CHAPTER I

1.0 INTRODUCTION

There has been an increasing demand for beef cattle and their products in Malaysia. The consumption of beef cattle is approximately 5.43 kg/capita/year and increasing every year (Appendix 1). This trend is mainly due to the increase in population size, higher standard of living in Malaysia, rising incomes, better socio economic condition and greater consumer demands. Malaysia only produces about 25 % of her own beef cattle even though the demand for local beef is very high. In 2008, population of cattle in Malaysia was approximately 847,757 and the number is increasing every year but still below the consumer demand (Appendix 2). In 2008, approximately 150,696 ton of beef was required per year (Appendix 3) and to accommodate the increasing demand, Malaysia has to spend RM 683.22 million yearly to import beef cattle from India, Australia and New Zealand (Appendix 4). To fulfill the demand, approximately 100,000 heads of cattle have to be slaughtered yearly in Malaysia.

There are many factors that contribute to the poor cattle production in Malaysia. The main factors include the limited available feed, shortage of feeder cattle, absence of suitable productive and adaptable breeds, shortage of land, lack of incentives for Malaysians to venture into animal husbandry and lack of knowledge in modern techniques for improving cattle reproductive performances and the number of calves born. Other factors include less government emphasis on animal production and low interest of investors in cattle rearing industry due to lower return investment (Zainur and Wan Zahari, 2005). After the economic crisis in 1997, Malaysian government started to realize the important of agriculture and had incorporated agricultural development plan as an integral component of industrial master plan. Under the National Agricultural

Policy 3 (NAP 3) (1998-2010), Malaysia gave very high priority to beef cattle production to meet self-sufficiency. Farmers are encouraged to change the cattle production system from backyard to commercial entity to achieve this target.

Farming systems that have been practiced by farmers and entrepreneurs to rear cattle in Malaysia include the intensive, semi-intensive and integration of livestock with plantation crops. Intensive system is where the reared cattle are kept in the shed all the time, constantly provided with sufficient quality feeds and forages, clean water and mineral supplements. Feeds, either as cut grass or concentrates or a mixture of cut grass and concentrates are supplied daily in the form of dry weight in a ratio of 3 - 5% of the animal's body weight (Zainur and Wan Zahari, 2005). Semi-intensive farming system is more practical in Malaysia. Cattle are allowed to graze freely either in a fenced pasture or in an open pasture for a fixed period during the day. The cattle are then herded back to the cattle sheds for supplementary feeding and shelter during the evening (Zainur and Wan Zahari, 2005). Integration farming system is cattle rearing with major plantation crops like oil palm, rubber, coconut and fruit orchards. Normally, these plantations are covered with undergrowth that required chemical control. Therefore, integration with livestock will reduce herbicide usage as well as fertilizer costs.

Malaysia has a number of beef cattle breeds including the indigenous Kedah-Kelantan (KK) cattle, their numerous crosses with exotic breeds and imported breed like Charolais and Brahman. Malaysia has produced a considerable number of beef cattle crosses through the effort of various agencies such as the Malaysian Agricultural Research and Development Institute (MARDI), Department of Veterinary Services (DVS), Federal Land Development Authority (FELDA), Rubber Industry Smallholders Development Authority (RISDA), private agencies such as Pahang Beef and other institutions (Zainur and Wan Zahari, 2005).

To date, many modern techniques are used to improve reproduction in cattle such as control internal drug release (CIDR) device to synchronize the estrus and artificial insemination (AI). Estrus synchronization and AI are reproductive management techniques that allow beef producers to enhance the reproductive efficiency and genetic composition of their cow population (Gordon, 2004).

Synchronization of estrus in cattle not only allows one to predict the time of estrus with reasonable accuracy but also will help the management to organize a proper breeding programme. Synchronization of estrus also reduces the time required for detection of estrus as well as facilitates timed artificial insemination without detection of estrus (Wahid *et al*., 2001). Estrus synchronization is a valuable management tool that has been used successfully to improve reproductive efficiency in ruminant. In small ruminant, estrus synchronization is used to advance the onset of estrus, decrease the length of the breeding period or shorten the postpartum interval. Estrus synchronization allows for the efficient application of reproductive technologies in commercial livestock operation. Application of estrus synchronization can increase profit through animal production, reduce prenatal mortality and it can improve herd management under various production systems.

Reproduction in animal is controlled by reproductive hormones such as progesterone, estradiol, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Normal secretion of reproductive hormone is very important to ensure the normal reproductive status of the animals. Abnormal estrus cycle may have resulted from asynchronous interactions between the reproductive hormones. Consequently, abnormal estrus cycle may result in low reproductive efficiency of the cows.

The main objectives of this study were:

1. To study the effect of farming system on body condition score (BCS) and fertility responses of synchronized cows.

2. To study the effect of breeds on body condition score (BCS) and fertility responses of synchronized cows.
3. To study the effect of body condition score (BCS) on fertility responses of synchronized cows.
4. To study the effect of days of postpartum interval on fertility responses of synchronized cows.
5. To study the hormone profiles of synchronized cows.

CHAPTER II

2.0 LITERATURE REVIEW

2.1 Breeds and type of beef cattle

Breeds or crossbreeds, used in a defined breeding program have a significant impact on the efficiency and profitability of the beef enterprise. Breed influences the important parameters of growth rate, reproductive efficiency, maternal ability, and end-product specifications. Additionally, nutritional requirements and production costs are related to traits such as mature cow size and growth rate that are largely determined by breed. Therefore, selecting appropriate breeds to be used in a crossbreeding program is an important decision for beef cattle producers (Zainur and Wan Zahari, 2005). There were only two kinds of wild cattle in prehistoric times, the Aurochs of Europe (*Bos taurus*) and Zebu cattle of Asia, India, and Africa (*Bos indicus*, the hump backed, and droopy eared cattle of tropics) (Thomas, 1998).

Malaysia has a number of beef breeds including the indigenous Kedah Kelantan (KK) cattle and their numerous crosses with exotic breeds. Malaysia has produced a considerable number of beef crosses though the effort of various agencies such as MARDI, DVS, FELDA, RISDA, private agencies such as Pahang Beef and other institutions (Zainur and Wan Zahari, 2005).

