CHAPTER II

Literature Review

I. Botanical background and chemical constituents of *Mucuna collettii*

Mucuna collettii is classified into the family Leguminosae. The local name is varied in various parts of Thailand such as Saba Ling (Kanchanaburi), Mak Ba Luem Dam (Sukhothai), Maba Maeng (Chiangmai), Yang Dam (Nakhornratchasima), Tao Hom and Han Hao Hom (Loei) (Boonkert *et al*, 1982; Plengklai, 1977).

Mucuna collettii is a large woody climber which it is 30-40 m long and scatters by streams in evergreen forest. The leaves are trifoliate; leaflets 11-22 by 5-10 cm, sparsely hairly, entire margin; petiole 5-10 cm long, base stout. Inflorescence is hanging on the stem up to 30 cm long with 5 sepals covers with brown rough hair and unites into a bell-shaped tube. The 5 petals are blackish-purple pea like shaped. The stamens are 2 bundles. Fruit is a linear-oblong pod up to 40 cm long. The seeds are hard and flattened. The blooming flowers occur during January-March (Phengklai, 1977). The botanical characteristics of this herb are shown in Figure 1.

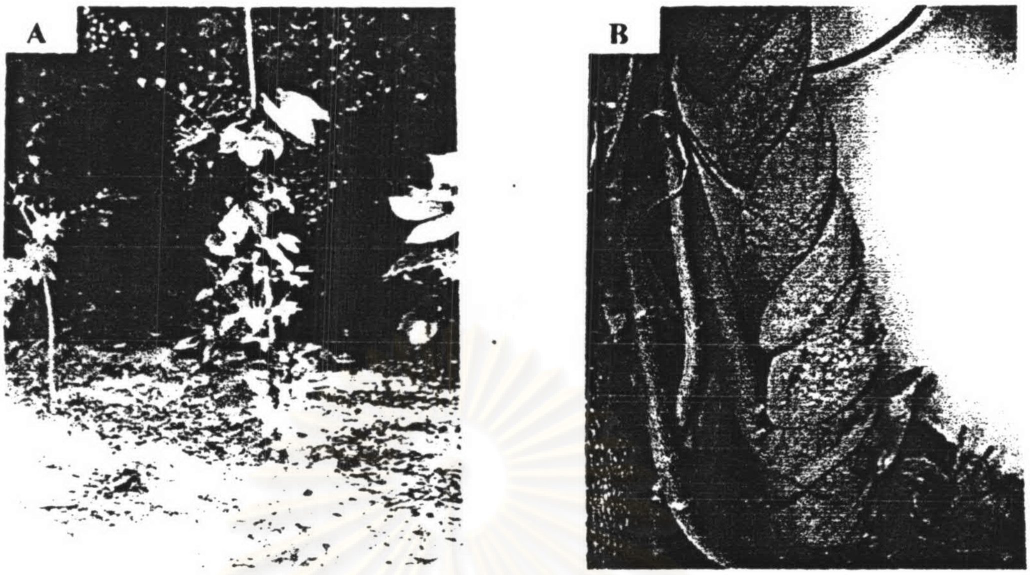


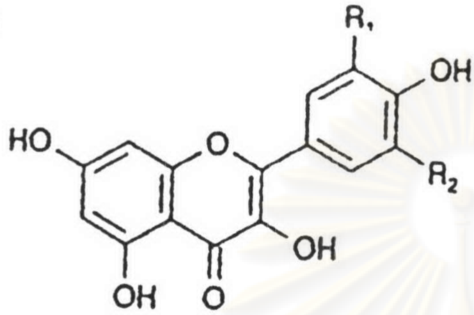
Figure 1 The botanical characteristics of *M. collettii*

The stem of *M. collettii* was found containing three isolated active compounds; kaempferol, quercetin and hopeaphenol. The structure of these active compounds is shown in Figure 2. Each chemical constituent could highly inhibit cyclic AMP phosphodiesterase enzyme and result in prolonged penile erection. The median inhibitory concentraion (IC_{50}) of kaempferol, quercetin and hopeaphenol for inhibiting cyclic AMP phosphodiesterase enzyme was 281.83, 80.91 and 22.75 μg/ml, respectively (Roengsamran *et al.*, 2001; Roengsamran *et al.*, 2003).

Kellis and Vickery (1984) reported that quercetin which is the one of flavones had a little inhibition of aromatization of androstenedione to E_2 in human placenta. The IC_{50} of quercetin for inhibiting aromatase enzyme was 12 μmol/l. In addition, quercetin significantly inhibited progesterone synthesis by inhibiting the reductive activity of 17β-hydroxysteroid dehydrogenase (HSD) in human granulosa-luteal cells.

