CHARPTER II

LITERATURE REVIEWS

Methamphetamine

Methamphetamine (METH), an illegal psychostimulants drug, is derived from amphetamine (Itzhak and Achat-Mendes, 2004; Barr, et al., 2006). Mechanism action of METH and amphetamine are similarity (Kish, 2008) (Figure 2). There are several names of METH such as speed, crystal, crank, go, ice (Derlet and Heischober, 1990; Albertson, Derlet and Van Hoozen, 1999; Golub, et al., 2005) and Ya-Ba (in Thailand) (Puthaviriyakorn, et al., 2002; Golub, et al., 2005). The chemical formula and the molecular mass of METH are $C_{10}H_{15}N$ and 149.24, respectively (Golub, et al., 2005). It can uptake into the body by smoking, injection, snorting and ingestion. METH is a lipophilic drug (Meredith, et al., 2005) which can transfer across the blood brain barrier. It has a psychological effect approximately 8-13 hours in the body (Barr, et al., 2006). But the other study has been reported that half-life of METH is about 10-30 hours depend on drug purity, amount of intake and elimination of urine pH (Russell, et al., 2008).

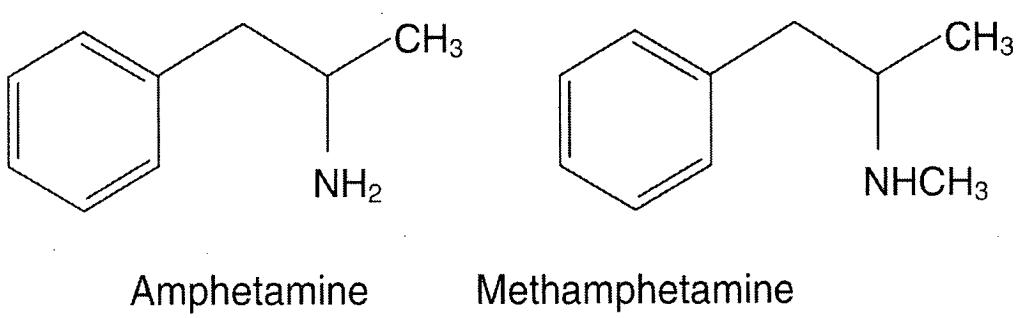

Figure 2 Differentiation of chemical structure between amphetamine and methamphetamine

Source: Golub, et al., 2005

Methamphetamine and behavior

In respiratory system, Intravenous or inhalation of METH can induce pulmonary edema, pulmonary hypertension, and pulmonary artery muscular hypertrophy with foreign body granuloma (Albertson, Derlet and Van Hoozen, 1999). In cardiovascular system, METH can induce cerebral hemorrhage and congestive heart failure, myocardial infarction and acute aortic dissection (Meredith, et al., 2005). Moreover, acute use of METH can induce hyperthermia, hypertension, increase heart and respiration rate and chest pain (Russell, et al., 2008) increase libido (Barr, et al., 2006) and chronic use of METH can cause neurotoxicity and neuron degeneration (Russell, et al., 2008).

Using MEHT is related to sexually transmitted disease such as gonorrhea, syphilis (Celentano, et al., 2008), hepatitis C and HIV. Besides, the fetal of maternal METH use during pregnancy shows prenatal complication such as fetal growth restriction, placental abruption, lower birth weight and cardiac defect (Meredith, et al., 2005).

Methamphetamine and neurotoxicity

METH is a central nervous system (CNS) stimulant (Derlet and Heischober, 1990, Itzhak and Achat-Mendes, 2004). The toxic of METH is mimic amphetamine because it have related compound (Alberson, Derlet and Hoozen, 1999).

METH induces the release of monoamine neurotransmitters consist of dopamine, serotonin and norepinephrine from the nerve ending. This causes an increase of extracellular monoamine neurotransmitters (Kish, 2008; Russell, et al., 2008). Besides, METH also blocked the neurotransmitter re-uptake (Figure 3). These causes induce euphoria, increasing of wakeful, increasing of sexual experience and hyperactivity (Meredith, et al., 2005; Russell, et al., 2008), depression (Sommers, Baskin and Baskin-Sommers, 2006), delirium, psychosis (Alberson, Derlet and Hoozen, 1999), paranoia and hallucination (Alberson, Derlet and Hoozen, 1999; Sommers, Baskin and Baskin-Sommers, 2006).

In human study, using of METH can cause neuronal damage, memory impairment (Itzhak and Achat-Mendes, 2004) and cognitive impairment (Hart, et al., 2012).

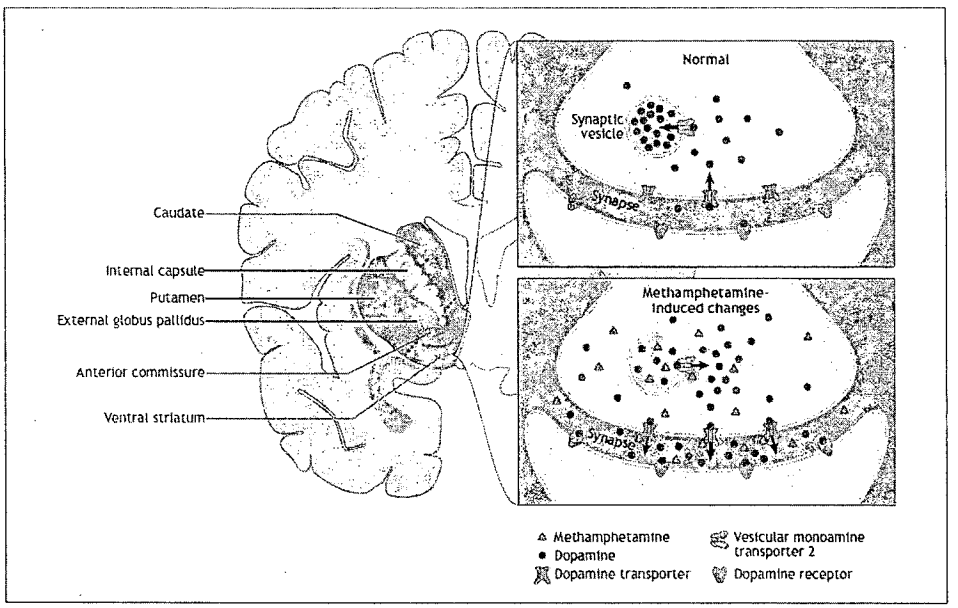

Figure 3 Methamphetamine causes release of monoamine neurotransmitters such as dopamine and blocks re-uptake of dopamine in pre-synaptic neuron

