2) Effect of kisspeptin and ubiquinone 10 on body weight and testicular measurements of pre-pubertal male rat (mean +SE):

Parameters / Groups	Total B.W (g)	Testis weight (g)	GSI %	Testis volume (cm3)	AGD (Mm)
Control	A 135.3±13.6	A 1.040±0.09	A 0.768±0.11	A 0.854±0.12	B 20.3±2.12
Group 1 (Kisspeptin 65µg/kg B.W.)	A 141.7±12.1	B 0.910±0.04	B 0.642±0.10	B 0.645±0.16	C 18.6±2.04
Group 2 (ubiquinone10 10mg/kg B.W.)	A 144.3±18.4	A 1.106±0.09	A 0782±0.03	A 0.977±0.14	A 21.6±1.32
Group 3 (Kisspeptin+ ubiquinone.)	A 138.8±15.1	A 1.090±0.06	A 0.785±0.07	A 0.817±0.11	B 20.3±1.94

LSD	11.635	0.137	0.105	0.169	1.098

N=6

Different letters represent significant differences at ($p \leq 0.05$).

4.3 Effect of kisspeptin and ubiquinone 10 on the total number of spermatid head, elongated spermatid and daily sperm production of prepubertal male rat:

Statistical analysis of variance of a total number of spermatid heads, elongated spermatid, and daily sperm production in the present study presented in table (4-3).

The result of a total number of sperm heads shows a significant decrease ($p \leq 0.05$) in rats treated with the kisspeptin group compare to other groups. in contrast, the coadministration of ubiquinone with kisspeptin significantly increases its value.

Regarding the elongated spermatid, there was a significant decrease ($p \leq 0.05$) in the group of rats treated with kisspeptin compared to the other groups. On the other hand, Kisspeptin coadministration with ubiquinone also resulted in a substantial increase in its value.

Concerning daily sperm production, the rats treated with kisspeptin only show a significant decrease ($p \leq 0.05$) in their value compared with other treated and control groups. while coadministration of kisspeptin and ubiquinone causes a significant increase ($p \leq 0.05$) in its value in comparison with kisspeptin only but it was non significantly different in the control group. ubiquinone alone reveals a significant increase in daily sperm production in comparison with all groups.

Table (4-3) Effect of kisspeptin and ubiquinone 10 on total number of spermatid head, elongated spermatid and daily sperm production of pre-pubertal male rat:

Parameters / Groups	Total number of spermatid head ($\times 10^6$ / ml)	Elongated spermatid ($\times 10^6$ / g of testis weight)	daily sperm production ($\times 10^6$ / g of testis weight)
Control	B 5.54 ±0.27	B 5.33±0.76	B 0.78±0.02
Group 1 (Kisspeptin 65µg/kg B.W)	C 1.94±0.31	C 2.13±0.84	C 0.35±0.07
Group 2 (ubiquinone10 10mg/kg B.W.)	A 7.58±0.49	A 6.85±0.39	A 1.12±0.03
Group 3 (Kisspeptin+ ubiquinone)	B 4.93±0.24	B 4.52±0.46	B 0.74±0.06
LSD	1.235	1.128	0.28

N=6

Different letters represent significant differences at ($p \leq 0.05$).

4.4 The Histological Examinations.

4.4.1 Histological Picture and Changes of the Testis:

Histological section of the testis of animals in the control group includes the normal structures of seminiferous tubules shows normal spermatogenic cells and supporting cells, (Figure 4-1).

Histological section of the testis of rats treated with (65µg/kg B.W of kisspeptin) reveals seminiferous tubule degeneration (increased intraepithelial gaps, sloughing and disruption of germinal epithelium, atrophied epithelial cells and placed irregularly on the basement membrane). There are a few spermatogonia, accumulation of pinkish edematous fluid between the seminiferous tubules and vascular engorgement, (Figure 4-2).

The Histological section of testis of animal rats with 10mg/kg B.W of ubiquinone, in this section shows normal structures of seminiferous tubules, which shows normal spermatogenic and supporting cells. ubiquinone 10 prevent the harmful effect of kisspeptin (Figure 4-3).

While other sections in the testis of animals treated with (65µg/kg B.W of kisspeptin and 10mg/kg B.W of ubiquinone 10) some of seminiferous tubules show decrease in number in spermatogonia, (Figur4-4).

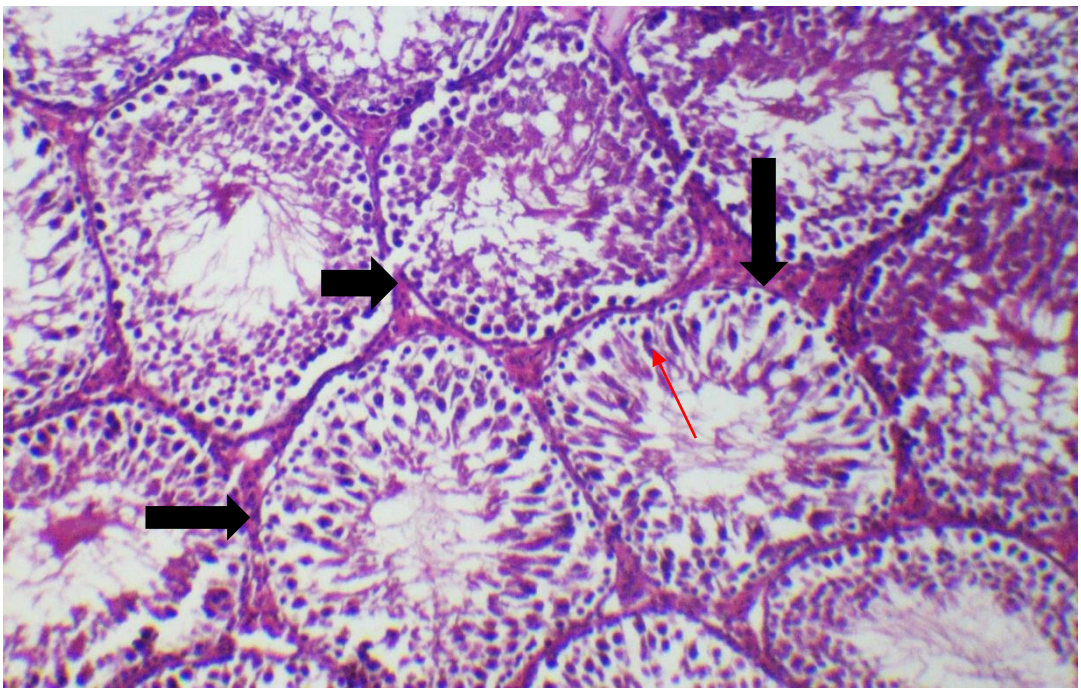

Figure 4-1: Light micrograph of testis of control rat showed the normal structures of seminiferous tubules (black arrow) contained spermatogenic cells in different stages of development (red arrow). H&E, 100x

