CHAPTER 2

LITERATURE REVIEW

2.1 Soybean

2.1.1 Taxonomy

Superkingdom : Eukaryota

Kingdom : Viridiplantae

Phylum : Magnoliophyta

Division : Tracheophyta

Subdivision : Spermatophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Genus : *Glycine*

Species : *Glycine max* (Olivia Scurek, 2006)

Figure 2.1 Soybean (*Glycine max*) (American Chemical Society, 2009)

2.1.2 Description and Functions

The Asians have consumed soy foods many centuries earlier than the rest of the world. It was perceived as a source of high-quality protein, low in saturated fat. Having many health benefits, soybeans have become increasingly popular among people (Messina, McCaskill-Stevens and Lampe, 2006). It can be grown in a variety of temperate climates. It has a unique benefit of improving soil quality because of its nitrogen-fixing ability. In the USA, soybean is grown more than any other crops after corn (Cooper *et al*., 2008).

Soy proteins are said to have hypocholesterolemic effects (Messina, 2010). Apart from being rich in phytoestrogens, soybeans are said to possess anti-cancer effects by inhibiting proteases, tyrosine kinases and DNA topoisomerases. Furthermore, they have active roles in antioxidation and immune response enhancement. (Shu *et al*., 2009).

2.2 Isoflavones and Phytoestrogens

Despite limited distribution in nature, isoflavones are found in relatively large amount in soybeans. Isoflavones that are found in soybeans are genistein, daidzein and glycitein. Traditional Asian soy foods contain around 3.5 mg of isoflavones. Isoflavones have short half-lives of 8 hours, and are excreted within a day after it is digested. Serum and urinary concentrations of isoflavones are significantly different due to individual variations in gut metabolism and biologic activity. This is one of the plausible explantions for inconsistent clinical trials. Isoflavones compete with endogenous estrogens to bind alpha and beta estrogen receptors (ER). Due of their preference to bind beta ER, they are sometimes called as selective estrogen receptor modulators (SERMs). (Messina, McCaskill-Stevens and Lampe, 2006).

The three soy isoflavones are genistein, daidzein and glycitein. Isoflavones have many biological functions such as increasing steroid clearance from circulation, increasing synthesis of sex hormone-binding globulin (SHBG) and inhibition of 17β-hydroxysteroid dehydrogenases (Shu *et al*., 2009). It has been mentioned that isoflavones are substances formed by the host tissue in response to physiological stimuli or infectious agents. Isoflavones accumulate to inhibit the growth of microorgansims. In order to increase the chance of survival of the soybean, isoflavones are in possession of properties like antifungal, antimicrobial, and antioxidant. Therefore, its concentrations largely increase in times of stress, and are under influence of environmental conditions. Moreover, isoflavone may improve bone mineral density which could benefit postmenopausal women.

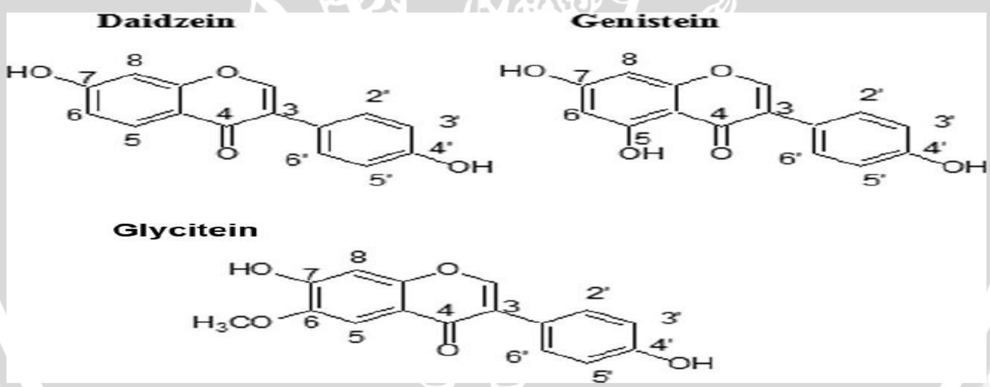

Figure 2.2 Isoflavones (Kalaiselvan *et al*., 2010)