The IC_{50} of quercetin for inhibiting 17β-HSD enzyme was 20 μmol/l (Whitehead and Lacey, 2003). However, there are no other reports on the effects of kaempferol and hopeaphenol in reproductive system.

A



R_1	R_2	Aglycone
OH	H	Quercetin
H	H	Kaempferol

B

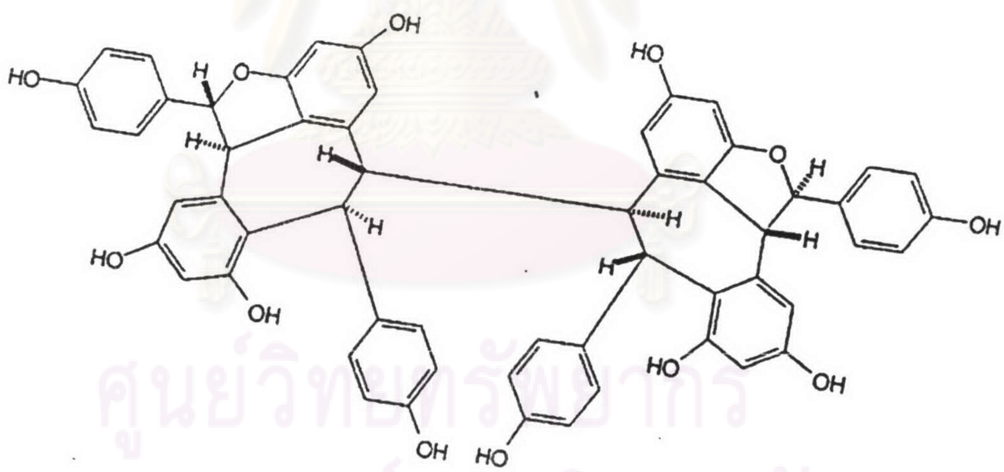


Figure 2 The structure of three isolated active compounds from the stem of *Mucuna collettii*; quercetin, kaempferol (**A**) and hopeaphenol (**B**).

II. The benefits of *M. collettii* according to Thai folklore remedy

As regards to Thai traditional medicine, it is beleived that *M. collettii* can improve the human physical appearances such as re-growing hair, promoting black hair, improving flexibility of the body and prolonging life after orally taking the mixture of *M. collettii* and honey (Suntara, 1931).

According to Suntara (1931), the ordinary dosage of *M. collettii* used in Thai folklore remedy was one-third of a fresh pepper seed for man and woman. Although, *M. collettii* has many advantages, but usage of *M. collettii* should be awareness.

As regards to Suntara (1931), the carefulness for using of *M. collettii* may be possible as follows;

1. Do not take *M. collettii* too high dosage or long-term, because it could make some effects such as headache, vomiting, malaise and so on.
2. Adult and pregnant female and adult male should avoid taking this herb because it could make hormonal imbalance.
3. Do not take this plant with sour or salty food.
4. Do not stay at the cold weather.

Nowadays, numbers of people who concern themselves with health promotion are increased. The way to promote health care and get rid of illness is usage of natural products (Sabcharoen, 1998). *M. collettii* is one of the popular herbs that Thai people used to improve their sexual functions, especially, in males (Roengsamran *et al.*, 2001).

Although, there were some published data of *M. collettii* on reproductive organs in male rats (Wuteeraphon *et al.*, 2001) and its chemical constituents from the stem (Roengsamran *et al.*, 2001; Roengsamran *et al.*, 2004), but there is no data of *M. collettii* on hormone-related reproductive functions. Thus, this study aimed to investigate the effects of *M. collettii* on hormone levels and reproductive organs in rats.

III. Regulation of the hypothalamic-pituitary-gonadal axis.

Regulation of reproductive system, the hypothalamic-pituitary-gonadal axis is the key control of gonadal hormones synthesis and secretion in female and male vertebrates. Thus, it is necessary to understand each part and function of hypothalamic-pituitary-gonadal axis.