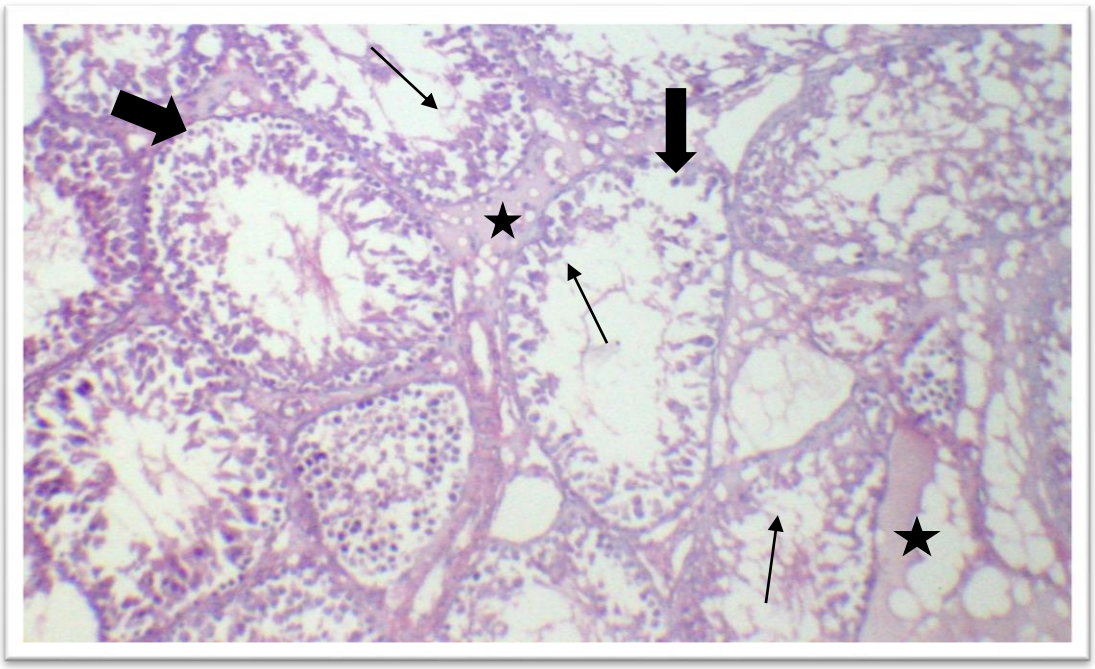

Figure 4.2. Light micrograph of histological changes in testis of rat treated with kisspeptin 65µg/kg B.W. showed decrease number of spermatogenic (thin arrows) and sloughing germinal epithelial (thick arrows) and pinkish edematous fluid between seminiferous tubules (star). H&E, 100x

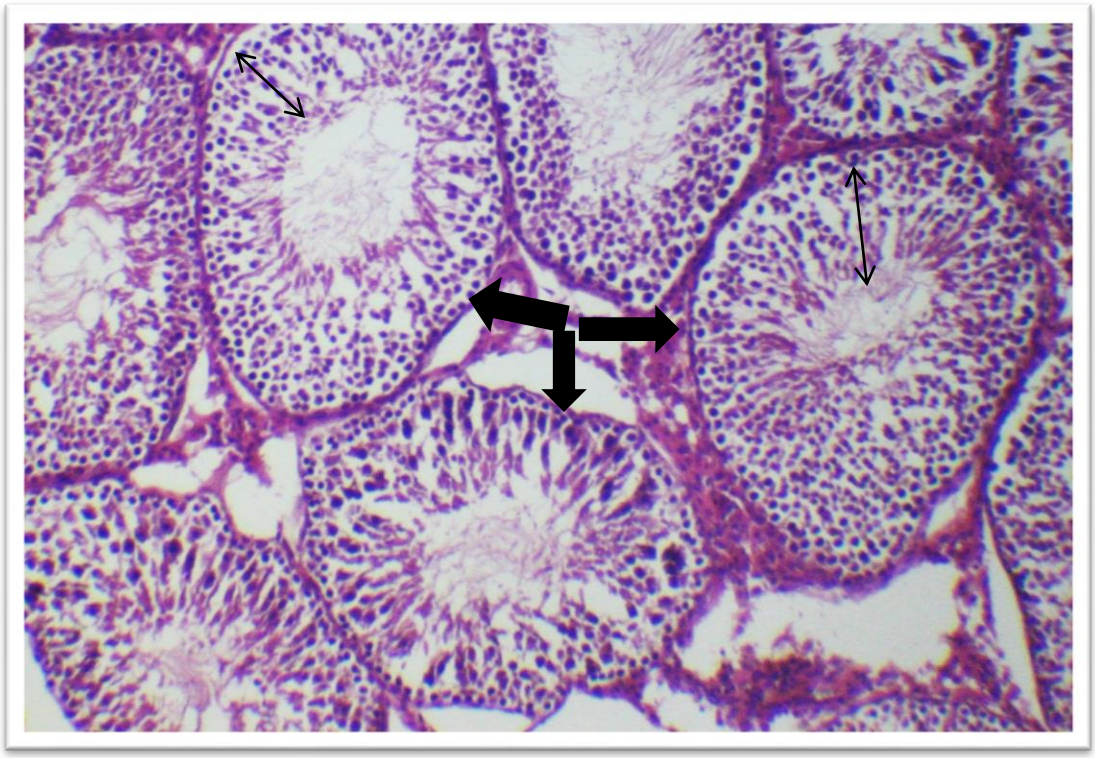

Figure 4-3: Light micrograph of histological changes in testis of treated with 10mg/kg B.W. of ubiquinone 10 showed the normal architecture of seminiferous tubules (thick black arrow) contained spermatogenic cells in different stages of development (thin black arrow). H&E, 100x

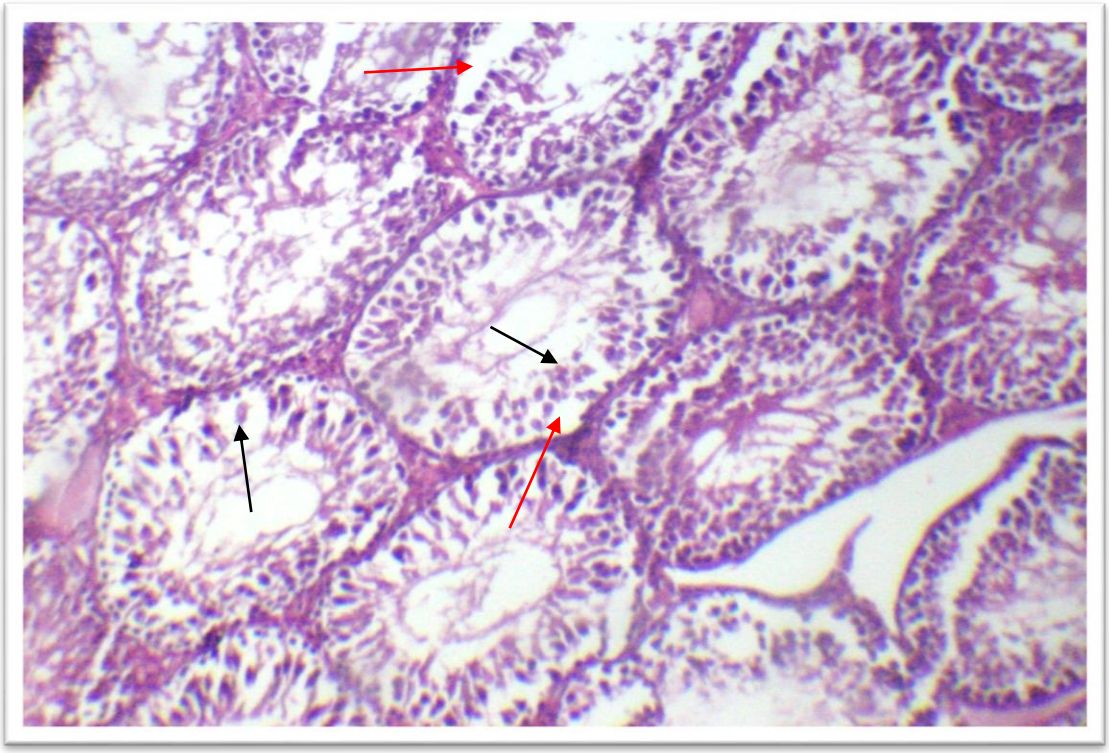

Figure 4.4 Light micrograph of histological changes in testis of treated with 65µg/kg B.W. of Kisspeptin+ 10mg/kg B.W of ubiquinone 10 showed some of seminiferous tubules (red arrows) with few numbers of spermatogonia (black arrow) H&E, 100x

4.4.2 Histological Picture and Changes of the Prostate:

Histological section of prostate of control rats shows normal prostatic gland and lobules containing mucous secretory units (acini), (Figure 4-5).