Due to its functions as antiestrogens, isoflavones may reduce risk of hormone-sensitive cancers. Asian women whose soy isoflavones intake is high have decrease risk of developing osteoporosis, cardiovascular disease, breast and uterine cancer. Furthermore, thereare reports, showing soy isoflavones consumption is associated with a decrease risk of endometrial cancer in postmenopausal women (Marini *et al*., 2008). On the other hand, some researches also state that isoflavones could be a risk factor for estrogen-sensitive breast cancer patients. Finally, due to its differences in clinical data, it is necessary to identify the contributing factors precisely (Messina, 2010).

Table 2.1 Contents of Isoflavones in Soy Foods (Clarkson *et al*., 2010)

Food	Mean mg isoflavones per 100 g of food
Soybeans (green, raw, edamame)	48.95
Soy flour (textured)	172.55
Soy protein isolate	91.05
Miso soup (mix, dry)	69.84
Tempeh	60.61
Soybeans (mature seeds, sprouted, raw)	34.39
Tofu (silken)	18.04
Tofu yogurt	16.30
Soy hot dog (frozen, unprepared)	1.00
Soy milk (original, vanilla)	10.73
Soy sauce (soy + wheat; shoyu)	1.18

2.3 Male Reproductive System

The main organs of the male reproductive system are the two oval-shaped testes. It also consists of accessory glands such as seminal vesicle and prostate. In addition, there exists a system of ducts, consisting epididymis, ductus deferens, ejaculatory ducts and urethra.

The scrotum is the pouch where the testes and its supporting structures lie. The pigmented thin skin of scrotum consists of rugae and sebaceous glands. It is incompletely divided into right and left compartments by a septum (Ellis and Mahadevan, 2010). The temperature within the scrotum is lower than normal body tempature because of the production and survival of the sperm. The skeletal muscles contract and relax according to the tempartures of the surroundings.

The testes are covered by the tunica albuginea, a dense white fibrous capule, and invaginated into the tunica vaginalis, a double serous covering. Each testis is divided into 200-300 lobules, each of which in turn contains one to three tightly coiled seminiferous tubules. One of the functions of the testes are sperm production and hormone secretion (Parkin, Logan and McCarthy, 2007). Two compartments of the testis are seminiferous tubules and the interstitium. In seminiferous tubules, there are two kinds of cells, namely germ cells and supporting Sertoli cells. Germ cells are in various stages of development such as spermatogonia, spermatocytes, spermatids and spermatozoa. The interstitium contains intertubular tissue, loose connective tissue, blood and lymphatic vessels, macrophages, fibroblasts, leukocytes and Leydig cells which are the main source of the male sex hormone testosterone (Akingbemi, 2005). LH receptors are expressed in Leydig cells while FSH receptors are found in Sertoli cells. Sertoli cells from the testes support and nourish spermatogenic cells. They regulate production of sperm by secretion of inhibin while leydig cells release testosterone that renders the development of musculine characteristics and promotes libido (Tortora and Derrickson, 2010).

2.3.1 Histology of Epididymis

The epididymis which is comma in shape, is found along the posterior border of the testis. The sinus epididymis seperates the epididymis from the testis. The epididymis can be divided into a head, a body and a tail which are also called caput, corpus and cauda respectively. However, some literatures mention the existence of two other regions known as the initial segment and intermediate zone (Robaire, Hinton and Orgebin-Crist, 2006). Tunica vaginalis also covers the epididymis, with the exception of the posterior margin. The process where sperm obtains the ability to mobilize and fertilize a secondary oocyte occurs in the epididymis. That is why it is also known as the site of sperm maturation. The ductus epididymis may store sperm until it is fertilized. Sperm are eventually phagocytized and reabsorbed when they do not undergo ejaculation after a certain period of time (Ellis and Mahadevan, 2010).