3.1 Hypothalamus

Hypothalamus is the basal part of diencephalon lying below thalamus as shown in Figure 3. It includes optic chiasma, tuber cinereum, infundibulum and mammillary bodies. Tuber cenereum is the part of third ventricle floor that extends downward to the infundibulum. The lower part of tuber cinereum which is richly supplied with blood vessels that drain into pituitary stalk. This is called a primary capillary plexus and then inturn empty into a secondary capillary plexus in the adenohypophysis via median eminence. The vascular system links between median eminence and pituitary gland is known as the hypothalamic-pituitary-portal system. Within hypothalamus are clusters of neurons. Neurosecretory nuclei of hypothalamus and preoptic area (POA) produce neurohormones that regulate hormonal secretions in pituitary gland as depicted in Figure 4. Axons from these nuclei travel either to median eminence or to pars nervosa. These neurohormones associated with median eminence and adenohypophysis are termed hypothalamic hypophysiotropic hormones and are identified either releasing hormones or releasing-inhibiting hormones, depending on whether they stimulate or inhibit hormone release from the adenohypophysis. Gonadotropin-releasing hormone (GnRH), a decapeptide, is assumed to be the most important final common mediator of all influences, external stimuli, e.g. light, pain and chemical substances and internal stimuli, e.g. emotions and feedback, on reproduction conveyed through the central nervous system as shown in Figure 5. In rats, most of the GnRH cells sending fibers to the median eminence are located in the POA. GnRH is released as a series of pulses into the portal vessels and binds to the gonadotrophs in the anterior pituitary gland or adenohypophysis and drives the Gn secretion in a similar pattern (Johnson and Everitt, 1995; Hadley, 2000; Norris, 1997; Turner and Bagnara, 1976).

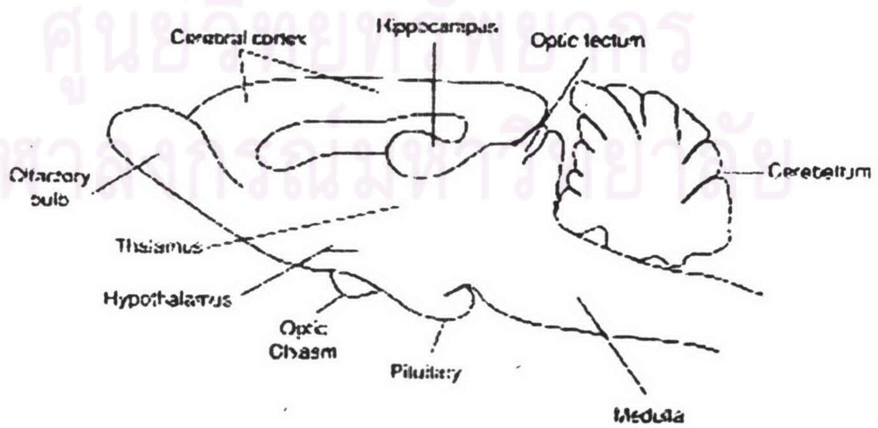


Figure 3 The brain of rat.

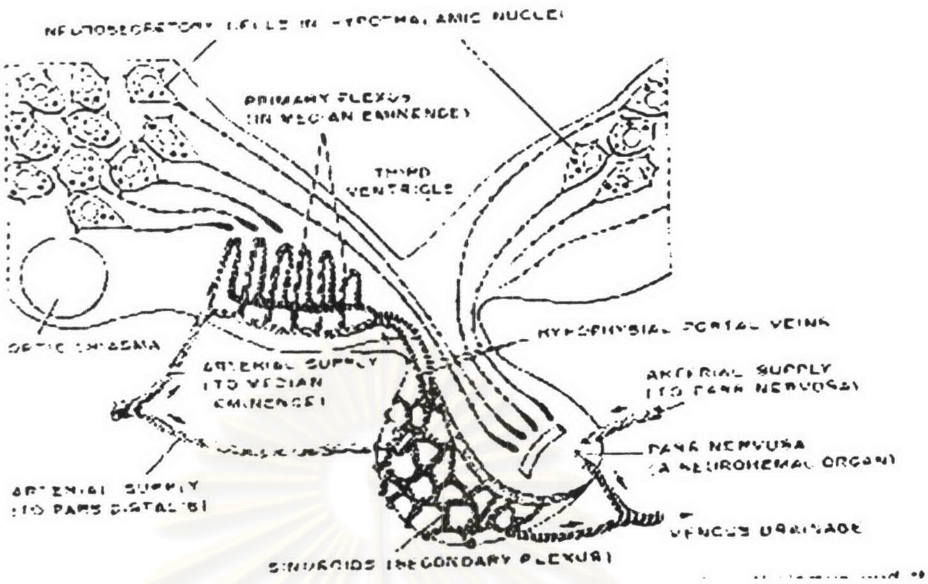


Figure 4 The hypothalamic-hypophysial-portal system.

3.2 Pituitary gland

Pituitary gland is derived from pituita that means slime or phlegm. Its alternate name is hypophysis which is derives from hypo that means under and physis that means growth. Pituitary gland of adult mammals locates ventral to the brain just posterior to the optic chiasma and it remains attached to hypothalamus by a stalk like connection as shown in Figure 4. It consists of two regions, the adenohypophysis and neurohypophysis or pars nervosa (Johnson and Everitt, 1995; Norris, 1997).

Adenohypophysis contains a variety of cell types that regulates the certain hormones in the body, gonadotroph which is the source of Gn granules and plays a role in Gn synthesis and secretion also locates in adenohypophysis, too (Johnson and Everitt, 1995; Norris, 1997; Kacsoh, 2000).