While some prostatic glands appear empty from the prostatic secretion in the light micrograph of the prostate removed from rats exposed to kisspeptin (65μg/kg B.W). There was also degeneration of epithelial cells and sloughing of some epithelia. also, show hyperplasia of glandular epithelial cells and of papillary projections toward the alveolar lumen, (Figure 4-6).

Histological section in the prostate of animals treated with 10mg/kg B.W of ubiquinone showed normal histological structures and prostatic secretions obviously filled the lumens, (Figure 4-7).

While other sections in the prostate of rats treated with (65μg/kg B.W of kisspeptin and 10mg/kg B.W of ubiquinone 10) showed normal prostatic glands lined by normal glandular epithelia and prostatic secretions filled the lumens, (Figure 4-8).

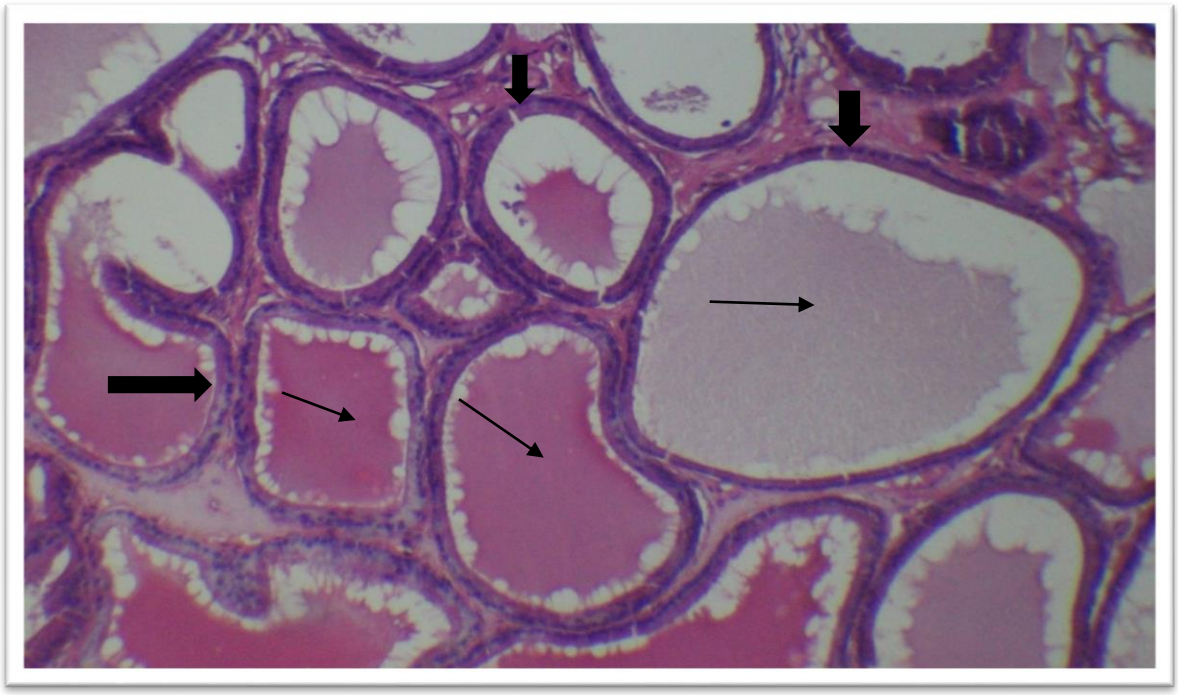

Figure 4-5: Light histological of prostate from control rats showed normal shape and size of prostatic gland lined by normal glandular epithelial (thick arrow) and filled with prostatic secretion (thin arrow). H&E, 100x

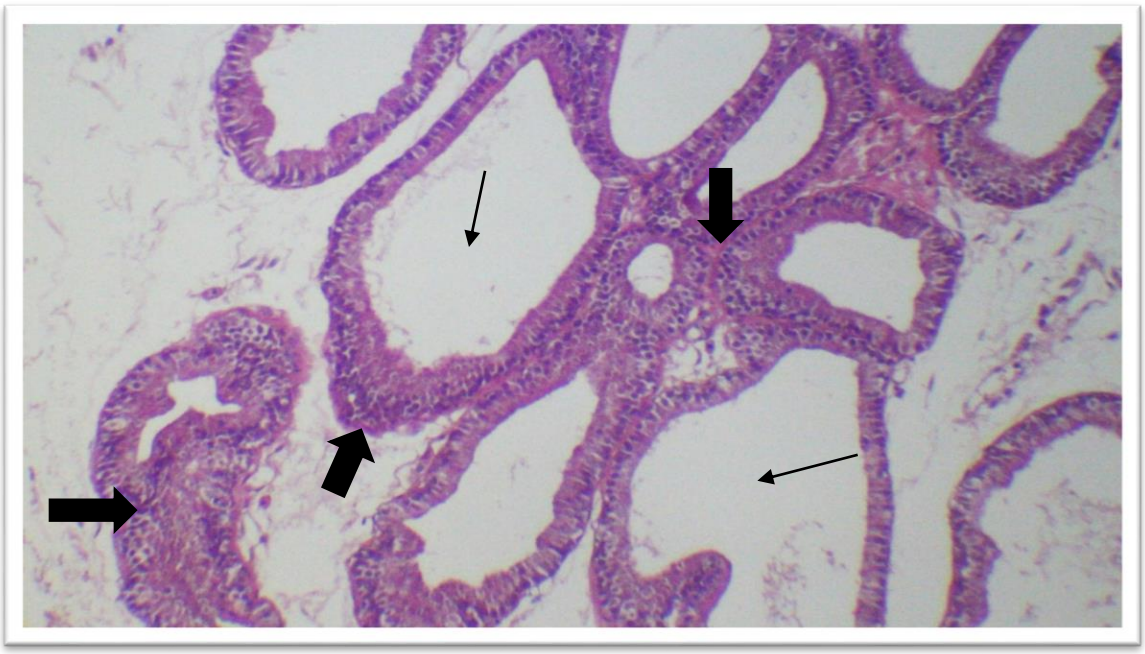

Figure 4-6: Light histological of prostate rat treated with 65µg/kg B. W of kisspeptin. showed hyperplasia of glandular epithelial cells (thick arrows) some prostatic acini appear empty from prostatic secretion (thin arrow). H&E, 100x