2.1.1 Kedah-Kelantan (KK)

Kedah Kelantan (KK) is the indigenous cattle breed of Malaysia and is mainly kept for meat production. Kedah Kelantan (KK) is popular among the smaller farm owners because of its small compact body, low maintenance requirement, excellent adaptation to the local climate and high resistant to various tropical diseases prevalent in this country. This breed can also fully sustain itself on the poor quality diets from local natural vegetation. Fertility rate of KK raised by small holders at FELDA was found to be between 46.3 to 52.5% (Zainur and Wan Zahari, 2005).

The majority of KK size are small by cattle standard. A mature animal (3 years old) is about 250 kg and 100 cm in height which is, not too big to handle. This is an important consideration for small holder farmers who have limited farm infrastructure. Kedah Kelantan (KK) has yellowish brown coats while some are with black coats. However, the color between the legs is much lighter and can be white. Kedah Kelantan (KK) also have small, sharp and bend inwards and upwards horn. Their overall growth rate is 0.36-0.44 kg per day under optimum condition (Zainur and Wan Zahari, 2005).

2.1.2 Charolais

The Charolais breed originated in the west-central to southeastern France, in the old French provinces of Charolles and neighboring Nievre. Charolais is very big in size and has good growth performance when compared to Kedah-Kelantan.

Charolais is white or creamy white in color, but the skin carries substantial pigmentation. The hair coats are usually short in summer but thicken and lengthen in cold weather. Charolais is a naturally horned beef animal. Charolais cattle are large with mature bulls weighing from 2,000 to over 2,500 pounds and cows weigh from 1,250 to over 2,000 pounds (Thomas, 1998).

This breed has been quite popular in the Australia where they are used for cross breeding. It has also become popular in the southern United States, where Charolais often crossed with ot her breeds have increasingly replaced Herefords. Despite their relatively northenly origin, Charolais tolerate heat well, and show good weight gains on even mediocre pasturage.

2.1.3 Brahman

Brahman originated from India, but those available in Malaysia are the American Brahman. There are not many pure Brahman in Malaysia, except in big breeding farm like MARDI, FELDA, DVS and Pahang Beef. Only the males are reared in this country for breeding purposes (Zainur and Wan Zahari, 2005).

In Malaysia the birth weight of Brahmans are at an average of 27 kg, and can reach up to 115 and 181 kg at 6 month and 1 year of age, respectively. Under optimum nutrition, pure Brahman cattle can achieve a maximum live weight of about 800 kg (Zainur and Wan Zahari, 2005). Brahman can be gray or red colour. Their tail switch is black, and have black pigmentation on their noses, tips of ears, and hooves. They are primarily a horned breed of cattle however there are some bloodlines of Brahman that without horns.

The Brahman is known for their extreme tolerance to heat conditions, and therefore is used in many tropical regions. They are also resistant to insects due to their thick layer of skin. Brahman cattle live longer than many other breeds, and still can produce calves at the age of 15 months/years old and older (Thomas, 1998).

2.2 The reproduction system of the cow

Cattle can reproduce at any time of the year. The cow must recover from calving before rebreed, and recovering period takes about 40 to 60 days. A young heifer will start to breed at any time of the year after becoming sexually mature (Thomas, 1998).

The reproductive system of the cows consists of two ovaries, oviducts, uterus, cervix, and vulva (Figure 2.1). The cow reproductive organs and their major function are tabulated in Table 2.1.

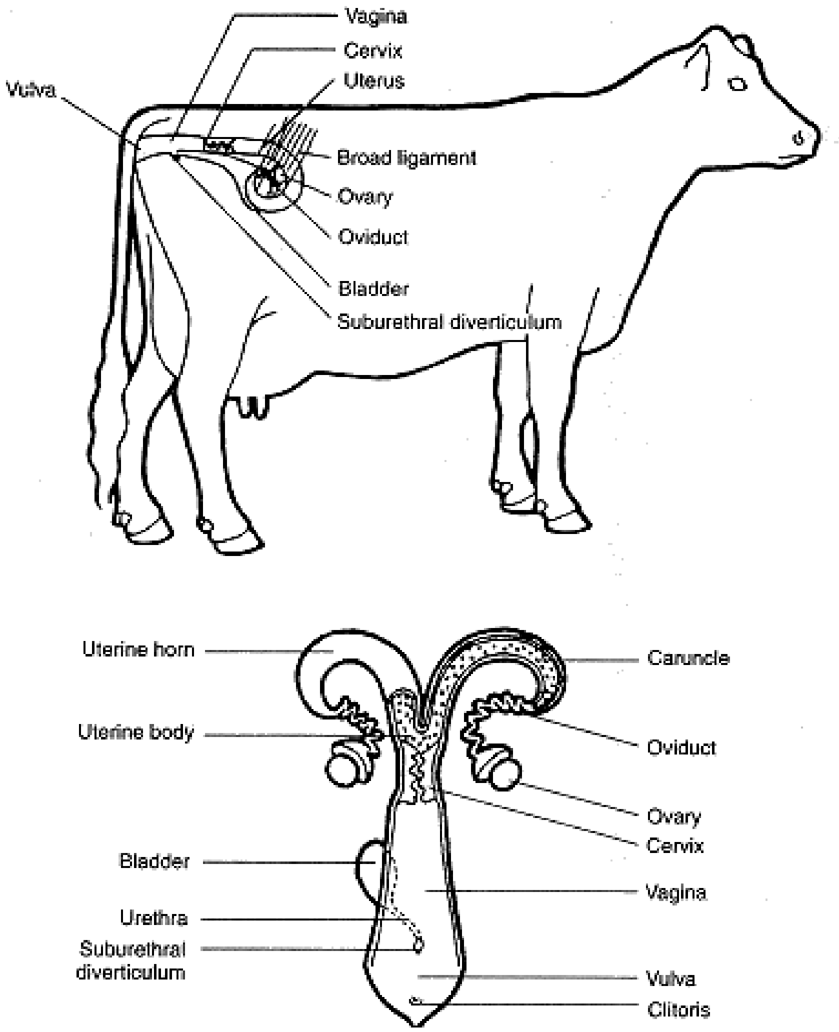

Figure 2.1 Reproductive system and associated parts of the urinary system of the cow (Source: Bearden and Fuquay, 2000).