Release of Gn (FSH and LH) is caused by GnRH. FSH and LH, which are secreted from gonadotroph in anterior pituitary gland, travel via bloodstream to gonads.

3.3 Gonad

3.3.1 Male gonad

The male reproductive system consists of a pair of testes, the testis is covered by mesothelium continuous with the visceral layer of tunica vaginalis. A thick dense connective tissue capsule, tunica albuginea, encloses the testis. The capsule is reflected into the median plane to form the mediasternum and septula to divide the testis into lobules to support the seminiferous tubules. The coiled seminiferous tubules are lined with multilayered seminiferous epithelium of spermatogenic cells and Sertoli cells. They rest on a basement membrane and are surrounded by a lamellated connective tissue with myoid elements. Leydig cells are found in the loose vascular connective tissue separating the tubules.

The testis has two important roles in producing spermatozoa and sex steroid hormones results from Gn activation. Spermatozoa develop within the seminiferous tubules in close association with Sertoli cells, while androgen is the most important male sex steroid hormones and its potent metabolite is testosterone which is produced by Leydig cells in the intertubular connective tissue (Aughey and Frye, 2001; Johnson and Everitt, 1995; Kacsoh, 2000).

3.3.2 Female gonad

The female reproductive system consists of a pair of ovaries. Each ovary is divided into an outer parenchymatous zone cortex and an inner vascular zone medulla. The cortex of each ovary is covered by a simple squamous or cuboidal epithelium, tunica albuginea is a layer of dense connective tissue beneath the epithelium. Deep to that is the ovarian stroma containing the various stages of follicle development. The ovarian stroma consists of spindle-shaped cells arranged in whorls surrounding the ovarian follicles.

The ovary has two important roles in producing oocytes and sex steroid hormones results from Gn activation. The sex hormones of females are estrogen and progesterone which are produced by maturing follicles before ovulation and the corpus luteum after ovulation in ovaries, respectively (Aughey and Frye, 2001; Johnson and Everitt, 1995; Kacsoh, 2000).

The hormones produced by gonads play an important role in supporting reproduction. In addition, these hormones can influence other physiologic functions such as mineral and electrolyte homeostasis, fat, protein and carbohydrate metabolism and muscle mass (Kacsoh, 2000).

3.4 General regulation of reproductive system

Hormonal feedback loops become very complex in neuroendocrine function. Two types of hormonal feedback have been described with respect to the length of the loop; 1) long-loop feedback, in which blood concentrations of sex steroid hormones from gonads act back upon anterior pituitary gland, hypothalamus or other parts of the brain to affect anterior pituitary gland, and 2) short-loop feedback, whereby FSH and LH from anterior pituitary gland act back upon the hypothalamus or other parts of the brain to affect anterior pituitary gland as shown in Figure 5. In both male and female, negative feedback effects of gonadal hormones on anterior pituitary gland and hypothalamus are similar. The negative feedback effect of E_2 and T is achieved by decreasing LH secretion via an effect on hypothalamus. E_2 and progesterone (P) (in females) and T (in males) also inhibit FSH secretion, but these effects are less than on LH. The more completely suppression of FSH results from the combined action of E_2 or P or T and inhibin. In females, E_2 has a dual function in regulation of Gn secretion. At the low levels, it has a negative feedback effect on Gn secretion. At the high levels, it shows a positive feedback effect. The positive feedback induces LH and FSH surge, then ovulation will be occurred afterward (Johnson and Everitt, 1995; Turner and Bagnara, 1976; Yen and Jaffe, 1986).