Source: Kish, 2008

Methamphetamine and reproductive system

METH is not only the central nervous system toxic stimulant but also reproductive toxicity. There are several studies of METH on the reproductive system.

In 1999, Yamamoto, et al. has studied the effect of METH on male mice. The study showed that METH at 15 mg/kg can induce a decrease of sperm motility (Yamamoto, Yamamoto and Hayase, 1999). In 2002, the study of Yamamoto, et al. in male mice showed that METH at 5, 10 and 15 mg/kg can induce apoptotic cell in the seminiferous tubules. Moreover, METH at 10 and 15 mg/kg can induce the fluctuation of serum testosterone concentration (Yamamoto, et al., 2002). In 2008, the previous study of Alavi, et al. in male rats showed that METH at 1, 5 and 10 mg/kg can induce a decrease of proliferation/apoptosis in spermatogonia (Alavi, Taghavi and Moallem, 2008). In addition, the previous study of Nudmamud-Thanoi and Thanoi in male rats showed that METH can induce abnormal sperm morphology, decrease of

sperm concentration and apoptotic cell in the seminiferous tubules (Nudmamud-Thanoi and Thanoi, 2011).

Male reproductive system

Testis

Testis is the male primary reproductive organ. Functions of the testis are sperm production and hormone production such as testosterone (endocrine) (Marieb, 2002). The human testis is rotational shape with diameter of 2.4x4 cm. Each testis is surrounded by a fibrous connective tissue called "tunica albuginea" (Holstein, Schulze and Davidoff, 2003). Tunica albuginea extend into the testis and divide it into the lobules which consist of seminiferous tubule and intertubular tissue (Holstein, Schulze and Davidoff, 2003; Marieb, 2002) (Figure 4).

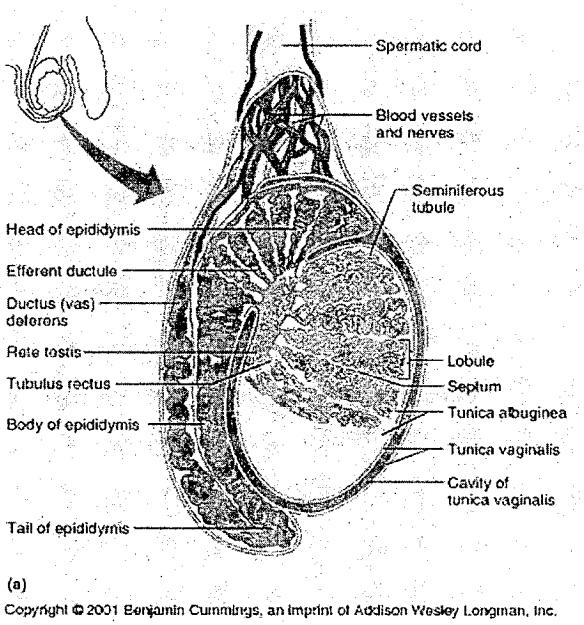

Figure 4 Seminiferous tubule and relative duct of male reproductive system

Source: http://legacy.owensboro.kctcs.edu/gcaplan/anat2/notes/APIINotes2%20male%20reproductive%20anatomy.htm

Seminiferous tubule

Seminiferous tubule consists of seminiferous epithelium that line on basement membrane and fluid-fill lumen. Seminiferous epithelium contains Sertoli cell and germ cells (Hess, 1999) (Figure 5).

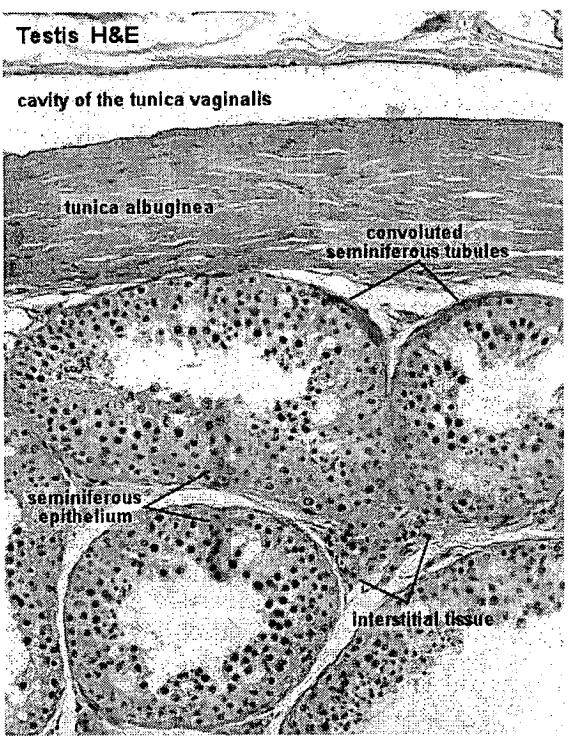

Figure 5 Seminiferous tubule

Source: http://legacy.owensboro.kctcs.edu/gcaplan/anat2/histology/histo%20b%20male%20reproductive.htm

Spermatogenesis

In the testis, there is the process of germ cell development and maturation into spermatid. It is called "spermatogenesis" (O'Donnell, et al., 1996; Collins, et al., 2003). The process of spermatogenesis begins from spermatogonia, the cell locates at basement membrane, divides into primary spermatocyte by mitotic division. After that, the primary spermatocyte will be divided into secondary spermatocyte by meiosis I division and moved from basement membrane to adluminal compartment. Then, the secondary spermatocyte will be quickly divided into round spermatid by meiosis II

division. Afterwards, the round spermatid will be differentiated into elongated spermatid by spermiogenesis (Holstein, Schulze and Davidoff 2003; Wang, et al., 2006; Walker, 2010) (Figure 6).