Table 2.1 Reproductive organs of cow with their major functions

Organ	Function(s)
Ovary	Production of oocyte, secretion of estrogen (Grafian follicle), production of progestine (corpus luteum).
Oviduct	Transport of gametes (sperm and oocyte), site for fertilization.
Uterus	Retain and nourishes the embryo and fetus.
Cervix	Prevent microbial contamination of the uterus, reservoir for semen and transport of sperm.
Vagina	Organ for copulation, site for semen deposit during natural mating, birth canal
Vulva	External opening to reproductive tract

(Source: Bearden and Fuquay, 2000)

2.2.1 Ovaries

The ovaries are paired and held near the kidneys. The ovaries are considered as a primary reproductive organ in the cow, because they produce gamete (the ovum) and female sex hormones (estrogen and progestin). These hormones play indispensable role in preparing the reproductive tract for pregnancy, maintaining and delivering the product of pregnancy (Salisbury *et al*., 1978). The cow is monotocous, normally giving birth to one young for each gestation period. Therefore, one ovum is produced during each estrus cycle (Bearden and Fuquay 2000).

Bearden and Fuquay (2000), described the ovary of the cow as an almond-shaped, but the shape is changed by growing follicle or copora lutea. The average size of the ovary is about 35 mm x 25 mm x 15 mm. The size is varied among cows, and active ovaries are larger than inactive ovaries.

Each ovary consists of a central region, the medulla, which is covered by a thick layer, cortex. Most of an important activity of the ovary occurs in the cortex. The egg mother cells, or oogonia, are found in the outside layer of the cortex, the germinal epithelium. In bovine, it has been estimated that the oogonia capable of forming up to 75,000 eggs, or ova. Throughout her lifetime, a normal productive cow would utilize less than 60 eggs. The oogonia ripen as necessary during the whole breeding life of the animal, and this ripening follows a sequence (Lindsay *et al*., 1982).

As the oogonia divide and ripen, they form follicle. Three successive stages of growth seen were in the ovarian follicle (Lindsay *et al*., 1982). These contain oocytes or potential ova, surrounded by a small number of follicular cells. Some of these never develop beyond these stages but some become secondary follicles in which the oogonia grow in size and form a membrane, the zona pellucida. The follicles at these stages become oval in shape and move from the periphery of the ovary into the body of the cortex toward the medullary region. The final stage of follicle development is the tertiary follicle, also called the Graafian follicle. The size of Graafian follicle can reach up to a centimeter or more in diameter. The follicular cells surrounding the oogonium gradually form an antrum or fluid filled space. The cell lining these spaces are called membrana granulosa. The liquid inside the antrum is rich in protein hormones, estrogen. As the follicle grows further and mature, it occupies the full extent of the cortex of the ovary and protrudes from it. Mature follicles in the ovaries have the appearance of blister due to their thin external walls and the mass of straw-coloured liquid in antrum. Within the follicle, the oogonium is embedded in a number of cells called the cumulus oophorus which protrudes into the antrum on the opposite side to the site which will rupture during ovulation. There is a further layer of cell, the corona immediately surrounding the ovary. The lining of the antrum consist of two layers of cortical cells called the theca external and theca internal. These are important layers that secrete the female hormone, estrogen (Lindsay *et al*., 1982).

In addition to the normally developing follicle, there are always degenerating follicles, atretic follicles, in the ovary. Atresia can affect all stages of the follicle development but particularly affect tertiary follicle. In the process of atresia, the ovum dies then the whole follicle collapse (Lindsay *et al*., 1982).

2.2.2 Oviduct

The oviducts also called fallopian tubes are a pair of convoluted tubes extending from the ovaries to and becoming continuous to the tips of the uterine horns. These tubes lie in a peritoneal fold derived from the lateral layer of the broad ligament. The oviducts average 12.4 cm long in calves, 20.4 cm in heifer, and 24.5 cm in grown cows. The maximum length of oviduct was found in 7 to 11 years old cows, with an approximately 20 to 35 cm as the range of oviduct length for cows (Salisbury *et al*., 1978). Lindsay *et al*. (1982) described the oviducts to consist of three distinct regions.

1. The fimbrium. The first of region is the fimbrium (infundibulum or funnel). It is a flared end of the fallopian tube with a trumpet-shaped structure. The paired fimbrias are extremely efficient in collecting ova shed from the ovary. In the experimental situation, ova which have been deposited into the body cavity are attracted by the fimbria and directed into the fallopian tube.

2. The ampulla. This is the body of tube, where fertilization of the ovum by the sperm take place. A paradox which has intrigued scientist for years is why the ovum is able to travel down the fallopian tube at the same time as sperm are able to travel up the tube. It is thought that there are specialized cells in the wall of the fallopian tube with minute hair like projections or cilia propel the ovum away from the ovary towards the uterus end of the tube. At the same time, secretions from the wall of the tube tend to flow towards the ovary at the end of the tube and out the fimbria. The sperm are thought to be too small to be affected by the beating of the cilia in the wall.

3. The isthmus. The third region of the fallopian tube which constitutes the uterine end. It becomes constricted at the point of connection with the uterus into a utero-tubal junction. This junction is subjected to the action of the hormones and by dilating or constricting, it can control the speed of the ovum passes from the fallopian tube into the uterus.

2.2.3 Uterus

The uterus extends from the utero-tubal junction to the cervix. In cow, it is a bicarbonate (two horned) structure. In the non pregnant animal, it length is normally from 35 cm to 60 cm. The major function of the uterus is retained and nourishes the embryo and foetus. Before the embryo attaches to the uterus, yolk within the embryo or from uterine milk which is secreted by glands in the mucosal layer of the uterus supply nutrient for the embryo. After attachment of the embryo to the uterus, nutrient and waste product are conveyed between maternal and embryonic and fetal blood by way of the placenta (Salisbury *et al*., 1978).

The walls of the uterus consist of three layers. There are serous membrane which lies the myometrium or muscular layer. The muscles of the uterus are capable of expansion and reformation. The uterus of non pregnant cow, for example, has a diameter of 5 cm or 6 cm. During gestation period, this small structure will expand to accommodate a calf which might attain a weight of 40/50 kg before parturition (Lindsay *et al*., 1982).