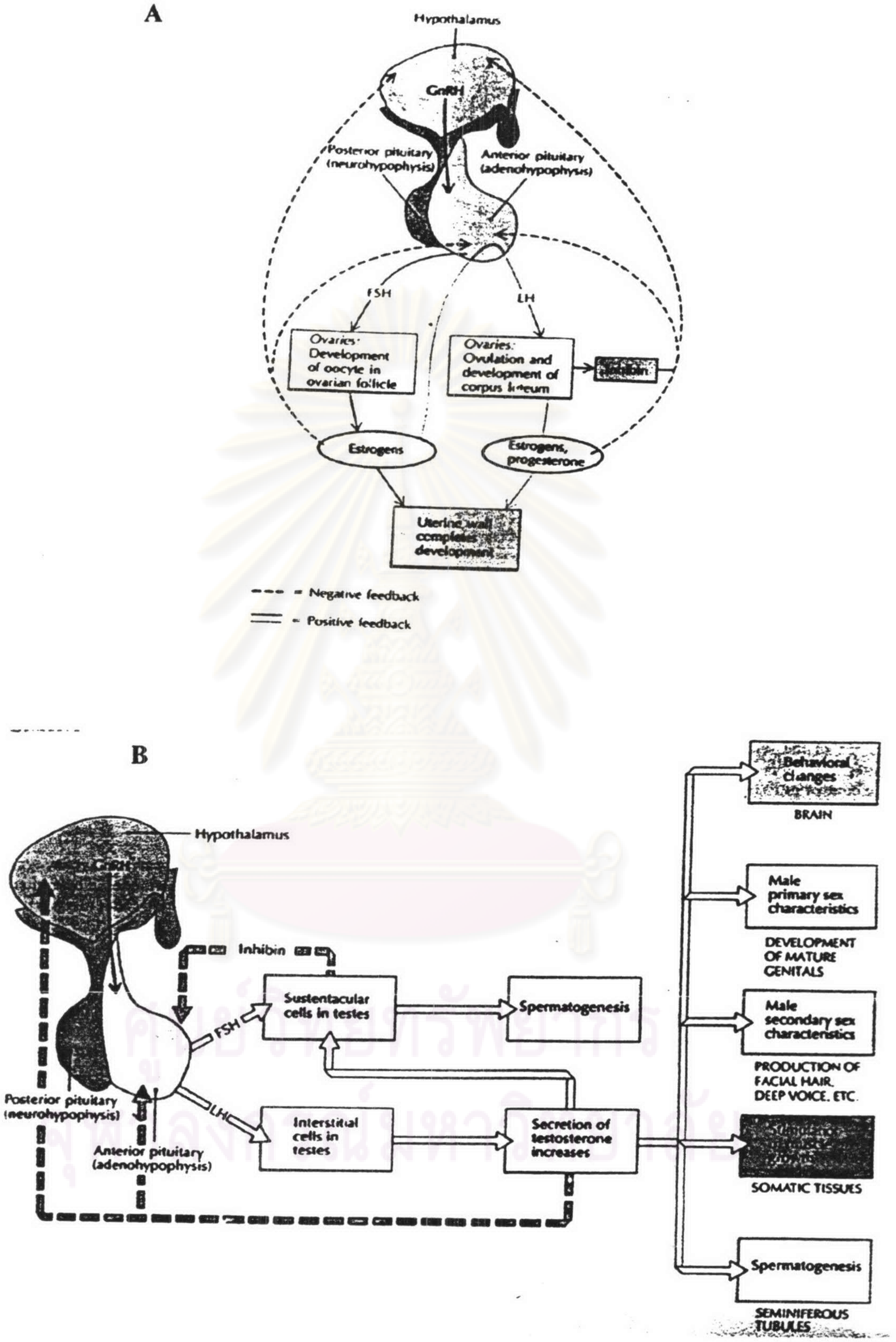


Figure 5 General principles of feedback control in reproductive system, in females (**A**) and in males (**B**).

3.5 Sex accessory ducts, organs and glands

Sex accesssory ducts, organs and glands in both sexes are the target organs of sex steroid hormones action. Therefore, changes of sex accessory ducts, organs and glands can be the supportive data related to the hormonal levels. Thus, some sex accessory ducts, organs and glands were used as the parameter in this study.

3.5.1 Male accessory ducts and organs

The male sex accessory ducts include the tubuli recti, rete testes, vasa efferentia, ductus epididymis, vas deferens, ejaculatory duct and urethra. These ducts serve to nurture and transport sperm. Sperm leave the seminiferous tubules and enter into tubuli recti, rete testes and vasa efferentia, respectively. The vasa efferentia then enter the epididymis (Jones, 1997).

Epididymis forms a semilunar head attached to the cranial pole of the testis, narrows to form the body which lies on the medial side and continues as the club-shape tail beyond the caudal pole of the testis. The single coiled epididymis tubule is lined by a pseudostratified columnar epithelium resting on a vascular lamina propria continuous with the supporting connective tissue. Luminal cytoplasm extends into the lumen as stereocilia. Sperm maturation and motilty are completely occurred in epididymis (Hebel and Stromberg, 1976; Johnson and Everitt, 1995).

Vas deferens or ductus deferens emerges from the medial side of the epididymal tail. It also runs cranially along the medial side of the testis and enters the abdominal cavity. This duct penetrates the dorsolateral lobe of prostate gland and opens dorsally into the urethra. The end of each vas deferens has an expanded portion, the ampulla, which is a reservior for sperm. Each vas deferens enters an ejaculatory duct and urethra, respectively (Hebel and Stromberg, 1976; Johnson and Everitt, 1995).

Urethra is a tube extending from urinary bladder through the floor of the pelvic cavity and the penile length to its external opening. Thus, urethra is a passageway of urine. However, the ejaculatory ducts enter the urethra, this tube also transport sperm to the outside (Jones, 1997).