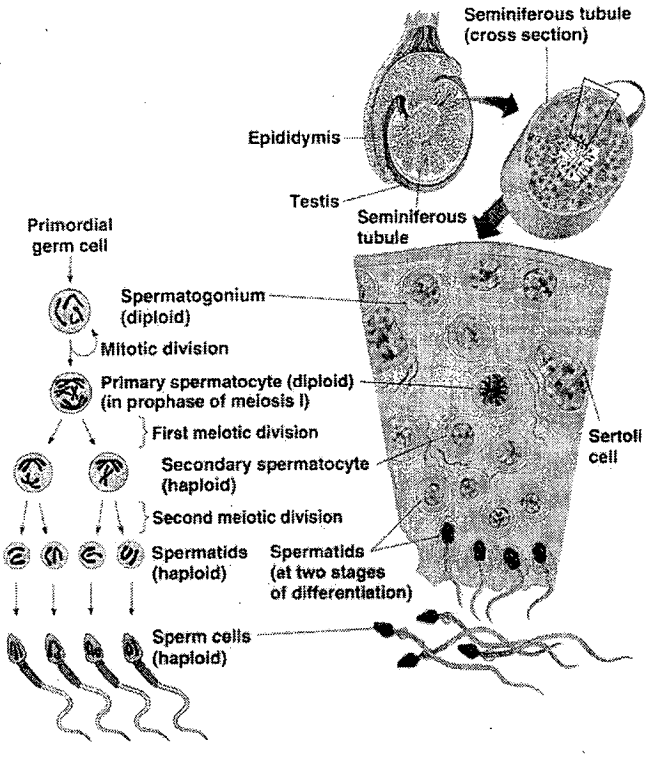

Figure 6 Spermatogenesis of seminiferous tubule

Source: http://ldysinger.stjohnsem.edu/ThM_599d_Beg/02_Biology/02_spermat-oogen.htm

The stage of the cycle of the seminiferous epithelium in the rat

The stage of the cycle of the seminiferous epithelium in the rat can be divided into 14 stages which each stage will be written by Roman numeral (stage I-XIV) (Figure 9). The first guide of the stage of seminiferous epithelium is spermatid following by spermatocyte, spermatogonia and the change of Sertoli cell, respectively. Among 14 stages of the seminiferous epithelium, the spermatogonia will be changed into spermatozoa when the process complete (Leblond and Clermont, 1952) (Figure 7).

The stages VII-VIII of the rat stage of seminiferous epithelium are the androgen-dependent stages because these stages show the highest levels of androgen receptor expression (Lue, et al., 2000). Besides, the expression of AR in the nucleus of Sertoli cell is highest at these stages (Hill, et al., 2004).

The previous study of O'Donnell, et al. (1996) demonstrated that testosterone is necessary for spermatogenesis at stage VII-VIII. Suppression of testosterone lead to round spermatid could not be change between stages VII-VIII.