2.2.4 Cervix

Salisbury *et al*. (1978) described the cervix as a thick-walled portion of the reproductive tract which is 5 cm to 10 cm from its junction with the uterus to the exterior of the animal where it is continuous with the thin walled vagina. Its total thickness is about 3.0 cm to 4.4 cm. The muscle layers of the cervix are heavy, and mucosa form numerous thick folds or rings. Opposite fold tend to overlap, forming a spiral passageway through the cervix. This makes possible tight closure of the cervix with a cervical plug that is formed during pregnancy. Like the uterus the cervix is capable of enormous expansion. At parturition, the cervix dilates, allowing the calf and fetal membranes to pass out. The mucosa of the cervix is rich with mucus-secreting cells which secrete much of the mucus that passes through the vulva during estrus.

The cervix contains a large number of glands which under the influence of hormones, particularly estrogen, produces large quantity of mucus. The mucus produce in the cervix flows toward the exterior into the vagina. The quality and quantity of mucus exert an enormous influence on the fertility of female. Ideally, the mucus at the time of ovulation should be clear and somewhat ropey in appearance. The ropey strands are thought to act as guides for sperm swimming up the tract and, in fact, direct the sperm to the walls of the cervix whence these strands originate. The net result of this guiding of the sperm is the built up of semen storage in the cervix. During pregnancy and when animal is not on heat, the cervix is normally closed. In this way it acts as important barrier for the uterus against infection (Bearden and Fuquay, 2000).

2.2.5 Vagina

The vagina is tubular in shape, thin walled and quite elastic. The length isa 25 cm to 30 cm in the cow and semen is deposited in the anterior end of the vagina, near the opening to the cervix, during natural mating. The vagina is the female organ of copulation. During pregnancy, uterus drops into the abdominal cavity, and the length of vagina may be twice. Vagina also has a small opening, which is connected to the bladder for the disposal of urine from the body (Bearden and Fuquay, 2000).

2.2.6 Vulva

The vulva or external genital, consists of the vestibule, the labia, and the clitoris. The vestibule is common to both the reproductive and urinary systems (Bearden and Fuquay, 2000). It is from 10 cm to 12 cm in length in cow and mare, half that length in sow and one-quarter that length in ewe and doe (Salisbury *et al*., 1978). The labia consists of the labia minora, inner folds or lips of the vulva, and labia majora, outer folds or lips of the vulva. The labia minora is homologous to the prepuce (sheath) in the male and not prominent in farm animals. The labia majora, homologous to the scrotum in the male, which is visible externally in the female reproductive system. In the cow, the labia majora is covered with fine hair up to the mucosa. The clitoris, homologous to the glans penis in the male, is located ventrally and about 1.0 cm inside the labia. It contains erectile tissue and is well supplied with sensory nerves, which erects during estrus. It is not prominent enough to be used in estrus or detection in most species. Vestibular glands, is located in the posterior part of the vestibule, are actived during estrus and secretes a lubricating mucus. These glands give moist appearance to the vulva to the cow during estrus (Lindsay *et al*., 1982).

2.3 The Reproductive system of the bull

The reproductive system of the bull consists of the testicles and secondary sex organs, which transport the sperm from the testicle and eventually deposits them in the female reproductive tract. The male reproductive organs are the epididymis, vas deferens, penis, and three accessory sex glands, the seminal vesicles, prostate and Cowper's gland (Bearden and Fuquay, 2000). This basic anatomy is illustrated in Figure 2.2. An overview of the bull reproductive organs and major function of these organs are shown in Table 2.2.