Scrotum is located ventrolateral to anus, testes and epididymis are easily palpable through the wall of the scrotal sac in the adult. The cremaster muscle is provided for regulation testicular temperature by upward or downward of the testes to the abdominal cavity (Hebel and Stromberg, 1976; Johnson and Everitt, 1995).

Penis consists of an outer fibroelastic connective tissue capsule, tunica albuginea, a glans and a prepuce. The shaft of the penis contains three spongy tissues filled with blood sinuses, 2 copora cavernosa which are formed by tunica albuginea and 1 corpus spongiosum. These spongy tissues fill with blood during erection (Hebel and Stromberg, 1976; Jones, 1997).

3.5.2 Male accessory glands

The male accessory glands include the seminal vesicles, prostate glands and bulbourethral glands. These glands secrete substances into ducts that join the sex accessory ducts (Jones, 1997).

Seminal vesicles are compound tubular glands with a fibromuscular capsule and elastic fibers in the connective tissue stroma. Lumen is lined by tall columnar secretory epithelium with surface blebs giving a ragged appearance to the cells. These glands attach the vas deferens to form the ejaculatory duct. These glands also secrete an alkaline, viscous fluid rich in fructose, and an important nutrient for sperm (Hebel and Stromberg, 1976; Jones, 1997).

Prostate gland is a single doughnut-shape. It lies below the urinary bladder and surrounds the prostatic urethra. This gland secretes the alkaline substances (Aughey and Frye, 2001; Jones, 1997).

Bulbourethral glands or Cowper's glands lie on each side of membranous urethra. Their ducts empty into the spongy urethra, these glands secrete mucus that lubricates the urethra during ejaculation (Aughey and Frye, 2001; Jones, 1997).

3.5.3 Female accessory ducts, organs and glands

Oviducts consist of infundibulum which is close to the ovary, ampulla which is the middle segment and isthmus which opens into the ipsi-lateral horn of the uterus. The epithelium is simple or pseudostratified columnar with secretory and ciliated cells which play important roles in ovum, sperm and embryo transportations (Aughey and Frye, 2001; Hebel and Stromberg, 1976; Jones, 1997).

Uterus is bicornuate with right and left horns (cornua), a body (corpus) and a neck (cervix). The uterine wall has three layers; endometrium, myometrium and perimetrium. The epithelial lining of endometrium is simple cuboidal or columnar shape. The lamina propria is a deep layer of vascular connective tissue, with simple tubular endometrial glands that opens into the uterine lumen. Endometrium is the layer prepared to receive embryo after fertilization. Myometrium is composed of a deep inner layer of circular smooth muscle and an outer layer of longitudinal smooth muscle and perimetrium is the outer loose connective tissue layer or serosa, which is continuous with the broad ligament (Aughey and Frye, 2001; Hebel and Stromberg, 1976).

Vagina is thin walled and opens above the anus and separately from urethral orifice. The vaginal walls consist of mucosa, muscularis and serosa. The vaginal epithelium undergoes cyclic changes. The vaginal wall has folds that allow it to stretch during mating or birth (Aughey and Frye, 2001; Hebel and Stromberg, 1976).

Vestibule is lined with stratified squamous epithelium. Cyclic changes are less evident than in the vagina (Aughey and Frye, 2001; Hebel and Stromberg, 1976).

The stratified squamous epithelium of vulva is continuous with the skin at the labia. Sebaceous and sweat glands are present in lamina propia. The clitoris consists of erectile tissues, corpus carvernosum clitoridis, a glans and a prepuce (Aughey and Frye, 2001; Hebel and Stromberg, 1976).

3.6 Spermatogenesis in rats

Spermatogenesis is the process to form the spermatozoa in the seminiferous tubules. It consists of three elements (Johnson and Everitt, 1995; Ross, Romrell and Kaye, 1995) as follows;

1. Mitotic proliferation, this period produces large numbers of cells. Spermatogonial stem cells were the reservior of self-regenerating stem cells with subtle morphologic differences which they have been described as types A_1, A_2, A_3, A_4, Intermediate and B. Type B spermatogonia divide to produce preleptotene primary spermatocytes.
2. Meiotic division, this is time to generate genetic diversity and halve the chromosome number. As the primary spermatogenic cells proceed through the first meiotic division, they pass through a series of the morphological distinct phases. The secondary spermatocytes produced by the first meiotic division immediately enter the second meiotic division and give rise to the haploid spermatid.

3. Spermiogenesis is the period to pack the chromosomes for effective delivery to the oocyte, the haploid spermatids undergo a maturation process that produces the mature sperm. .