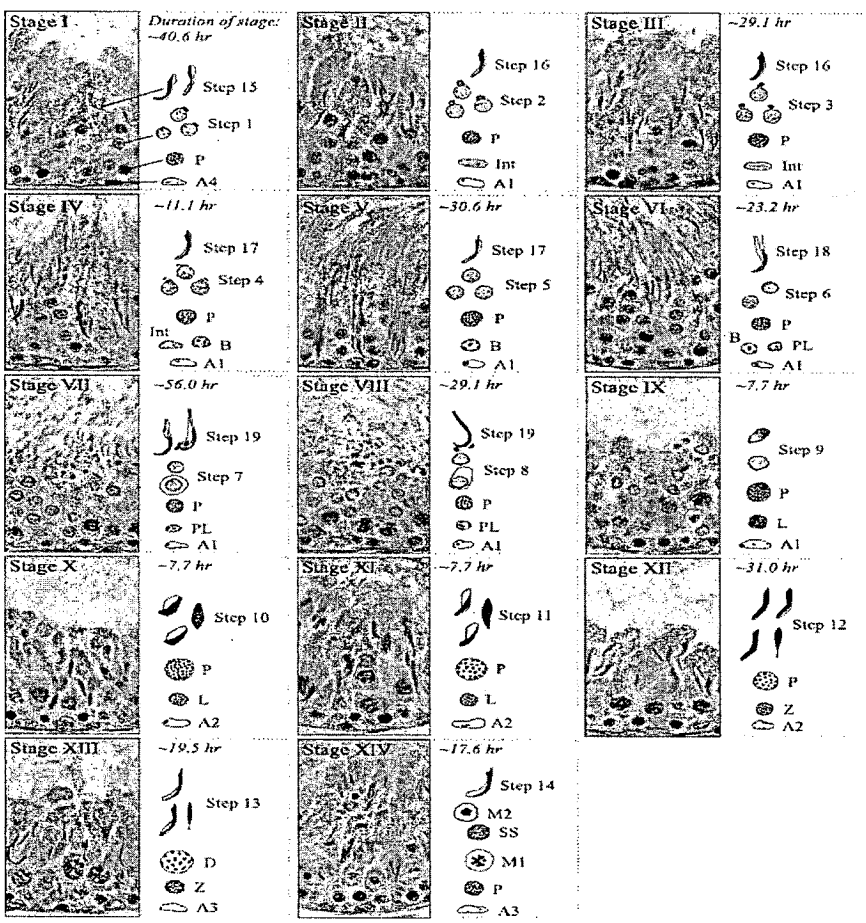

Figure 7 The stage of the seminiferous epithelium in the adult male rat

Source: Mruk, Silvestrini and Cheng, 2008

Sertoli cell

Sertoli cell is the supporting cell for spermatogenesis (O'Donnell, et al., 1996). Sertoli cell is located on the basement membrane of seminiferous tubule. Its nucleus is large and irregular shape (Zhu, et al., 2000). Sertoli cell is the somatic cell that reaches from the basement membrane to the lumen and surrounds the germ cell in the seminiferous tubule. Moreover, the adjacent Sertoli cell forms to be blood-testis barrier (BTB) by tight junction to select the proper nutrient and growth factor for the germ cell (Walker, 2010) (Figure 8).

Leydig cell

Leydig cell is an oval or round nucleus cell located interstitial tissue of testis (Teerds, et al., 1998). The function of Leydig cell is production and secretion of male sex hormone called androgens (Holstein, Schulze and Davidoff, 2003) under the regulation by luteinizing hormone (LH) (O'Donnell, et al., 1996; Pakarainen, et al., 2004; Holdcraft and Braun, 2004) (Figure 8).

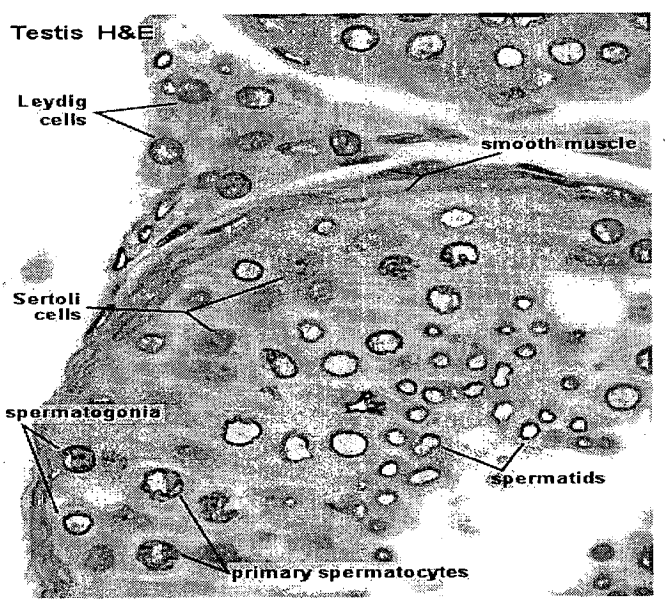

Figure 8 Leydig cells and Sertoli cells

Source: http://embryology.med.unsw.edu.au/embryology/index.php?title=Spermatozoa_Development

Semen analysis

Semen analysis is sperm quality measurement including sperm motility, sperm morphology, sperm concentration and seminal fluid parameter such as volume of semen and pH. It is used to assess sperm quality and their fertility potential (Rrumbullaku, 2012; WHO, 2010) (Table 1).

Table 1 Normal criteria for human semen analysis of WHO in 1992

	Standard tests
Volume	At least 2.0 ml
pH	Between 7.2-8.0
Sperm concentration	At least $20x10^6$ spermatozoa/ml
Total sperm count	$40x10^6$ spermatozoa per ejaculate or more
Sperm motility	At least 50% with forward progression (categories a and b) or 25% or more with rapid progression (category a) within 60 minutes of ejaculation
Sperm morphology	30% normal form or more
Sperm viability	75% or more live, i.e., excluding dye
White blood cells	Fewer than $1x10^6$/ml
Immunobead test	Fewer than 20% spermatozoa with adherent particles
MAR test	Fewer than 10% spermatozoa with adherent particles

Sperm motility

Sperm motility is a method to assess characteristic of sperm movement. This method is performed under bright field microscope (Rrumbullaku, 2012) or phase-contrast microscope as soon as possible after ejaculation. The assessment of sperm motility is related to pregnancy rates (WHO, 2010) and fertilizing ability (Shibahara, et al., 1997; Hirano, et al., 2001). The sperm motility can be divided into the grade consist of

(a) Rapid progressive motility

(b) Slow progressive motility

(c) Non-progressive motility

(d) immotility (Rrumbullaku, 2012, WHO, 2010).

The previous study of Yamamoto et al. indicates that the sperm motility of male mice in the control group was 79.9±2.57% (Yamamoto, et al., 1999). The previous study of El-Demerdash indicated that the sperm motility of male rat in the control group was 59.6±2.76% (El-Demerdash, et al., 2004).

Sperm morphology

The human sperm morphology has three regions consist of head, midpiece and tail. It has an oval head shape with diameter is 4-5 μm that contains DNA and cover by acrosome (Marieb, 2002; Holstein, Schulze and Davidoff, 2003). The midpiece of sperm contains centriole and mitochondria form a spiral around it (Holstein, Schulze and Davidoff, 2003). The tail of sperm contains filament which come from centriole of midpiece (Marieb, 2002) (Figure 9).