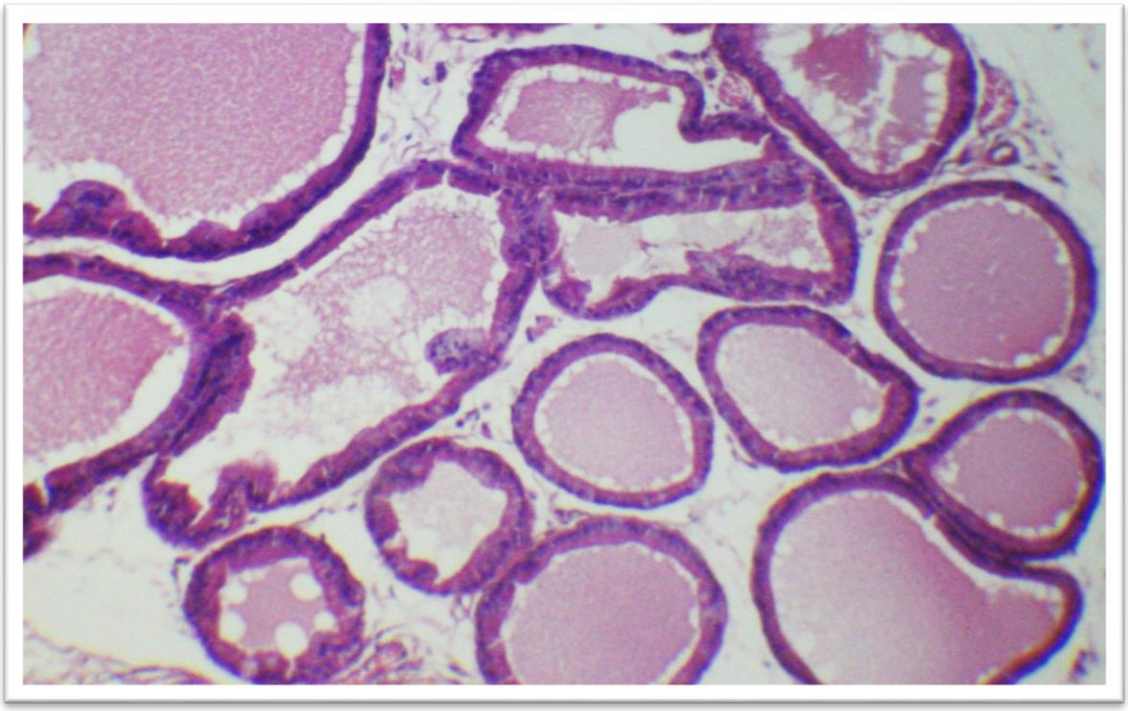

Figure 4-7: Light histological of prostate from rats received ubiquinone showed normal shape and size of lobule with prostatic fluid. H&E, 100x

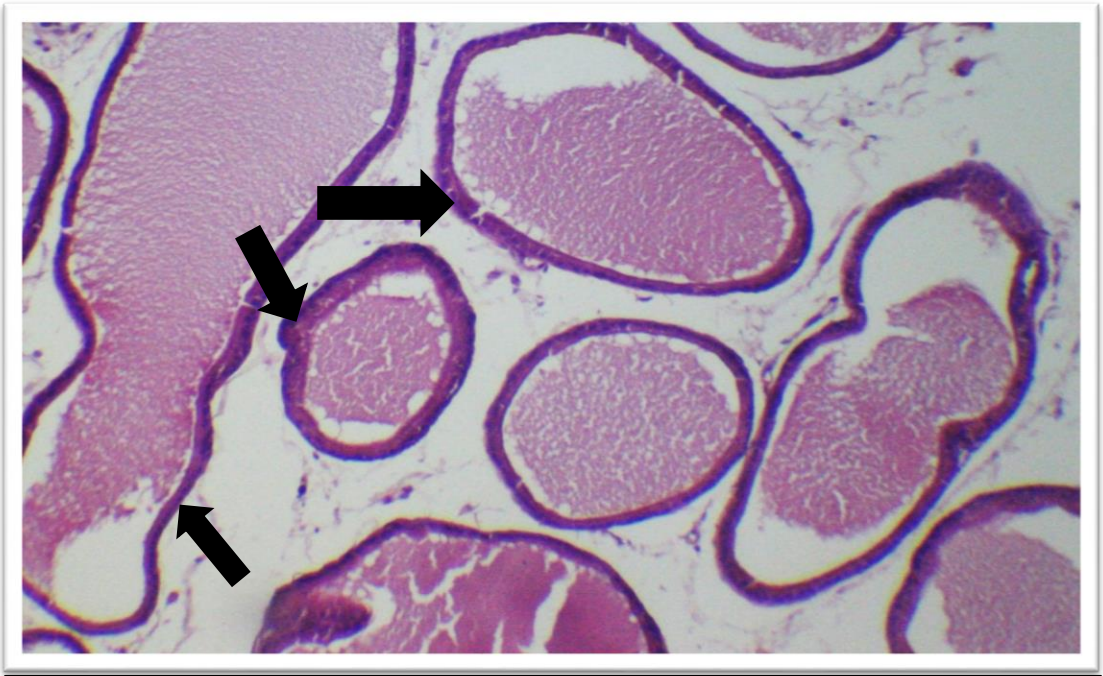

Figure 4-8: Light histological of prostate rat treated with 65µg/kg B. W of kisspeptin + 10mg/kg B.W of ubiquinone showed normal glandular epithelial cells (thick arrows). H&E, 100x

Chapter Five

Discussion

5.Discussion:

5.1 Effect of Kisspeptin and Protective role of Ubiquinone 10 on FSH, LH, Testosterone and Inhibin B:

The current study found that there was a significant decrease ($p \leq 0.05$) in FSH levels in male rats treated with kisspeptin when compared to other groups in the prepubertal stage. This study agreed with Husam, (2019) who pointed that chronic subcutaneous injection of 50nmol/day for 13 days of kisspeptin 54 produced a significant decrease in plasma FSH when compared to control group. While this result disagreed the findings of (Ramzan and Qureshi, 2011) who observed no influence on FSH levels after Kisspeptin administration intraperitoneally at different dosage concentrations (1 μg,1 ng, and 10 pg) in prepubertal male rats, twice daily for 12 days.

Hormonal intervention at this critical period causes an imbalance in hormone function. As a result, it was likely that pulsatile kisspeptin input altered the pattern of GnRH release in terms of frequency and pulse amplitude. The reduced level of FSH might be attributable to the inhibitory action of inhibin, which was considerably increased after kisspeptin administration. Or it might be the result of long-term usage of kisspeptin, which had a desensitizing impact on the hypothalamus and pituitary gland (Aytürk *et. al*., 2017 and Husam, 2019).

In terms of LH levels, the results show a significant decrease ($p \leq 0.05$) in LH levels in male rats treated with kisspeptin compared to treated and control groups, which was consistent with (Ramzan and Qureshi., 2011); Ramzan *et. al*., (2013); Aytürk *et.al*., (2017); Husam., (2019) and Ramzan *et.al*., (2021) who reported LH concentration was reduced in kisspeptin group compared to control group.

This reduction might be due to prolonged kisspeptins administration desensitized the hypothalamic–gonadal–pituitary axis (Aytürk *et.al*.,2017). or perhaps the substantial decrease in LH and testosterone concentrations, it is reasonable to assume that the cellular degeneration was caused by a decrease in plasma testosterone concentration. Exogenous kisspeptin concentrations higher than normal may also had a negative feedback impact on either the GnRH secreting neurons or directly on the pituitary due to Kiss1r expression (Kotani *et.al*., 2001), suppressing LH secretion and a consequent in a reduction in gonadal steroids. The existence of a functional kisspeptin receptor on the pituitary and kisspeptin release in the hypophyseal portal blood implies that kisspeptin may operate directly on the pituitary to control gonadotropin production (Smith *et.al*., 2008).