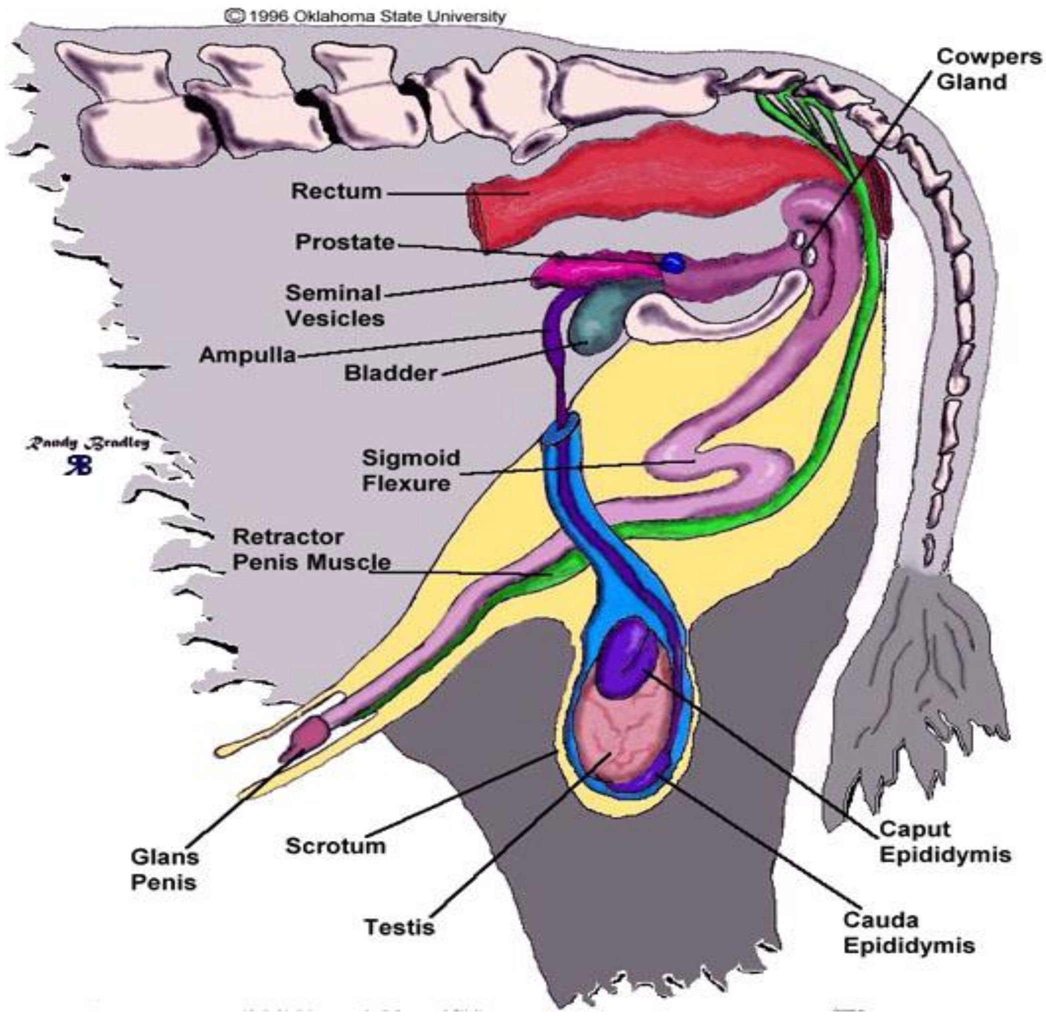

Figure 2.2 Reproductive system of bull (Source: Oklahoma State University, 1996)

Table 2.2 Reproductive organs of the male with their major function

Reproductive Organs	Function(s)
Testes	Production of sperm and androgens
Scrotum	Support and protect the testes, temperature control of the testes
Spermatic cord	Support of the testes, temperature control of the testes
Epididymis	Storage , maturation and transport of the sperm
Vas deferens	Transport of the sperm
Urethra	Transport of semen
Penis	Male organ of copulation
Prepuce	Encloses free end of the penis

(Source: Bearden and Fuquay, 2000)

2.3.1 Testes

The testes are the primary organs of reproduction in males (Bearden and Fuquay, 2000). Testes are considered as the primary sex organ because they produce male gametes (sperm) and male sex hormone (androgen). Testes differ from ovaries in that all potential gametes are not present at birth. Germ cells, located in the seminiferous tubules, undergo continual cell divisions, forming new sperm throughout the normal reproductive life of the male.

The testis of the bull is 10 cm to 13 cm long, 5 cm to 6.5 cm wide and weighs 300 gm to 400 gm. It is covered by a thin layer of elastic connective tissue, the tunica albuginia (Salisbury *et al*., 1978). The testis of bull is similar size with boars, but is smaller in rams, bucks, and stallions. Testes also differ from ovaries in that they do not remain in the body cavity. They descend from their site of origin, near the kidneys, down through the inguinal canals into the scrotum (Bearden and Fuquay, 2000).

2.3.2 Scrotum and spermatic cord

The scrotum is two-lobed sac which enclosed the testes. It is located in the inguinal region between the rear legs of most species. The scrotum has the same embryonic origin as the labia majora in the female. It is composed of an outer layer of think skin with numerous large sweat and sebaceous glands. This outer layer is lined with smooth muscle fibers, the tunica dartos, which is interspersed with connective tissue. The tunica dartos divides the scrotum into two pouches, and attached to the tunica vaginalis at the bottom of each pouch (Bearden and Fuquay, 2000).

The spermatic cord connects the testis to its life support mechanisms, the convoluted testicular arteries and surrounding venus plexus, and nerve trunks. In addition, the spermatic cord is composed of smooth muscle fibers, connective tissue, and a portion of the vas deferens. Both the spermatic cords and scrotum contribute to support the testes. Also, they have a joint function in regulating the temperature of the testes.

Salisbury *et al*. (1978) described the role of the scrotum and spermatic cord in temperature control of the testes which involved drawing of the testes away from the body as ambient temperature rises. Two smooth muscles are involved, the tunica darto, and the tunica vaginalis. The spermatic cord is sensitive to temperature. During cold weather, contraction of these muscles causes the scrotum to pucker and the spermatic cords to shorten, drawing the testes closer to the body. During hot weather, these muscles relax, permitting the scrotum to stretch and the spermatic cord to lengthen. Thus, the testes swing down away from the body. These muscles do not respond to changes in temperature until near the age of puberty. They are sensitized by testosterone to respond to the ambient temperature changes.

Actual cooling of testes occurs by two mechanisms. The skin of the scrotum has both sweat and sebaceous glands which are more active during hot weather.

Evaporation of the secretions from these glands cools the scrotum and thus the testes. The external scrotum has been observed to be 2^{o} C to 5^{o} C cooler than the temperature inside the testes. As the scrotum stretches during hot weather, more surface area is provided for cooling by evaporation. In addition, cooling of testis also occurs through heat exchange in the circulatory system. As arteries transporting blood at internal body temperature transcend the spermatic cord, their convoluted folds pass through a network of veins, the pampiniform venous plexus, transporting cooler blood back towards the heart. With this counter current mechanism, some cooling of arterial blood occurs before it reaches the testes. The lengthening of the cord during hot weather provides more surface area for this heat exchange (Bearden and Fuquay, 2000).

2.3.3 Epididymis

The Epididymis, the first external duct leading from the testis, is fused longitudinally to the surface of the testis and encased in the tunica vaginalis with the testis (Bearden and Fuquay, 2000). The single convoluted duct is covered with an extension of the tunica albuginea. The caput (head) of the epididymis is a flattened area at the apex of the testis, where 12 to 15 small ducts, the vasa efferentia, merge into a single duct. The corpus (body) extending along the longitudinal axis of the testis is a single duct which becomes continuous with the cauda (tail). The total length of this convoluted duct is about 34 meters in the bull and longer in the ram, boar, and stallion. The lumen of the cauda is wider than the lumen of the corpus. The structure of the epididymis and other external ducts (vas deferens and urethra) are similar to that of the tubular portion of the female tract. The tunica serosa (outer layer) is followed by a smooth muscle layer (middle) and an epithelial layer (innermost) (Salisbury *et al*., 1978).

The epididymis has a number of important functions in male reproductive physiology. First, it serves to transport sperm. In sexually active males, time involved in sperm transport is 1 to 9 days in boars, 13 to 15 days in rams, and 9 to 11 days in bulls. Frequent ejaculation has been reported to speed up sperm transport by 10 % to 20 % (Lindsay *et al*., 1982).

Secondly, the epididymis will increase sperm concentration. When sperm enters the epididymis from the testis of the bull or ram, sperm concentration increases about $4x10^9$ (4 billion) sperm per ml. Sperm concentration present as fluids, which suspend sperm in the testes, and the fluid are absorbed by the epithelial cells of the epididymis. Absorption of these fluids occurs principally in the caput and proximal end of the corpus (Salisbury *et al.,* 1978).