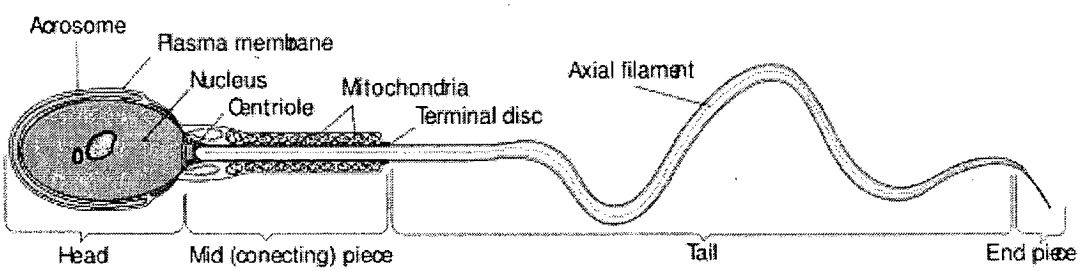

Figure 9 Human spermatozoon

Source: http://commons.wikimedia.org/wiki/File:Human_spermatozoa.png

Sperm morphology technique is used to evaluate form of sperm due to sperm is sensitive to reproductive toxicant (Inveresk research, 2000). The sperm morphology is predictor of fertilizing ability of sperm and used to assess function of reproductive tract (Rrumbullaku, 2012). Abnormal spermatozoa may occur during sperm production or during storage in the epididymis (Inveresk research, 2000). World Health Organization

(WHO) has categorized human spermatozoa defect consist of head defects, neck and midpiece defects, principal piece defects or tail defects and excess residual cytoplasm (WHO, 2010) (Figure 10).

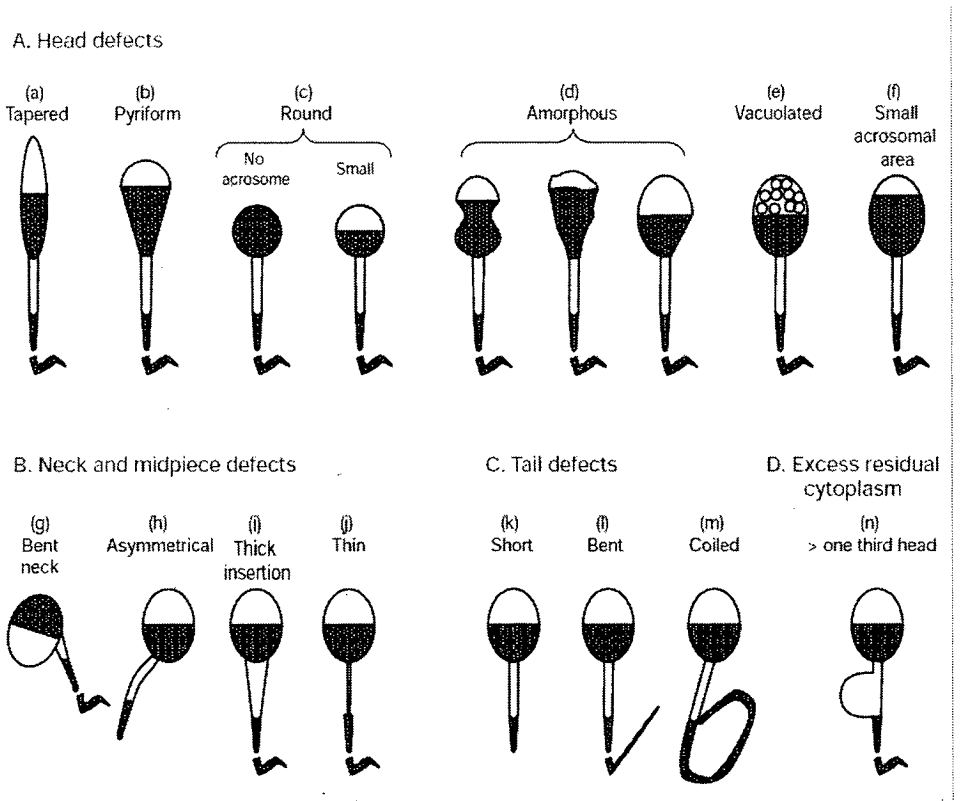

Figure 10 Schematic drawing of some abnormal human spermatozoa

Source: WHO, 2010

Rat sperm morphology

The rat sperm consist of head, midpiece and tail. The rat sperm has a hook head shape which contains a dense nucleus and acrosome in tip. The midpiece consists of sheath of mitrochondria and centrioles. The tail of sperm consists of filament. When spermatozoon is mature, the tail can vibrate for short time (Figure 11). The rat abnormal sperm morphology consist of headless sperm, flattened sperm, pinhead sperm, bent neck sperm, bent tail sperm and multiple abnormalities sperm (Inveresk research, 2000).

The study of Nudmamud-Thanoi and Thanoi in male rat indicated that the sperm morphology in control group was 94.50±2.17% (Nudmamud-Thanoi and Thanoi, 2011). The previous study of El-Demerdash indicated that abnormal sperm morphology of male rat in the control group was 12.7±0.34% (El-Demerdash, et al., 2004).