Regarding testosterone levels, the results revealed a significant reduction ($p \leq 0.05$) in testosterone levels in male rats treated with kisspeptin compare with other groups. This finding is similar to the findings of (Ramzan and Qureshi., 2011); Ramzan *et.al*., (2013); Aytürk *et.al*., (2017); Husam., (2019) and Ramzan *et.al*,.(2021). Who found a reduction in testosterone following continuous administration of Kisspeptin. Furthermore, these results were consistent with those of Ohga *et.al*. (2013), who reported that testosterone levels were decreased following 13 days of administration kisspeptin-54. Similarly, Thompson *et.al*. (2006) found that continuous subcutaneous injection of kisspeptin-54 resulted in a trend for total and free testosterone decrease with no statistical significance.

This reduction might be attributed to a substantial fall in testosterone concentration following kisspeptin therapy, which could be attributed to a significantly reduced LH concentration because testosterone production is regulated by LH. This suggests that the rapid drop in testosterone levels was most likely caused by active inhibition of gonadal testosterone production.

chronic kisspeptin usage may had a desensitizing impact on the hypothalamus and pituitary gland, affecting gonadal production of sex hormones (Husam,2019).

While the present study found a substantial increase in inhibin B in male rats treated with kisspeptin compared to other groups. This result contradicts (Thompson *et.al*., 2009) who spotted that continuous kisspeptin-54 administration leads to a significant decrease in circulating inhibin B after 12 days of continuous administration, as well as Aytürk *et.al*., (2017); Tariq and Shabab, (2013) who revealed that continuous infusions of Kisspeptin led to decrease inhibin B level in mice.

This rise in Inhibin B levels in the present study may be related to a reduction in follicle-stimulating hormone (FSH), which may have a direct influence on Sertoli cells, leading to higher levels of Inhibin B. Plasma Inhibin B, which is mostly generated by Sertoli cells, typically corresponds with Sertoli cell number (Ramaswamy *et.al*., 1999 and Sharpe *et.al*., 1999). Inhibin B has been considered as a sensitive endocrine marker reporting spermatogenesis status (Pierik *et.al*., 1998). FSH, a hormone generated and released by the anterior pituitary, stimulates spermatogenesis by acting on Sertoli cells in the seminiferous tubules (Meeker *et.al*.,2007). FSH and Inhibin B both are more sensitive than either alone in predicting the histological state of the testis and the presence of sperm in bioptic tissue (Barbotin *et.al*.,2015).

The present study pointed that coadministration of kisspeptin with ubiquinone leads to improvements in FSH, LH, testosterone and inhibin B levels.

Ubiquinone 10 is a natural antioxidant that is essential to the electron transport chain (Nohl *et.al*.,1999), Ubiquinone 10 may protect the testis from oxidative stress by reducing lipid peroxidation and increasing antioxidant enzyme activity. This, in turn, can prevent oxidative damage and keep Leydig cells working to safeguard testosterone secretion (Palmeira *et.al*.,2001). The primary function of ubiquinone10 in the testis was to raise the amounts of Ubiquinone10 and its reduced form ubiquinol in the semen (Balercia.,2004). Ubiquinol was a powerful fat-soluble antioxidant that had the ability to replenish other antioxidants such as vitamins E and C (Turunen *et.al*.,2004). ubiquinone 10 removes peroxyl radicals produced by the lipid peroxidation process (Potgieter *et.al*.,2013). also, it lowers follicle-stimulating hormone and luteinizing hormone levels (Safarinejad.,2009; Mancini and Balercia.,2011). Coadministration of Ubiquinone-10 with procarbazine improves hormone levels as well as testicular catalase activity, glutathione, and superoxide dismutase levels according to recent studies (Hassan and Jasim.,2020).

5.2 Effect of Kisspeptin and Protective role of Ubiquinone 10 on total body weight, testis weight, gonadal somatic index, testis volume and AGD:

In the present study, results showed that there was no effect on total body weight in male rats treated with kisspeptin compared to other groups in the prepubertal stage, which was consistent with the findings of Ramazan et al., (2021), who recorded no effect on body weight after intraperitoneal administration of kisspeptin for 12 days.

This might be because the exposure time did not act on the growth hormone, all animals go through an equal stage of growth, and kisspeptin acts on the gonads rather than the growth hormones.

Concerning testis weight, male rats treated with kisspeptin had a significant reduction ($p \leq 0.05$) in testis weight when compared to treated and control groups. This is consistent with the findings of Husam., (2019) who reported that a chronic subcutaneous injection of kisspeptin-54 at a daily dose of 50 nmol resulted in a substantial decrease in testis weight. Furthermore, this result agreed with (Thompson *et.al*., 2006) who revealed a significant decrease in testicular weight in their trial of injecting kisspeptin-54 for thirteen days with a daily dosage of 50 nmol when compared to the control group. Long-term subcutaneous injection of 50 nmol of Kisspeptin-54 for 13 days resulted in a considerable decrease in testicular weight in adult male rats.

This decrease may be due to kisspeptin's desensitizing effect on the hypothalamus and pituitary, which then impairs gonadal activity, rather than kisspeptin's direct action on the gonads (Husam.,2019),Or that kisspeptin may had a direct impact on the gonads in addition to the expected indirect effects via HPG axis regulation (Papaoiconomou *et.al*.,2011), It was probable that the weight of the testis was significantly reduced due to extensive degeneration of the seminiferous tubules, which resulted in damage to both germ and Sertoli cells (Thompson *et.al* .,2006, Papaoiconomou *et.al*.,2011).

In regard to gonadal somatic index and testis volume, male rats treated with kisspeptin had a significant reduction ($p \leq 0.05$) when compared to other groups. This finding contradicts the findings of (Ramzan *et.al*., 2021) who found no impact on the gonadal somatic index or testicular volume following intraperitoneal administration of a 1g kisspeptin dosage for 12 days.

This decrease in the present study may be due to testicular atrophy, a condition that causes shrinkage of the size of the testicle. Conversely, the small size of the testicles in many cases may be associated with a lack of testosterone within them; thus, hormonal imbalances, particularly of the

pituitary gland, are one of the main reasons for the small size of the testicles (Thompson *et.al*., 2009; Ramzan and Qureshi, 2013). it might also be related to testicular degeneration, which lead to a reduction in the number of sperms and, as a result, a decrease in the size and weight of the testicle (Thompson *et.al*.,2006). Ramzan and Qureshi., (2011) found a reduction in testosterone and LH levels, which may lead to a fall in the gonadal somatic index.

Also, the results of this study revealed a significant decrease ($p \leq 0.05$) in AGD in kisspeptin-treated rats when compared to treated groups and control. This finding was consistent with the result of (Funes *et.al*., 2003) and Seminara *et.al*., (2003) who reported that GKirKO males exhibited a substantial decrease in the anogenital distance in global knockout mice models. Furthermore, as compared to control mice, the knockout males exhibited a significant reduction in testicular size.

This decrease may be due to the kisspeptin-related reduction of testosterone levels in male rats during the critical developmental stage because testicular growth was highly promoted by testosterone (Eisenberg *et.al*.,2012 and Ramzan *et.al*.,2021). Anogenital distance (AGD), a sexual dimorphism measure of genital development, was an indicator for endocrine disturbance in animal research and may be shorter in infant males with genital abnormalities. There was relationship between genital development and anogenital distance (Eisenberg *et.al*.,2011). Shorter male AGD is linked with cryptorchidism and hypospadias, decreased sperm count, and infertility in rats and humans. AGD is influenced by hormones throughout fetal development and has long been utilized as an early indicator of reproductive toxicity. Multiple human research had shown that males exposed to environmental anti-androgens had a shorter AGD, which was consistent with animal studies. For these reasons, AGD has been regarded as a singularly important valuable metric in endocrine disruptor research (Swan and Kristensen, 2018).