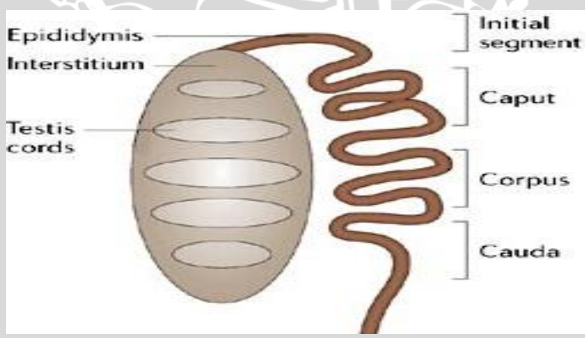

Figure 2.3 Epididymis (Wilhelm and Koopman, 2006)

The lining epitheliium of the epididymis is pseudo-stratified columnar. Stercocilia are seen on the outer surface of the epithelium towards the lumen. The intertubular area is filled with connective tissue and smooth muscle. Several types of cells are found in the epithelium of the epididymis. These include principal cells, apical cells, narrow cell, clear cells, basal cells and halo cells.

Principle cells are the major type of cells in the epididymis as they constitute around 65% to 80% of the cell population. They can be found in all regions of the duct with differences in structure and functions. They play an important role in the synthesis of proteins which are either stored in the cells or endocytosed into the luminal compartment. Apical cells and narrow cells are mainly found in the initial segment and intermediate zone. However, the presence of apical cells in other segments are reported in aging rats. These cells are located apically with spherical nucleus. Their functions are still unknown. As the name applies, narrow cells are narrower than the principal cells. They contain cup-shaped vesicles for endocytosis and secreting H+ ions into the lumen. The large clear cells are found only in the caput, corpus and cauda regions of the epididymis.The activity of these endocytic cells are much greater than in the principal cells.

Basal cells are present closely to the basement membrane where the thin processes of the cells are extended (Figure 2.4). They are often describe to possess immunlogic functions. On the other hand, halo cells are suggested as lymphocytes or monocytes. These small cells usually lie at the base of the epithelium and contain granules (Robaire, Hinton and Orgebin-Crist, 2006).

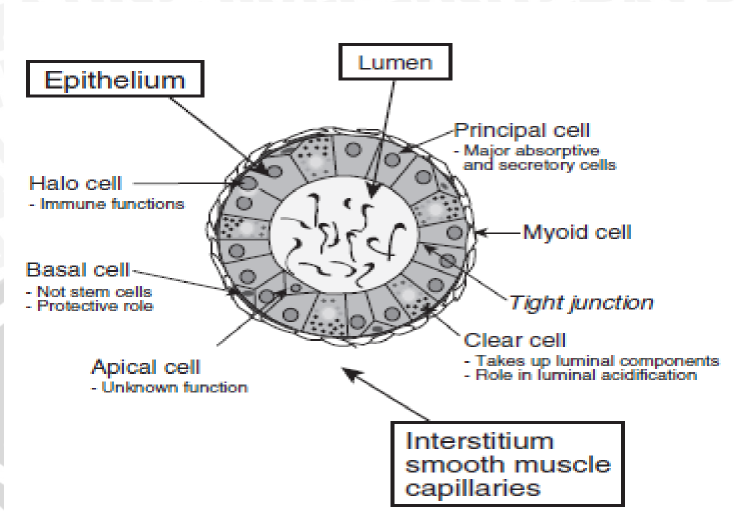

Figure 2.4 Cell Types in Epididymis (Robaire, Hinton and Orgebin-Crist, 2006)

2.3.2 Formation of Vacuoles in Epithelial Cells

Cells maintain their normal homeostasis within a range of physiologic parameters. If they encounter stresses or noxious stimuli, they undergo a process called adapation where they attain a new steady state for viability and function. The basic adaptive responses are hypertrophy, hyperplasia, atrophy and metaplasia. However, if the ability of adaptation is exceeded or the stimuli are extremely harmful, cell injury ensues (Kemp, Burns and Brown, 2008).