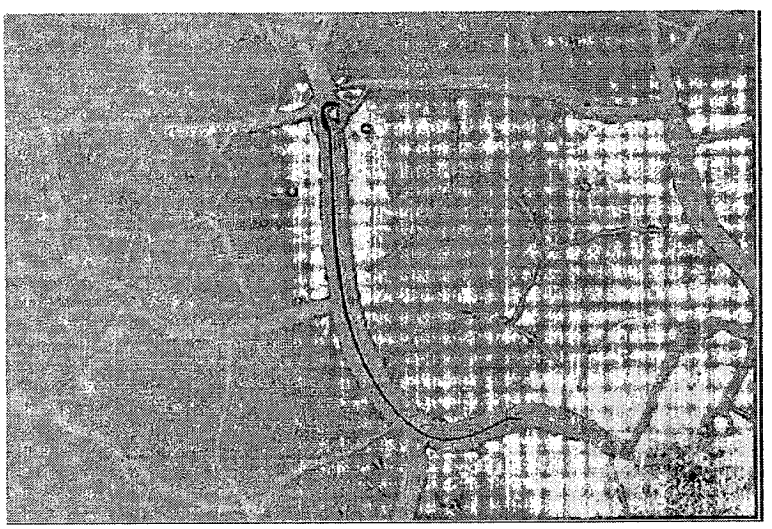

Figure 11 Normal rat sperm morphology

Source: Inveresk research, 2000

Sperm concentration

Sperm concentration is a method to evaluate number of spermatozoa that refer spermatogenesis (Rrumbullaku, 2012), predictor of conception, time to pregnancy and pregnancy rate (WHO, 2010). Reduction of sperm concentration is related to fertility (Holstein, Schulze and Davidoff, 2003). Spermatozoa are counted under bright field microscope by using hemacytometer (Rrumbullaku, 2012) (Figure 12). The normal sperm concentration of human is at least $20x10^6$ spermatozoa/ml (Cooper, et al., 2010; Rrumbullaku, 2012).

In male rat, the previous study of Nudmamud-Thanoi and Thanoi indicated that the sperm concentration in the control group was $185.00±9.67x10^6$ cells/ml (Nudmamud-Thanoi and Thanoi, 2011).

The study of Al-Akhras indicated that normal sperm concentration in male rat was 113.2±15.471x10^6 cells/ml (Al-Akhras, et al., 2006). The previous study of El-Demerdash indicated that sperm concentration of male rat in the control group was 172±29.8x10^6 cells/ml (El-Demerdash, et al., 2004).

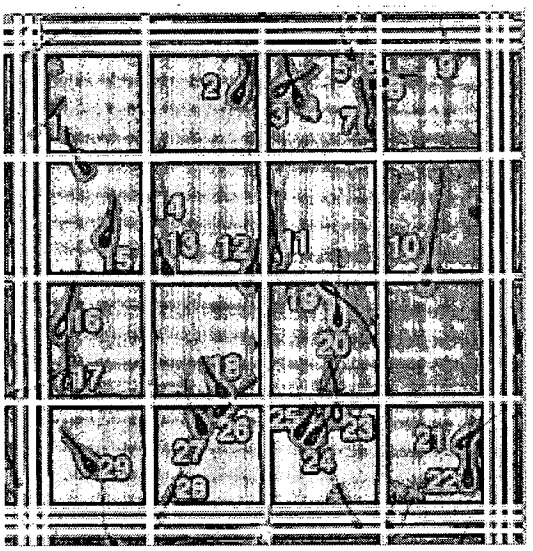

Figure 12 Sperm were counted by hemacytometer under bright field microscope. The number is counted sperm and asterisk is uncounted sperm

Source: http://www.stallionai.co.uk/services/semen-evaluation/

The hypothalamic pituitary gonadal axis

The function of hypothalamic pituitary gonadal (HPG) axis is important for reproduction (Asimakopoulos, 2012). Hypothalamus secrets gonadrotropin releasing hormone (GnRH) to pituitary gland and stimulate pituitary gland to secrets follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both FSH and LH are necessary for gonad (Asimakopoulos, 2012).

FSH is released to Sertoli cells for stimulate proliferation of Sertoli cells during prepubertal development (Holdcraft and Braun, 2004). Then, FSH stimulates germ cells proliferation via Sertoli cells. In the hypogonadal mice with lacking FSH, germ cells cannot progress meiosis (O'Shaughnessy, et al., 2010). LH regulates production of testosterone in Leydig cells in the testis (Holdcraft and Braun, 2004) (Figure 13).

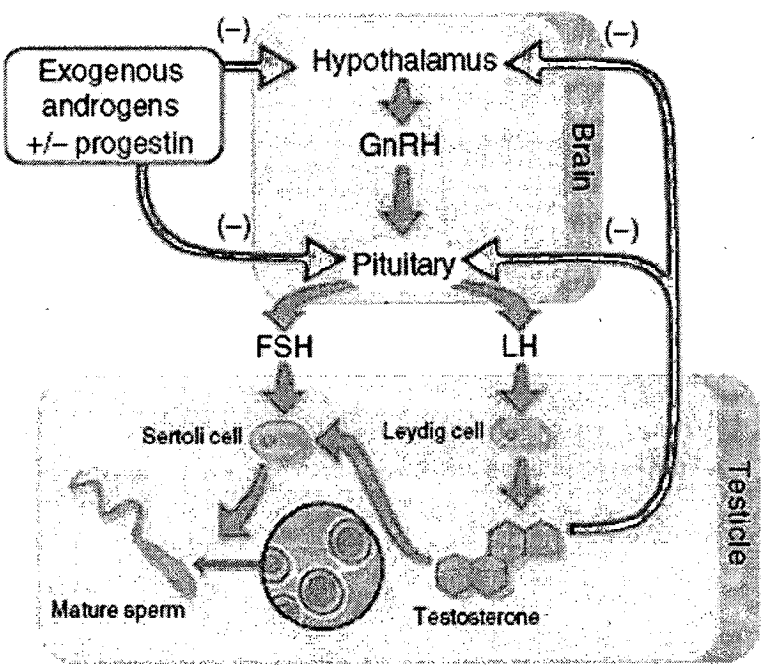

Figure 13 The hypothalamic pituitary gonadal axis

Source: Roth and Amory, 2011

Hormonal regulation of spermatogenesis

Steroidogenesis

Steroidogenesis is the process to synthesize steroid hormone by cholesterol (Stocco, 2001; Hu, et al., 2010). The source of cholesterol is acetate, low density lipoprotein (LDL) and high density lipoprotein (HDL), and lipid droplet (Hu, et al., 2010; Zaidi, Shen and Azhar, 2012). Steroidogenesis is controlled by peptide hormone. Function of steroid hormone is several controls of physiology such as reproductive

system, maintenance of secondary sex characteristic, blood balance, stress and neuronal function (Hu, et al., 2010).