Coadministration of kisspeptin with ubiquinone leads to enhancement of its value body weight and testicular measurements compare with the control group. This improvement might be attributed to hormonal improvements or because ubiquinone 10 had antioxidant characteristics after treatment, with levels of ubiquinone10 increasing in seminal plasma and sperm cells (Balercia *et.al*., 2004). This results in an inhibitory decrease of kisspeptin on the gonads.

5.3 Effect of Kisspeptin and Productive role of ubiquinone 10 on the total number of spermatid heads, elongated spermatid and daily sperm production.

In the current study, our results showed that there was a significant decrease ($p \leq 0.05$) in the total number of spermatid heads in male rats treated with kisspeptin compare with other groups in the prepubertal stage.

This study agrees with the findings of Aytürk *et.al*., (2017), who found that administering kisspeptin 54 (50 nmol/day) for 13 days using mini-osmotic pumps implanted subcutaneously in the interscapular area of male rats significantly lower sperm count. This result is also consistent with Husam, (2019), who reported that a chronic subcutaneous injection of kisspeptin 54 at a daily dosage of 50 nmol resulted in a substantial reduction in sperm count.

This reduction of sperm count might be due to direct desensitization of the HPG axis (Aytürk *et.al*., 2017), or it could be due to kisspeptin's desensitizing impact on the hypothalamus and pituitary gland, which then affects gonadal functions rather than kisspeptin's direct influence on the gonads (Husam.,2019), or the testicular degeneration observed after prolonged kisspeptin treatment is most likely due to changes in testicular blood flow. Apoptosis is caused by short-term flow decreases in spermatogonia and early spermatocytes (Bergh *et.al*.,2001).

Concerning elongated spermatid, the results revealed a significant decrease ($p \leq 0.05$) in elongated spermatid in male rats treated with kisspeptin when compared to other groups. This result agrees with previous studies by Ramzan and Qureshi, (2011); Ramzan *et.al*., (2013); Zou *et.al*., (2019) and Ramzan *et.al*., (2021) who revealed a significant decrease in elongated spermatid following administration of kisspeptin.

This reduction might be attributed to data at the cellular level that totally supports the suppression of testosterone and LH concentrations, mediated by kisspeptin at the spermatogenic cycle, resulting in excessive degradation of the seminiferous epithelium. Currently, a limited number of elongated and spherical spermatids were persistently seen, indicating a halt in germ cell maturation (Ramzan *et.al*.,2021).Or that LH withdrawal suppresses spermatogenesis, resulting in significant decreases in the numbers of pachytene spermatocytes, preleptotene spermatocytes, spermatogonia, and round spermatids while elongated spermatids remained undetectable (McLachlan *et.al*., 1994), perhaps because acute removal of testosterone causes a unique degeneration pattern of the spermatogenic cell. A substantial drop in elongated spermatid and spermatid heads was seen at day 8 and beyond, since the animals tested were prepubertal and did not have these cells before that age (McLachlan *et.al*., 1994).

Regarding daily sperm production, the results showed that there was a significant decrease ($p \leq 0.05$) in daily sperm production in male rats treated with kisspeptin compare with other groups. This result agreed with Ramzan and Qureshi., (2011); Ramzan *et.al*., (2013) and Ramzan *et.al*., (2021) who revealed a significant decline in daily sperm production following 12 days of continuous kisspeptin administration.

This reduction may be related to this degeneration, which had been linked to a decrease in plasma testosterone. The tendency toward decreased testosterone levels, on the other hand, may indicate a deficit in the

steroidogenic activity of the Leydig cells (Thompson *et.al*., 2009), Or because substantially decreased serum hormone levels (LH, FSH, and testosterone levels), testis weight through direct desensitization of the HPG axis (Aytürk *et.al*.2017). perhaps due to significant declines in germ cells, daily sperm production, total Sertoli cell support capacity, Sertoli efficiency, and meiotic index, as well as an increase in the coefficient of mitosis, suggested germ cell loss, which may have happened via apoptotic processes (Ramzan *et.al*.,2021).

Coadministration in the present study of kisspeptin and ubiquinone resulted in a significant enhancement in the total sperm count, elongated spermatid, and daily sperm production, the improvements might be attributed to ubiquinone 10 capacity to serve as an antioxidant, energy booster, membrane stabilizer, and regulator of mitochondrial permeability transition pores (Turunen *et.al*.,2004). The mitochondria of the mid-piece contain the bulk of ubiquinone 10 in sperm cells, and energy-dependent functions in the sperm cell are dependent on ubiquinone 10 availability (Lewin and Lavon.,1997). ubiquinone 10 levels in the seminal fluid are directly related to sperm parameters (Mancini *et.al*.,2005). Exogenous ubiquinone 10 therapy elevates both ubiquinone and ubiquinol levels in the sperm and might be beneficial in improving sperm kinetic features in individuals with idiopathic asthenozoospermia due to its participation in mitochondrial bioenergetics and antioxidant properties (Balercia *et.al*.,2004 and Balericia *et.al*.,2009).

According to the studies, supplementing with Ubiquinol help to increase sperm count and motility (Thakur *et.al*.,2015). Reduced oxidative damage in the presence of the lipophilic antioxidant ubiquinol might explain why testicular cells produce normal levels of testosterone. Flagella that had a long expose structure are more susceptible to oxidative destruction. By conserving flagella size, which assists in sperm motility, ubiquinol may have

reduced oxidative stress and averted oxidative damage to the flagella structure, which aids in sperm motility (Sharma,1996).

5.4 Histopathological Study:

5.4.1 Histopathological Effect of Kisspeptin and Ubiquinone 10 on Testis Tissue

Testis of male rats treated with kisspeptin applied in the present study showed seminiferous tubule degeneration (increased intraepithelial gaps, atrophied epithelial cells, sloughing and disruption of germinal epithelium, and placed irregularly on the basement membrane). There were a little number of spermatogonia, accumulation of pinkish edematous fluid between the seminiferous tubules and vascular engorgement.

These histological results, which were similar to those reported by (Matsui *et.al*., 2004); Thompson *et.al*., (2009) and Hameed *et.al*., (2013) may be attributable to kisspeptin effects on the HPG axis, which were considered to be mediated via hypothalamic GnRH (Murphy.,2005), or due to the continued injection of kisspeptin-54 may result in a reduction in gonadotropins (Husam.,2019). Another explanation for these histological alterations was that the testicular degeneration found following prolonged kisspeptin treatment might be due to changes in testicular blood flow. Short-term flow reductions cause apoptosis in spermatogonia and early spermatocytes (Bergh *et.al*.,2001), with moderate, long-term blood flow decreases causing localized damage to the seminiferous tubules and spermatogenesis disruption (Markey *et.al*.,1994). Or as a result of considerably lower serum hormone levels (FSH, LH, and testosterone levels), testis weight and sperm count owing to direct desensitization of the HPG axis (Aytürk *et.al*.,2017).

5.4.2 Histopathological Effect of Kisspeptin and Ubiquinone 10 on Prostate Tissue

The light microscopy of a prostate excised from male rats treated to kisspeptin (65µg/kg B.W) revealed some prostatic glands appear empty from prostatic secretion. There was also epithelial cell degeneration and sloughing of some epithelia, in addition, hyperplasia of glandular epithelial cells and of papillary projections toward the alveolar lumen. these findings were similar to those obtained by (Ramzan *et.al*., 2013) who found degenerative effects of continuous administration of Kisspeptin 10 in rat seminal vesicles and prepubertal prostate gland.