Cell injury develops if cells are so severely stressed that they are no longer able to adapt. Moreover, it may result when cells are exposed to damaging agents or suffer from intrinsic abnormalities. Various harmful stimuli affect many metabolic pathways and cellular organelles. Injury may progress through a reversible stage where it can become irreversible with continuing damage and eventually result in cell death (Kumar *et al*.,2010).

Formation of vacuoles in cellular level can be seen as a type of cell injury in smooth endoplasmic reticulum , rough endoplasmic reticulum and mitochondria. This cell injury is caused due to the disruption of energy metabolism in which the reaction chain involving Na^+, K^+-ATPase fails. This renders water to flow into the cell and the cisterns of the rough endoplasmic reticulum. It gives rise to cytoplasmic degeneration with formation of vacuoles when accompanied by swelling of the smooth endoplasmic reticulum and mitochondria. It is recognizable under the light microscope (Riede and Werner, 2004).

The epididymis relies on testosterone to maintain its structure and functions. It has been shown that testosterone withdrawal may affect the epididymis. It causes a decrease in epididymal weight, luminal diameter and epithelial cell height. In this testosterone-deprived state, the function of cells in epididymal epithelium becomes compromised. It results in decrease content of endoplasmic reticulum, loss of apical microvilli from their surface, lysosome accumululation, vacuolization and increased endocytosis. Furthermore, androgen receptors in epididymis and 5-alpha reductase activity are both decreased in this state (Robaire, Hinton and Orgebin-Crist, 2006). The epididymis plays an important role in sperm transport, concentration, storage and maturation which are important processes for male fertility, and the absence of these functions might significantly affect male infertility (Arroteia *et al*., 2012).

2.3.3 Spermatogenesis

It is the process of production of sperm (Figure 2.5). It starts from the mitosis of spermatogonia until sperm are released into the lumen of seminiferous tubules. It does not occur until puberty when the seminiferous tubules and testes become enlarge due to the influence of androgens. Spermatogonia possess diploid number of chromosomes. After mitosis, they give rise to primary spermatocytes which also are diploid. These primary spermatocytes undergo meiosis. Following meiosis I, there are secondary spermatocytes which have reduced or haploid number of chromosomes. Four spermatids are produced after completion of meiosis II. Finally, these spermatids undergo the final stage of spermatogenesis, known as spermiogenesis that results in mature spermatozoa. Each sperm has three distinct parts: a head, a middle piece and a tail. The nucleus lies in the head while the mitochondria in the middle piece provides energy for the movement of the tail (Mader, 2004).