Testosterone is an sex steroid hormone which synthesized from cholesterol (Hu, et al., 2010). For testosterone synthesize, firstly, the cholesterol is changed into pregnenolone by cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc). The P450scc is an enzyme in mitochondria. After that, pregnenolone is converted by 2 pathways consist of Δ5 and Δ4 pathways which the end product of 2 pathways is testosterone (Marieb, 2002; Hu, et al., 2010).

The Δ4 pathway, pregnenolone is converted into progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD). Then, progesterone is changed into 17α-hydroxyprogesterone by CYP17 (P450c17) and androstenedione by 17, 20 lyase, repectively. The androstenedione is changed by 17β-hydroxysteroid dehydrogenases (17β-HSD) into testosterone (Hu, et al., 2010) (Figure 14).

In the Δ5 pathway, pregnenolone is changed into 17α-hydroxypregnenolone and dyhydroepiandrosterone (DHEA) by CYP17 (P450c17) and 17, 12 lyase, respectively. DHEA can be changed into testosterone by androstenedione or androstenediol. DHEA is changed into androstenedione by 3β-HSD. Then, androstenedione is changed into testosterone by 17β-HSD. Moreover, DHEA can be changed into testosterone through androstenediol by 17β-HSD. Then, androstenediol is changed into testosterone by 3β-HSD (Hu, et al., 2010) (Figure 14).

In men, testosterone level in the testis is 340-2000 nM but in the serum is 8.7-35 nM (Walker, 2010). The study of Kirschner et al. that used a double isotope derivative method following gas-liquid chromatography to measure plasma testosterone level in men between 18-44 years old. The result indicated that range plasma testosterone level was 0.44-1.30 μg per 100 ml and the mean value with standard deviation was 0.74±0.26 μg per 100 ml (Kirschner, Lipsett and Collins, 1965). The previous study of Yamamoto et al. showed that serum testosterone concentration of male mice was 8.92±0.84 ng/ml (Yamamoto, Yamamoto and Hayase, 1999). The study of Lamia, et al. (2008) that measured testosterone level in male rat by electrochemiluminescence indicated that plasma testosterone level was 5.50±1.32 ng/ml and 5.82±0.73 ng/ml in 2 control groups (30 days and 60 days). Normal range

of serum testosterone in male rat is 0.66-5.4 ng/ml and mean value is 3.06 ng/ml (DRG., 2010).

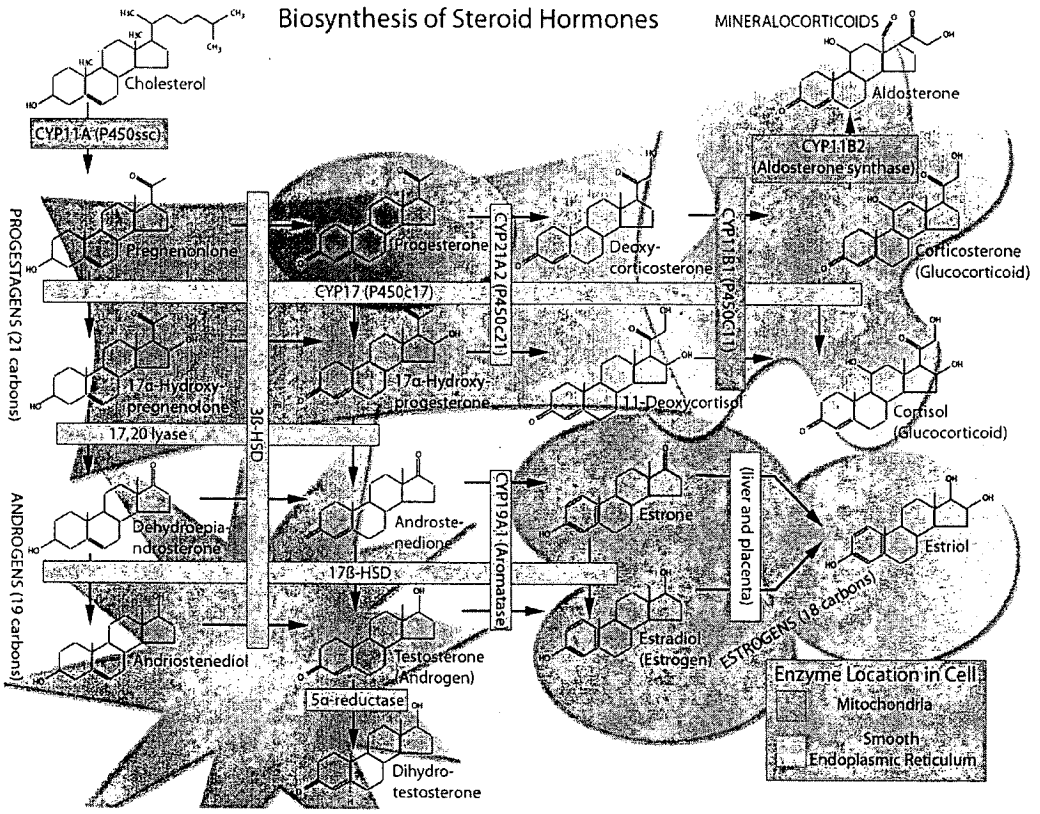

Figure 14 Testosterone synthesis

Source: Hu, et al., 2010

Androgen

Androgen, mainly testosterone, is a hormone that important for spermatogenesis within the seminiferous tubule and male fertility. Besides, testosterone also stimulates sexual behavior, male secondary sex characteristics and genital organ (Holstein, Schulze and Davidoff, 2003). Regulation of spermatogenesis by testosterone is not understood (Fix, et al., 2004). Testosterone acts via androgen receptor (AR) that express in Leydig cells, Sertoli cells and peritubular cells (Walker, 2009).