The degenerative changes observed in the prostate may be caused by a decrease in testosterone hormone. Testosterone is necessary for the preservation of the height of the mucosal epithelium, which is required for the generation of continuous prostate secretions (Fawcett *et.al*.,1986). The presence of circulating androgens is required for the development and active secretion of accessory reproductive tissues (Brandes.,1974 and Higgins *et.al*.,1976) ,Because prostatic epithelial height was known to be androgen-dependent (Gonzales *et.al*., 2005), prostate secretory activity was a sensitive, androgen-dependent function. Decreased plasma testosterone levels (Ramzan and Qureshi.,2011) may cause atrophic alterations, resulting in decreased secretion.

Coadministration of kisspeptin and ubiquinone resulted in a significant improvement in the studied parameters, the improvements in testicular and prostate detected in the present study could be attributed to the antioxidant properties of ubiquinone 10.

the most important role of ubiquinone 10 in the mitochondrial respiratory chain and its antioxidant properties, this improvement could be due to adequate ubiquinone 10 levels being required for proper spermatozoa

function. Mitochondrial dysfunction in spermatozoa, in particular, has been linked to decrease sperm motility (Pereira *et.al*.,2017), exogenous ubiquinone 10 supplementations may raise its levels in seminal plasma and improve sperm function (Balercia *et.al*.,2009).

To our knowledge, the results of the present study is first record according to relationship between interaction effects of ubiquinone and kisspeptin.

Chapter Six

Conclusions& Recommendations

Chapter Six----------Conclusions & Recommendations

6.1 Conclusions:

It has been concluded from the current study that:

1. Kisspeptin cause harmful effects on reproductive efficacy of male rat during prepubertal period via Its effect on reproductive hormones (FSH, LH, Testosterone, Inhibin B)and testicular measurements(Testis weight, Gonadosomatic index %, Testis volume, anogenital distance)) and also, kisspeptin had a negative effect on reproductive efficacy of male rat during prepubertal period via the effect on sperm analysis (total spermatid heads, elongated spermatid, daily sperm production) and it also, causes a change in the architecture of the testicle and prostate.
2. Coadministration of ubiquinone with kisspeptin leads to ameliorating the harmful effect of kisspeptin by enhancing reproductive hormones (FSH, LH, Testosterone, Inhibin B) and improves the testicle measurements (Testis weight, Gonadosomatic index %, Testis volume, anogenital distance). also, the present study showed coadministration of ubiquinone with kisspeptin ameliorates the negative effect of kisspeptin alone on reproductive efficacy by improving sperm analysis (total spermatid heads, elongated spermatid, daily sperm production) and enhancing testicular and prostate tissues of male rats).

6.2 Recommendations:

From the results of the present study we recommend the followings:

1. Avoid using kisspeptin during the prepubertal stage on males.
2. Use ubiquinone as a supporter of reproductive efficacy in males.
3. Conduct a study to investigating the effect of kisspeptin on adult male rats.
4. Conduct a study to investigating the effect of kisspeptin on females.

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Appendix

Appendix

Appendix I

Below is the composition of our pellets for rats. However we do not prepare this ourselves, but buy it from a commercial producer.

number	contents	Values %
1.	Crude Protein	23%
2.	Crude Fat	3.0%
3.	Crude Fiber	7.0%
4.	Acid Insoluble A	8%
5.	Calcium	1-2.5 %
6.	Phosphorus	0.9%
7.	Sodium	0.5-1%
8.	Moisture	12%

Ingredients: Corn, Soybean pulp, Sunflower seed meal, Shorts, Bonquality flour, Alfalfa pellets, Molases, Meat and bone meal, Poultry meal, Sepiolite, inorganic DCP, Marble dust, vitamins, minerals.

Appendix II

3.3.1 Hormones Assay (Enzyme-Linked Immunosorbent Assay "ELISA")

3.3.1.1 Estimation of Follicle Stimulating Hormone (FSH) Concentration (µlU/ml)

Procedure of the test:

The test procedure was done according to the following steps:

1. FSH-enzyme conjugate solution was prepared by diluting 1ml of

FSH enzyme conjugate with 11ml of total FSH conjugate buffer in

a suitable container.

2. Wash solution was prepared by diluting 20ml of concentrated wash solution with 980ml of distilled water to final volume of 1000ml.

3. Substrate solution was mixed solution A and B.

4. Desired number of microplate wells was secured in the holder.

5. The serum (50µl) of the standard and treated group was dispensed into the assigned wells (all samples were run in duplicate

concurrently so that all conditions of testing were the same).

6. FSH enzyme conjugate solution (100µl) was added to all well.

7. Microplate thoroughly was gently mixed and covered for 20-30

seconds. It is important to have a complete mixing in this step.

8. Microplate was incubated for 60 minutes at room temperature.

9. Contents of the wells were drawn by manual plate washer and the

wells were rinsed 3 times with diluted wash solution (300µl per

well).

10. 100 µl of substrate solution was added to each well.

11. Microplate was incubated for 15 minutes at room temperature.

12. The enzymatic reaction was stopped by adding 50µl of stop

solution to each well.

13. The absorbance (OD) of each well was determined at 450nm with a microplate reader.

Appendix III

3.3.1.2 Estimation of Luteinizing Hormone (LH) concentration (ng/ml):

Procedure of the test:

The test procedure was done according to the following steps:

1. LH-enzyme conjugate solution was prepared by diluting 1ml of

LH enzyme conjugate with 11ml of total LH conjugate buffer in a

suitable container.

2. Wash solution was prepared by diluting 20ml of concentrated

wash solution with 980ml of distilled water to final volume of

1000ml.

3. Substrate solution was mixed solution A and B.

4. Desired number of microplate wells was secured in the holder.

5. The serum (50µl) of the standard and treated group were dispensed into the assigned wells (all samples were run in duplicate concurrently so that all conditions of testing were the same).

6. LH enzyme conjugate solution (100µl) was added to all well.

7. Microplate thoroughly was gently mixed and covered for 20-30 seconds. It is important to have a complete mixing in this step.

8. Microplate was incubated for 60 minutes at room temperature.

9. Contents of the wells were drawn by manual plate washer and the wells were rinsed 3 times with diluted wash solution (300µl per well).

10. 100 µl of substrate solution was added to each well.

11. Microplate was incubated for 15 minutes at room temperature.

12. The enzymatic reaction was stopped by adding 50µl of stop solution to each well.

13. The absorbance (OD) of each well was determined at 450nm with a microplate reader.

appendix IV

3.3.1.3 Estimation of Testosterone (T) Concentration (ng/ml):

Procedure of the test:

1) Secure the desired number of microliter wells in the holder.

2) Dispense 25 µl of each standard, controls and samples with new disposable tips into appropriate wells.

3) Dispense 200 µl enzyme conjugate into each well.

4) Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

5) Incubate for 60 minutes at room temperature.

6) Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution (400 µl per well). Strike the wells

sharply on absorbent paper to remove residual droplets.

7) Add 200 µl of substrate solution to each well.

8) Incubate for 15 minutes at room temperature.

9) Stop the enzymatic reaction by adding 100 µl of stop solution to

each well.

10) Read the OD at 450±10 nm with a microtiter plate reader within 10 minutes after adding the stop solution.