The third function of the epididymis is storage of sperm. Most are stored in the cauda of the epididymis where concentrated sperm are packed into the wide lumen. The epididymis of a mature bull may contain 50 to 74 billion sperm. Capacities of sperm in other species are not reported. Conditions are optimum in the cauda for preserving the viability of sperm for an extended period. Low pH, high viscosity, high carbon dioxide concentration, high potassium-to-sodium ratio, testosterone level, and probably other factors contribute to a lower metabolic rate and longevity life of sperm. If the epididymis is ligated to prevent entry of new sperm and removal of old, sperm remained alive and fertile for about 60 days. On the other hand, after a long period of sexual rest, the first few ejaculates may contain a high percentage of non-fertile sperm (Bearden and Fuquay, 2000).

The fourth function of the epididymis is maturation of sperm. When recently formed sperm enter the caput from the vasa efferentia, they have the ability of neither motility nor fertility. As the aperm pass through the epididymis, they gain the ability to be both motile and fertile. If the cauda is ligated at each end, fertilizing ability of the sperm nearest to the corpus increases in for up to 25 days. During the same period, sperm nearest to the vasa deferens exhibited reduce fertilizing ability. Therefore, it appears that sperm gain ability to be fertile in the cauda and start to age and deteriorate if they are not removed (Bearden and Fuquay, 2000)

2.3.4 Vas deferens and urethra

The vas deferens is a pair of ducts with one leading from the distal end of the cauda of each epididymis. Initially, supported by folds of the peritoneum, pass along the spermatic cord, through the inguinal canal to the pelvic region, where it merges with the urethra near the opening to the bladder (Bearden and Fuquay, 2000). The wall of vas deferens has a thick layer of smooth muscles and appears to have single function of sperm transport. The enlarged end of the vas deferens near the urethra is the *ampulla*. Researchers have suggested that ampullae serve as a short term storage depot for semen. However, sperm aged quickly in the ampullae. It seems more likely that sperm may pool in the ampullae during ejaculation before being expelled into the urethra (Salisbury *et al*., 1978).

The urethra is a single duct which extends from the junction of the ampullae to the end of the penis. It serves as an excretory duct for both urine and semen. During ejaculation in bull and ram, there is a complete mixing of sperm with fluids from the accessory glands. However in boars, mixing is not as complete. The ejaculate may contain sperm-free and sperm-rich segments (Bearden and Fuquay, 2000). .

2.3.5 Penis

The penis is an organ of copulation in males. It forms dorsally around the urethra from the point where the urethra leaves the pelvis, with the external urethral orifice at the free end of the penis (Bearden and Fuquay, 2000). The penis of the adult bull is about 1 m long and 2.5 cm in diameter. It is cylindrical and tapers somewhat from the root to the free end. It has very little erectile tissue, except in the root, thus it becomes more rigid does not enlarge much during erection (Salisbury *et al*., 1978).

Bulls, boars, and rams have a sigmoid flexure, an S-shaped bend in the penis which permits complete retraction into the body. These three species and the stallion have retractor penis muscles, a pair of smooth muscles which will relax to permit extension of the penis and contract to draw the penis back into the body. These retractor penis muscles arise from the vertebrae in the coccygeal region and fuse to the ventral penis just anterior to the sigmoid flexure. The glans penis, the free end of the penis, is well supplied with sensory nerves and homologous to the clitoris in the female. In most species, the penis is fibroelastic, containing small amounts of erectile tissue (Bearden and Fuquay, 2000).

2.3.6 Prepuce

The prepuce (sheath) is an invagination of skin which completely enclosed the free end of the penis. It has the same embryonic origin as the labia minora in the female. It can be divided into a prepenile portion, which is the outer fold, and the penile portion, or the inner folds. The orifice of the prepuce is surrounded by long and tough preputial hairs (Bearden and Fuquay, 2000).

2.4 Regulation of reproduction in cow

2.4.1 Estrus cycle

The estrus cycle is defined as time between periods of estrus. Cows come into estrus approximately every 21 days (range 18 to 24 days) during which time they are receptive to the bull (Bearden and Fuquay, 2000). Estrus lasts 18 hours in cows and only 6 to 12 hours in heifers. The best time to inseminate the cow is 12 to 24 hours after the onset of estrus, that is, towards the end or just after the end of estrus. In practice, if first estrus is observed in the morning, insemination is carried out in the afternoon.

However, if the first estrus is observed in the afternoon, insemination is carried out on the following morning (Walker *et al*., 1994).

2.4.2 Puberty

Traditional definition of puberty is the process of acquiring reproductive competence and/or the ability to accomplish reproduction successfully (Senger, 2003). Specifically, the physiological onset of puberty may be defined as the stage of development when the female first expresses estrus and ovulates (Bearden and Fuquay, 2000). It should not be considered as sexually matured. If animals are bred at puberty, a high percentage will have difficulty with parturition. Most breeds of sheep will reach puberty when they are 40% to 50% of their mature weight, but breeding is recommended until they are about 65% of their mature weight. Dairy cows reach puberty at 35% to 45% of their mature weight but breeding is not recommended until they are about 55% of their mature weight (Bearden and Fuquay, 2000). Puberty occurs when gonadotropins (FSH and LH) are produced at high enough levels to initiate follicle growth, oocyte maturation, and ovulation. Follicle growth can be detected several months before puberty. As puberty approaches, pulse-like releases of gonadotropins become more frequent and of higher amplitude. When they approach adult levels, they stimulate resumption of oocyte maturation and ovulation occurs (Hafez and Hafez, 2000).

Puberty is affected by genetics, age and environmental factors (Taylor, 1994). Genetic factors can be seen by comparing of breeds within a species. An average age of females at puberty is 4 to 7 months for sows, 5 to 7 months for does, 6 to 9 months for ewes, 8 to 11 months for European-type dairy cows, 10 to 15 months for European-type beef cows, 17 to 27 months for Zebu-type cows, and 15 to 24 months for mares. Weight of females at puberty for breeds within a given species is depended on the mature size of the breed in question. Jerseys reach puberty at about 8 months old with the weight of

160 kg, while Holsteins, at 11 months old with the weight of 270 kg (Bearden and Fuquay, 2000).