Testosterone level is regulated by AR in the Leydig cells via autocrine feedback or suppression of LH synthesis and secretion in the anterior pituitary gland (Holdcraft and Braun, 2004). After Leydig cell is stimulated by luteinizing hormone (LH), Testosterone is produced and secreted. Then, testosterone will bind the AR in Sertoli cell lead to spermatogenesis is initiated and maintained. Moreover, germ cell apoptosis will be inhibited (Dohle, Smit and Weber, 2003).

Testosterone and AR expression are required for 3 major processes in Sertoli cell consist of formation of BTB by adjacent Sertoli cell, maintaining the attachment of Sertoli cells and round spermatid and the release of spermatozoa from Sertoli cells (Walker, 2010). Sertoli cell is the major target for testosterone (Walker, 2009). There are two pathways consist of classical and non-classical pathways (Walker, 2009; Walker, 2010; Walker, 2011).

In classical pathway, testosterone diffuses through plasma membrane and binds to AR in cytoplasm. After that, AR is released from heat shock protein (HSP) and translocated to the nucleus where it binds to specific DNA sequence called androgen response element (AREs). The AR binds to DNA recruit co-activator and stimulates gene transcription (Walker and Cheng, 2005; Walker, 2009; Walker, 2010) (Figure 15).

In non-classical pathway, testosterone diffuses plasma membrane and binds to AR in cytoplasm. After that, the AR interacts with Src by phosphorylation of Src. Then, Src activates EGFR. Then MAP kinase including RAF and ERK will be activated. Afterwards, $p90^{RSK}$ and CREB will be activated, respectively. The $p90^{RSK}$ phosphorylate CREB which becomes activated transcription factor and mediated transcription of CREB-response gene. Phosphorylation of CREB maintains Sertoli cell to support survival of spermatocytes (Walker, 2010) (Figure 15).

Testosterone is important for differentiation of round to elongated spermatid. Suppression of intratesticular testosterone cause round spermatid can't differentiate into elongated spermatid at stage VII-VIII of seminiferous tubule (O'Donnell, et al., 1996) and meiosis was halted (Walker, 2010). Moreover, mature spermatozoa unable to release form Sertoli cell (Walker, 2011).

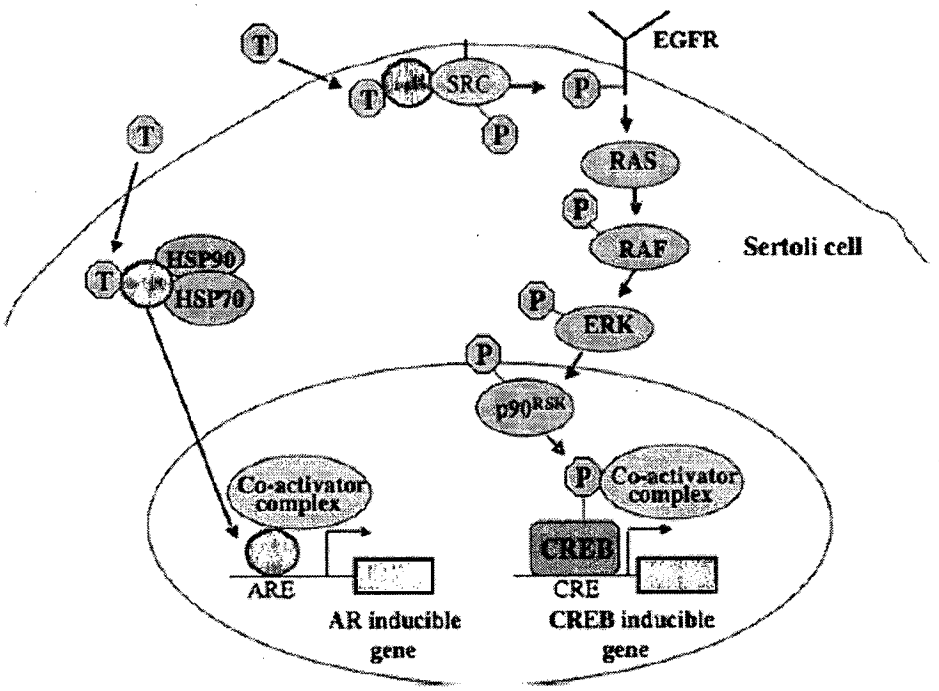

Figure 15 Classical (left) and non-classical (right) testosterone pathways in spermatogenesis

Source: Walker, 2009

Androgen receptor

Androgen is not only necessary for male fertility but androgen receptor (AR) is also important for male fertility too (Xu, et al., 2007). AR is a nuclear receptor superfamily (Patrao, et al., 2009). AR expression is detected in the Leydig cells, Sertoli cells and peritubular myoid cells. But the expression of AR in germ cell is still debate (Wang, et al., 2006; Walker, 2011). There are some studies indicated that AR is expressed in the germ cell such as spermatogonia, spermatocyte, elongated spermatid at stage XI (Collins, et al., 2003) and round spermatid (Xu, et al., 2007).

AR expression in the Sertoli cells is highest at stage VII-VIII (Holdcraft and Braun, 2004). AR expression in Sertoli cells is necessary for spermatogenesis particularly in meiosis division of primary spermatocyte and spermiation of spermatid. The action of AR in Sertoli cell for normal spermatogenesis is unknown but the previous study

indicated that it may maintain structure of Sertoli cell lead to environment of seminiferous epithelium is proper for development of germ cell (Wang, et al., 2006). The previous study of Xu et al. indicated that male mice lacking androgen receptor in Leydig cell are azoospermia (Xu, et al., 2007).