3.7 Folliculogenesis in rats

Folliculogenesis is the process of follicle development, which it is generated the mature follicles in the area of ovarian cortex to ovulation. The growing follicles are further subcategorized as primordial, primary, secondary and mature or Graafian follicles. The histological identify the continuum of folliculogenesis were as follows (Johnson and Everitt, 1995);

A primordial follicle consists of an immature ovum, the primary oocyte, surrounded by a single layer of flattened granulosa cells. The primary oocyte has a little cytoplasm and a large nucleus with a prominent nucleolus and finely dispersed chromatin. When the follicles are stimulated to be developed, a primordial follicle enlarges to form a primary follicle. Entering the stage of primary follicle, the primary oocyte enlarges because of an increase in the volume of the ooplasm. The flattened follicular cells become cuboidal shape and increase in number. The primary oocyte continues to enlarge until it reaches as size that is about three times that of the primordial oocyte. The granulosa cells proliferate to form the epithelium around the oocyte. Between the oocyte and the granulosa cells there is the homogeneous glycoprotein layer, zona pellucida which it is synthesized by the granulosa cells.

With further development, the primary follicle gradually changes in to a secondary follicle. Spaces between granulosa cells occur, widen, and eventually merge forming the antrum. The follicle becomes a secondary follicle. The antrum is filled with follicular fluid. Membrana granulosa which it is formed by the arrangement of granulosa cells in a stratified manner along the basement membrane occurs while the antrum enlarges. The granulosa cells surrounding the oocyte form the corona radiata and the cumulus oophorus which it is resulted form the continuous membrana granulosa surrounding the oocyte is presented. Early in the stage of the secondary follicle, the adjacent stromal cells organize into a capsule or the theca folliculi, which it is separated from the membrena granulosa by the basement membrane. Then the theca folliculi differentiates into two layers; an inner layer, the theca interna, and an outer layer, the theca externa. The theca interna contains enlarged stromal cells and numberous capillaries. The theca externa is composed of closely packed collagen fibers and small fusiform cells.

The mature (Graafian) follicle is a transparent vesicle that protrudes from the surface of the ovary. As a result of the accumulation of follicular fluid, the antrum increases greatly in size, and the membrana granulose becomes thinner. The oocyte adheres to the follicular epithelium through a pedicle by granulosa cells.

After ovulation, the follicular wall is thrown into folds and transformed into a tempory endocrine gland, the corpus luteum. The corpus luteum is surrounded by a layer of connective tissue. The parenchyma of the gland consists mainly of two cell types; granulosa lutien cells and theca lutien cells.

3.8 Reproductive cycles in rats

Reproductive cycles in mammals can be separated into 2 types; the menstrual cycle which it is exhibited in seasonal reproductive cycles and the estrous cycle which it is exhibited in most mammals. The female animals that show the estrous cycle are sexually receptive to males only around the time of ovulation (Jones, 1997; Johnson and Everitt, 1995).

The estrous cycle of the rat is very short, 4-5 days, although the timing of the cycle may be influenced by external factors such as light, temperature, nutritional status and social relationships. The cycle consists of 4 stages (Norris, 1997; Turner and Bagnara, 1976) as follows;

1. Estrus, this is the period of heat and copulation. Heat is permitted only this time, this condition lasts from 9-15 hours. Ovulation occurs in this period under the control of LH and FSH surges, a dozen or more ovarian follicles grow rapidly and mature. The uteri undergo progressive enlargement and become distended owing to the accumulation of luminal fluid. Cornified cells, which were produced by the actions of estrogen during proestrus, appear in the superficial layers of vagina. Vaginal smear reveals cornified cells (Co) as shown in Figure 6
2. Metestrus, this period occurs shortly after ovulation and it is the intermediate between estrus and diestrus. The period lasts for 10-14 hours. The ovaries maintain corpus luteum and small follicles. The uteri have reduced in

vascularity. Vaginal smear exposes many leukocytes (L) with a few cornified cells as depicted in Figure 6.

3. Diestrus, this is the longest period of estrous cycle. It lasts for 60-70 hours during the functional regression of corpus luteum occurs. This evidence of corpus lutea cause a slight increase in plasma progesterone, which it was induced by LH. The uteri are small, anemia and only slightly contractile. The vaginal mucosa is thin and leukocytes migrate through it. Vaginal smear appears entirely of leukocytes (L) as illustrated in Figure 6.
4. Proestrus, the levels of plasma estrogens reach a peak in this period. This result is ongoing stimulation the LH surge accompanied by a small surge of FSH and is followed rapidly by a marked surge of progesterone. Fluid accumulates in the uteri. Vaginal smear represents dominantly nucleated epithelial cells (O) as shown in Figure 6.