2.4.3 Period of the estrus cycle

The estrus cycle can be divided into four period according to certain visible and invisible changes that occur during the cycle: estrus, metestrus, diestrus, and proestrus (Table 2.3) (Salisbury *et al*., 1978). This period occurs in cyclic and sequential manner, except for anestrus (absence of cycling) in seasonal breeding, during pregnancy and in the early postpartum period (Bearden and Fuquay, 2000).

Table 2.3 Primary characteristic of the periods of the estrus cycle in the cow

Period	**Day(s)**	**Principle Features**
Estrus	1	Behavioral sign of estrus ovulation
Metestrus	2-4	Corpus luteum formation
Diestrus	5-16	Corpus luteum function
Proestrus	17-21	Rapid follicle growth

(Source: Bearden and Fuquay, 2000)

1. Proestrus

   The proestrus period occurs about 2 to 3 days prior to the onset of estrus in the cow and is characterized by the follicular growth and estrogen (estradiol) production. Rising level of estrogen causes the blood supply to the reproductive tract to increase resulting in swelling of the entire tract. Glands of the cervix and vagina are stimulated to increase secretory activities yielding a thin vaginal discharge.

2. Estrus

   Estrus is a period of sexual desire at the end of proestrus. These behavioral manifestations are a result of estrogen acting on the central nervous system. During this time, the cow becomes very restless, may shout, lose appetite, and drop in milk production. Blood supply to the reproductive tract increases and glandular secretion is stimulated, yielding a clear viscid mucus from the vulva. After about 14 to 18 hours, these clinical signs of estrus begin to disappear.

The cow is different from most other animals because of very short period of sexual receptivity that is ready to accept the bull or to be mounted by the bulls. Further, cows do not ovulate until 12 to 16 hours after the end of estrus. Consequently, signs of estrus activities in cows can be easily missed, unless the signs are observed frequently.

3. Metestrus

Metestrus is a period immediately after estrus (the period of sexual receptivity and activity) is metestrus. The cow does not ovulate until after she goes out of estrus. Therefore, metestrus is the period in which ovulation occurs. As the egg is released from the follicle (the process of ovulation), blood and luteal cells fill the follicular cavity and begin to organize and develop to form the corpus luteum (CL). Metestrus lasts only about 2 to 3 days. It is not uncommon to observe a blood stained mucus discharge during this period. It results from blood engorgement of the reproductive tract tissues which occurs during estrus. It indicates that the cow is likely on heat in the last1 or 2 days.

During this time, the egg is being gathered in the infundibulum of the oviduct and travels toward the uterus (roughly 5 to 7 days). Fertilization (union of the sperm and egg) will occur in the upper 1/3 to 1/2 of the oviduct.

4. Diestrus

This is the lengthiest period in the estrus cycle. Diestrus is the period when corpus luteum (CL) starts to function. Formation of a CL begins immediately after ovulation. Whether the cow is pregnant or not, the CL continues to develop into a fully functional organ and secretes large amounts of progesterone. If a fertilized egg reaches the uterus, the CL will be maintained throughout the pregnancy. On the other hand, if the egg which reaches the uterus is not fertilized, the CL will remain functional only up to about 17 days or 18 days after which time it will degenerate thereby initiating a new estrus cycle.

In summary, the estrus cycle can be divided into four phases: the estrogenic phases (proestrus and estrus) and the progestational phases (metestrus and diestrus). Estrogen dominates only about 4 days of the 21 day cycle, whereas, progesterone dominates about 17 days. A fundamental understanding of the cow's reproductive cycle is essential for reproductive management. It is the basis for understanding where, when, and how to apply a technology such as milk progesterone assay (Table 2.4).

Table 2.4 Summary of timing of events in the estrus cycle (day of estrus cycle)

Events	Cow	Ewe	Sow	Mare
Proestrus	0-1	0-1	0-2	0-12
Oestrus	1-3	1-2	2-4	12-14
Metestrus	4-16	4-14	4-15	14-26
Diestrus	17-21	15-17	15-21	26-28

(Source: Bearden and Fuquay, 2000)

2.4.4 Hormonal control of the estrus cycle

Regulation of the estrus cycle involves a reciprocal interaction between reproductive hormones of the hypothalamus, anterior pituitary, and ovaries. An interaction between the uterus and ovaries is also important. Prostaglandin (PGF2α) from uterus is the natural luteolysin that causes regression of the corpus luteum and cessation of progesterone production. Removal of the uterus during diestrus will greatly prolong the life of the corpus luteum and lengthen the estrus cycle (Bearden and Fuquay, 2000).

Concentrations of gonadotropins and ovarian steroids have been monitored in a cow through out the estrus cycle (Figure 2.3). Similarities are more marked than differences when these species are compared. Progesterone concentrations are high during diestrus. Drop in progesterone level indicates the start of proestrus which followes by dramatic surges in this hormone near the start of estrus. Small surges of FSH and estradiol are observed again in metestrus and mid-diestrus. A surge in prolactin occurs in late estrus. With a knowledge of the circulating concentrations of these hormones during the estrus cycle with an understanding of the mechanisms of their release, how their receptors are regulated, and their physiological actions, logical sequence for hormonal regulation of the estrus cycle can be set fort (Bearden and Fuquay, 2000).