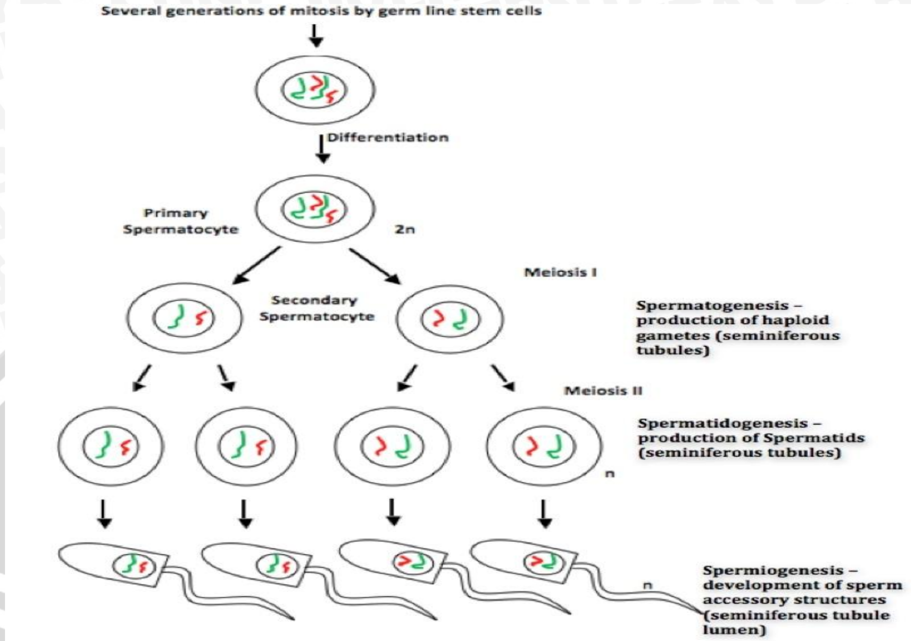

Figure 2.5 Spermatogenesis (Studart, 2013)

2.3.4 Testosterone

Apart from mediating the proper differentiation of the male genital system during fetal development, the functional growth of penis, prostate, seminal vesicle, epididymis,vas deferens, and the development of male secondary sexual characteristics have been largely influenced by androgens. Three primary steroids contributing to these functions are testosterone, dihydrotestosterone and estradiol. Among them, testosterone is the most crucial androgen.

Testosterone is produced from Leydig cells in the testes. Regulation of reproductive functions is primarily done by the hypothalamus, adenohypophysis and gonads. Gonadotropin-releasing hormone (GnRH) which are secreted from the hypothalmus binds to gonadotropins from the anterior pituitary gland. This results in stimulation of the release of follicle stimulating hormone (FSH) and lutenizing hormone (LH) into the blood. While FSH acts on the seminiferous tubules of the testes to initiate spermatogenesis, LH induces testosterone secretion from the leydig cells. Testosterone encourges the maturation of the male reproductive organs and elicits feedback from the hypothalamic-pituitary-testicular axis (Sherwood, 2006).

Testosterone concentrations alters during an individual's life-time. It peaks during puberty but declines in the course of aging. There is an increase in the prevalence of low testosterone levels in men from 20% to 50% from the age of 60 to 90 (Fernandez-Balsells *et al*., 2010). Low testosterone level with old age is asscociated with muscle loss and chronic dieseases such as rheumatoid arthritis, diabetes, cancer, acquired immunodeficiency syndrome (AIDS). Moreover, certain drugs such as chemotherapeutic agents,steroids and opioids might render low levels of testosterone. The fall in free testosterone (FT) levels is greater than that of total testosterone (TT) levels since there is a rise in sex hormone binding globulin (SHBG) with age (Srinivas-Shankar and Frederick, 2009).

Moreover, testosterone plays important roles in a wide range of regulations of several systems throughout human entire life. Besides its involvement in physiologic functions and behaviors such as erectile quality, its level increases during sleep and decreases during waking (Andersen and Tufik, 2008). Testosterone also mediates immunosuppression indirectly by increasing the release of corticosterone (CORT) (Roberts *et al*., 2009). Since the disorder of erectile capacity caused by low levels of testosterone can be reversible, a certain number of recent developments make use of testosterone therapy to treat erectile dysfunction (ED) in aging men (Jockenhovel *et al*.,2009).

2.3.5 Estrogen

Estrogens have many biological functions, starting from gene expression to the whole individual. Estrogens play vital roles in female sexual development. They mediate reproductive cyclicity, pregnancy, lactation, development and manintenance of the reproductive tract and secondary sexual characteristics. Furthermore, they regulate cell proliferation in cells containing estrogen receptors (ERs). ERs are found in the female reproductive organs, the male reproductive organs, the mammary gland, the brain, the thyroid gland, the skeletal and cardiovascular systems. Estrogen exposure throughout a woman's life constitutes a major risk factor for breast cancer (Vandenberg, 2009).