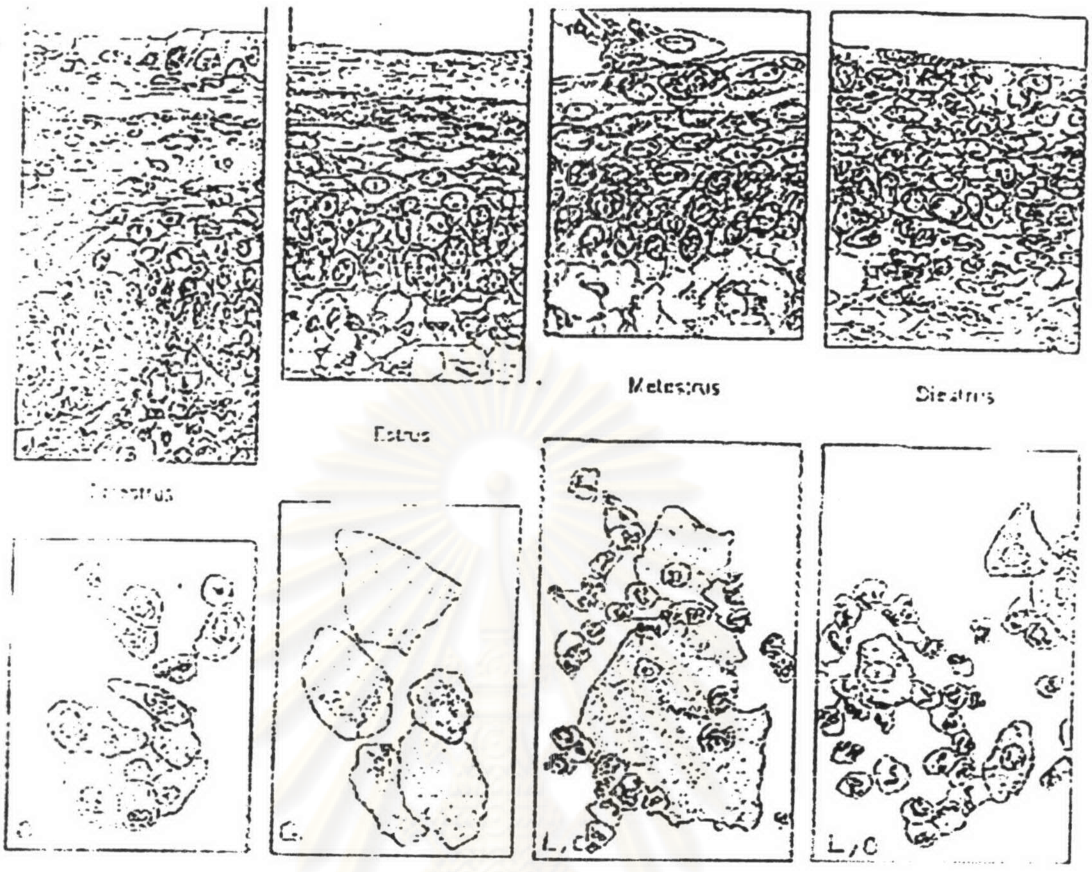


Figure 6 Types of cells in the different stages of estrus cycle by vaginal smear.

IV. Effect of exogenous testosterone administration on the pituitary-gonadal axis

Exogenous testosterone propionate administration, approximately 300 μg/100gBW/day in 0.2 ml of sesame oil or more could suppress serum Gn levels in both male and female rats (Beyer *et al.*, 1974; Gay and Bogdanove, 1969; Ramirez and McCann, 1965; Swerdloff and Walsh, 1973; Wierman *et al.*, 1990). In addition, testosterone propionate administration at least 50 μg/300gBW/day could gain the weight of epididymis and seminal vesicle, increase serum testosterone levels and decrease testicular testosterone levels by dose-dependent in male rats (Gay and Bogdanove, 1969; Ramirez and McCann, 1965; Swerdloff and Walsh, 1973; van Roijen *et al.*, 1997). Moreover, testosterone propionate at least 500 μg/200gBW/day could decrease ovarian weight (Beyer *et al.*, 1974), increase uterine weight (Garcia and Rochefort, 1977; Ruth and Ruth, 1975; Schmidt *et al.*, 1976) and produce unestrous cycles in normal female rats (Beyer *et al.*, 1974). Therefore, testosterone propionate at the dosage of 600 μg/100gBW/day in 0.2 ml of sesame oil was chosen to ensure the suppression of exogenous testosterone propionate on pituitary-gonadal axis in this study.

From the above mentioned, the administration of *M. collettii* may disturb the pituitary-gonadal axis. To clarify the indirect effect of *M. collettii* on gonad through the hypothalamic-pituitary-gonadal axis, serum LH, FSH, E_2 and T levels were determined in this study. To clarify the direct effect of *M. collettii* on gonad, changes of testes, epididymis and seminal vesicle in males, and changes of ovaries and uteri and vaginal cytology in females were also determined in this study.