Appendix V

3.3.1.4 Estimation of rat inhibin B (INH-B) Concentration (ng/ml) by using appropriate the enzyme–linked immunosorbent assay (ELISA) (Elisys UNO Japane):

Procedure of the Test.

1.add standard: set standard wells, testing sample wells. add standard 50 µl to standard well.

2.add sample: set blank wells separately (blank comparison well not add sample and HRP-Conjugte reagent, other each step operation is cleare).

testing sample well. add sample dilution 40 µl to the testing sample well, then add testing sample 10 µl (sample final dilution is 5-fold), add sample to wells, don't touch the well wall as far as possible, and gently mix.

3.add enzyme: add HRP-conjugate reagent 100 µl to each well, except blank well.

4.Incubate: after closing plate with closure plate membrane, incubate for 60min at 37C^{o}

5.configurate liquid:20-fold wash solution diluted 20-fold with distilled water and reserve.

6.washing: uncover closure plate membrane, discard liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

7.Color: add Chromogen solution A 50 μl and chromogen solution B to each well, evade the light preservation for 15min at 37C^{o}

8.Stop the reaction: add stop solution 50 μl to each well, stop the reaction (the blue color change to yellow color).

9.assay: take blank well as zero, read absorbance at 450nm after adding stop solution and within 15 min.

الخلاصة

أجريت هذه الدراسة للتحري في آثار إعطاء كيسببتين بصورة نبضية لمدة 12 يومًا على الكفاءة التكاثرية في ذكور الجرذان قبيل مرحلة البلوغ الجنسي ولتقييم الدور الوقائي لل ايبيكواينون -10. ستة وثلاثون من ذكور الجرذان مقسمة إلى أربع مجموعات لكل مجموعة 9 حيوانات تم التعامل معها على النحو التالي: مجموعة السيطرة حقنت DMSO داخل البريتون مرتين يوميا. مجموعة كيسببتين عولجت بالكيسببتين بطريقة نبضية (65 ميكروغرام / كيلوغرام من وزن الجسم) داخل البرتون مرتين يوميًا ، مجموعة ايبيكواينون عولجت بال ايبيكواينون -10 (10ملغم / كغم من وزن الجسم) عن طريق الفم ، مرتين يوميًا ومجموعة كيسببتين و ايبيكواينون عولجت بالكيسببتن بطريقة نبضية (65 ميكروغرام / كغم من وزن الجسم) داخل البريتون مع ايبيكواينون -10 (10ملغم / كغم من وزن الجسم) عن طريق الفم مرتين يومياً ، واستمرت جميع العلاجات لمدة 12 يوماً ، وتم رعاية الحيوانات حتى اليوم الستين من العمر ، ثم تم قياس وزن الجسم والمسافة الشرجية التناسلية ،

تم أخذ عينات من الدم عن طريق القلب لتقييم (هرمون المنبة للجريب، الهرمون اللوتيني، هرومن الشحمون الخصوي،Inhibin B) ، ثم تم التضحية بالحيوانات تحت التخدير لتشريحها وجمع (الخصى والبروستات) لقياس قياسات الخصية، تحليل الحيوانات المنوية وللدراسة النسجية.

أظهرت الذتائج انخفاضًا معنويًا ($P\leq0.05$) في مستويات الهرمون المنبه للجريب والهرمون اللوتيني وهرمون الشحمون الخصوي في ذكور الجرذان التي عولجت بالكيسببتين مقارنة بالمجموعات الأخرى، بينما أظهر مستوى Inhibin B زيادة معنوية ($P\leq0.05$) في ذكور الجرذان التي عولجت بالكيسببتين مقارنة بالحيوانات في المجموعات الأخرى. يؤدي التناول المتزامن للكيسببتين مع ايبيكواينون إلى تعزيز هذه الهرمونات التناسلية (الهرمون المنبه للجريب، الهرمون اللوتيني، هرمون الشحمون الخصوي،Inhibin B) .

أظهرت نتيجة وزن الجسم وقياسات الخصية عدم وجود تأثير معنوي ($P \leq 0.05$) على وزن الجسم في ذكور الجرذان المعالجة بالكيسببتين، بينما كان هناك انخفاض معنوي في وزن الخصية، ومؤشر الاقناد، وحجم الخصية، والمسافة الشرجية التناسلية عند ذكور الجرذان التي عولجت بالكيسببتين مقارنة مع الحيوانات في مجموعات السيطرة وكيسببتين وايبيكواينون وكيسببتين مع ايبيكواينون. يؤدي التناول المتزامن للكيسببتين مع ايبيكواينون إلى تحسن كبير في قياسات الخصية (وزن الخصية، مؤشر الاقناد، حجم الخصية والمسافة الشرجية التناسلية).

أظهرت نتيجة تحليل الحيوانات المنوية أن مجموع رؤوس الحيوانات المنوية واورمات النطف مستطالة الرأس والإنتاج اليومي للحيوانات المنوية انخفض بشكل كبير ($p \leq 0.05$) في مجموعة كيسببتين مقارنة مع المجموعات الأخرى، يؤدي التناول المتزامن للكيسببتين مع ايبيكواينون إلى تحسن كبير في مجموع رؤوس الحيوانات المنوية، واورمات النطف مستطالة الرأس والإنتاج اليومي للحيوانات المنوية.

أظهرت الدراسة النسجية لخصية الحيوانات المعالجة بالكيسببتين بالمقارنة مع حيوانات السيطرة تنكس النبيبات المنوية (انسلاخ وتمزق الظهارة الجرثومية ، وزيادة الفجوات داخل الظهارة ، وضمور الخلايا الظهارية ووضعها بشكل غير منتظم على الغشاء القاعدي) وتراكم السائل الوذمي الوردي بين الأنابيب المنوية ، واحتقان الأوعية الدموية وهناك عدد قليل من الحيوانات المنوية ،

أيضا بروستات الحيوانات التي عولجت بالكيسبتين مقارنة مع حيوانات السيطرة ، اظهرت تنكس الخلايا الظهارية وانسلاخ بعض الخلايا الظهارية ، وتضخم الخلايا الظهارية الغدية والنتوءات الحليمية تجاه التجويف السنخي ، كما ان بعض الغدد البروستاتية تبدو فارغة من إفراز البروستات. بينما اظهر التناول المتزامن للكيسبتيين مع ايبيكواينون إلى تحسن في انسجة الخصية والبروستات.

ومع ذلك، عند استخدام ايبيكواينون وحده، لاحظنا جميع المؤشرات المدروسة بالقرب من الحالات الطبيعية.

في الختام، من نتائجنا، نستنتج أن الكيسبتين يؤدي إلى آثار ضارة على الهرمونات التناسلية، وقياسات الخصية، وتحليل الحيوانات المنوية، وبنية الخصية والبروستات عند حقنها في مرحلة ما قبل البلوغ. بينما يؤدي التناول المتزامن للكيسبتين مع ايبيكواينون إلى تحسين كل هذه المعلمات المدروسة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء / كلية الطب البيطري
فرع الفسلجة والكيمياء الحياتية والادوية

دور الابيكواينين-10 والكيسبيتين-10 في التغيرات التكاثرية لذكور الجرذ خلال مرحلة ماقبل البلوغ الجنسي

رسالة مقدمة الى

مجلس كلية الطب البيطري جامعة كربلاء – وهي جزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري / الفسلجة

من قبل

آمال عبد السجاد كريم

بكالوريوس طب وجراحة بيطرية– كلية الطب البيطري– جامعة كربلاء

2018-2017

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