Estrogen is irrversibly converted from testosterone by aromatase that is present in the endoplasmic reticulum of of cells of mammalian testes. The testes possess high affinity estrogen receptors that mediate the effects of estrogen (Carreau, Bouraima-Lelong and Delalande, 2012). Estrogen has been suggested to play a very complex role in the development of the male reproductive system. Moreover, it is said that estrogen plays a regulatory role in the testes since it is synthesized in the testicular cells. Adverse effects of spermatogenesis and steroidogenesis is obeserved when there is no ER. Xenoestrogens can bind and activate ERs, and affect gene expression in the testes (Akingbemi, 2005).

2.4 Endorine Disruptors

The USA Envrionmental Protection Agency (EPA) defined endocrine disruptors as an exogenous agent that hinders with synthesis, secretion, transport, metabolism,binding, action, or elimination of natural blood-borne hormones that are present in the body and take responsiblity for homeostasis, reproduction, and developmental process. Endocrine disruptors are a group of chemicals that act as agonists and antagonists if the estrogen receoptors (ERs), androgen receptor, thyroid hormone receptor, and others.

Also being called endocrine-disrupting compounds (EDC), endocrine disruptors affect several the reproductive system and energy balance system thoroughout life. However, most of effects of EDCs are seen duirng neonatal period and adulthood. Since both of the systems are regulated by feedback mechanisms between peripheral tissues and the pituitary gland, hypothalamus and CNS, EDCs could affect any of those sites directly or secondarily through changes in messengers. This disorders may include abnormalities of sexual differentiation, infertility or subfertility and some neoplasia, obesity and metabolic syndrome. Furthermore, EDCs are reported to cause lipid accumulation in differentiated adipocytes (Bourguignon and Parent, 2010).

Although there have been substantial advances, several controversies have sprung up concerning endocrine disruptors (Vandenberg, 2009).

2.5 Infertility

According to the World Health Organization (WHO), infertility is defined as the failure to conceive in a couple, having sexual intercourse without conception in a period of two years. Infertility is a major clinical problem, affectiong couples psychosocially (Agarwal, Makker and Sharma , 2008). It is estimated that 15% of couples are infertile, affecting 60-80 million couples every year. Infertility can be categorized as either primary or secondary depending on the occurence of pregnency. Male infertility , a multifactorial syndrome, accounts for half of the overall infertility. The cause of infertility is unknown in more than half of the infertile man. However, it is thought to be caused by either genetic conditions or non-genetic conditions. Genetic causes include chromosomal disorders, mitochondrial DNA (mDNA) mutations, monogenic disorders, and endocrine disorders while non-genetic conditions range from testicular maldescence, hypogonadotrophic hypogonadism, structural anomalies of the male genital tract, impotency, previous scrotal or inguinal surgery, varicoceles, medications, exposure to chemicals, environmental causes immunological factors to chronic infections (Poongothai, Gopenath and Manonayaki, 2009).

Evidences suggest that there have been some associations between some of the causes of inferitily and certain pathologic condtions. Varicoceles have very damaging effects of male fertility in obese men, causing increased germ cell apoptosis, decreased spem motility and testicular atrophy. Furthermore, dyslipidmemia can render negative effects on sperm quality and fertility while ejaculatory dysfunction may present as common etiology of male infertilty in diabetic men (Kasturi, Tannir and Brannigan, 2008). Apart from these conventional causes of male infertility, oxidative stress has been described as a powerful mechanism that can render sperm damage, and eventually, male fertility (Makker, Agarwal and Sharma, 2009).

Male infertiltiy could be diagnosed by semen analysis that measures various aspects of sperm, including concentration, motility and morphology. The concentrations of motile spermatozoa in the semen may indicate the ability to conceive a child. According to WHO, a normal fertile male should have 20 miliion sperms in one mililitre of semen. Infertile men may also have abnormal sperm conditions in the seminogram, such as azoospermia, oligospermia, astheonspermia, teratospermia, necrospermia and pyospermia. However, nowadays, infertility in couples may be overcome by the use of artifical reproduction techiques, for example intracytoplasmic sperm injection (ICSI) (Poongothai, Gopenath and Manonayaki, 2009).