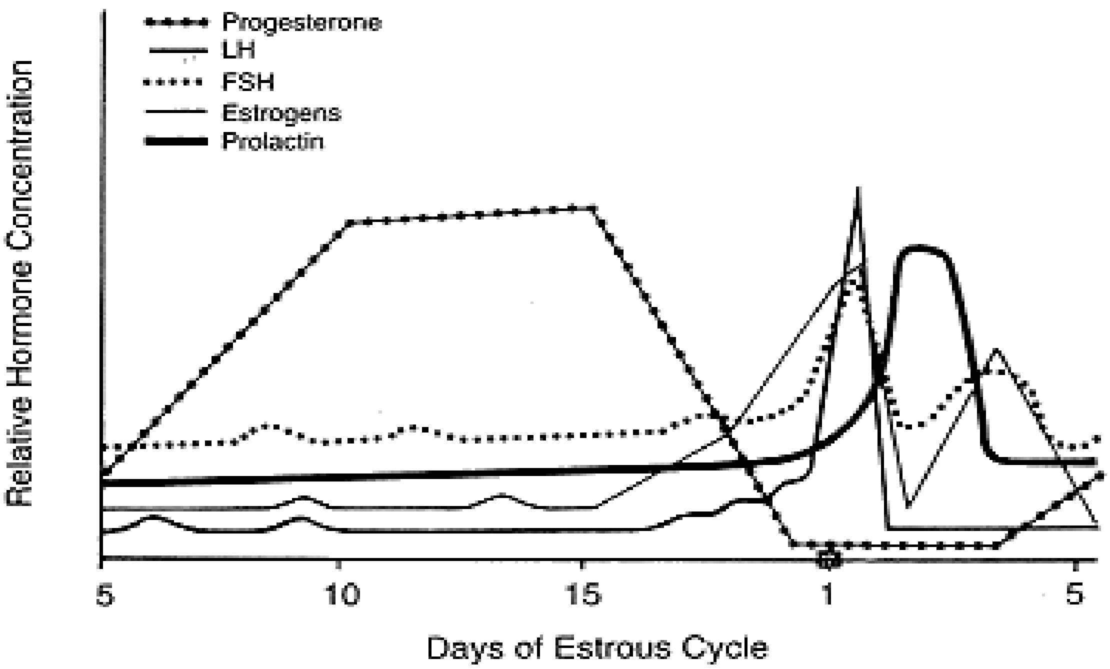

Figure 2.3 Hormonal changes in the peripheral plasma during estrus cycle of cow (Source: Bearden and Fuquay, 2000)

Progesterone has a dominant role in regulating the estrus cycle. During diestrus with the corpus luteum functioning, high concentrations of progesterone inhibit the release of FSH and LH through its negative feedback control of the hypothalamus and anterior pituitary. High concentration of progesterone also inhibits behavioral estrus. Likewise, during pregnancy, high concentrations of progesterone inhibit the release of gonadotropic hormones as well as behavioral estrus. Small episodic increases in LH that occurs during diestrus may be a factor in maintaining the function of the corpus luteum. Mid-diestrus increases the follicle growth and estrogen level, preceded by an increase in FSH, which is small when compared to the changes that occur during estrus. If females do not become pregnant during the preceding estrus, PGF2α will be released from the uterus and transported to the ovary. PGF2α causes regression of the corpus luteum 10 to 14 days after its formation (Lindsay *et al*., 1982).

Drop in progesterone level inhibits the hypothalamus from its negative feedback. With the removal of this inhibition, pulses of GnRH, initiate the released of FSH which stimulates rapid follicle growth and increases the secretion of estradiol.

The sensitivity of the anterior pituitary to GnRH increases through up-regulation of the GnRH receptors by the more frequent pulses of GnRH. Likewise, the increasing concentrations of FSH and estradiol will up-regulate ovarian receptors for FSH and LH. Increased released of FSH and LH may not be reflected in circulating blood, due to increase binding of these hormones to receptors in granulose and the follicle cells (Bearden and Fuquay, 2000).

Two to three days after the drop in progesterone, estradiol reaches the threshold concentration which stimulates (through a positive feedback control on the hypothalamus) preovulatory surge of GnRH, FSH, and LH. The preovulatory surge of FSH stimulates more rapid growth of the follicle and greater secrection of estradiol. This high concentration of estradiol is necessary for the female to exhibit behavioral signs of estrus. Stop of estradiol inhibition likely modulates the release of FSH during estrus, thereby preventing overstimulation of the ovaries. Inhibits effect on FSH may also be a factor in the atreia of follicles that growing but do not reach maturity necessary for ovulation. The preovulatory surge of LH stimulates final maturation of the oocyte and ovulation. The preovulatory surge of LH occurs during early estrus and lasts for 6 to 10 hours in most species. However, in mare, the surge may last for several days. Ovulation follows the preovulatory LH surge about 24 to 30 hours in cows and ewes, 30 to 36 hours in does, and 40 to 45 hours in sows (Bearden and Fuquay, 2000).

2.5 Estrus synchronization

Estrus synchronization involves controlling or manipulating the estrus cycle so that females in a herd express estrus at approximately the same time (Taylor, 1994). Synchronization of estrus in cattle not only allow one to predict the time of estrus with reasonable accuracy but also help the management to plan and organize a proper breeding programme. Synchronization of estrus also reduces the time required for detection of estrus as well as facilitates for timed artificial insemination without detection of estrus (Wahid *et al*., 2001). Other benefit from estrus synchronization include: a) a potential for faster genetic improvement by breeding to genetically superior sires, resulting in more productive calves, b) the breeding season could be shortened by several weeks, c) a potential to shorten calving period and d) a more uniform calf crop (Gordon, 2004).

Experiments on estrus synchronization in cattle started in 1940. The most frequent means of attempting to synchronize estrus in cattle has involved treatment with some form of progesterone in order to mimic the activity of corpus luteum. Synchronization involves injection of progesterone, feeding synthetic form of progesterone (oral progesterone), implanting silicone rubber capsule of progesterone under the skin, or inserting intra-vaginal sponges or coils containing progesterone (Hunter, 1982).

Synchronization is to control both luteal and follicular growth. There are two methods to control the length of the estrus cycle: 1) administration of $PGF_{2\alpha}$, causing a regression of the corpus luteum (CL) thus shortening the cycle and 2) administration of progesterone to delay the onset of estrus following either natural or induced luteolysis. The next approach in ovulation control is to manipulate the ovulatory follicle by administering GnRH, which should causes ovulation of the dominant follicle (Peeler *et al*., 2004).

There are several protocols available for synchronizing estrus in cows. Traditional protocols are designed to mimic or control the corpus luteum on the ovary. New protocols have been designed to control ovulation and/or the follicular waves that occur in the ovary during the 21 days of estrus cycle. There are three primary groups of products used to synchronize estrus or ovulation in cattle: prostaglandins, progestins, and gonadotropins. Prostaglandin products have the trade names of Lutalyse, Estrumate, and in-synch. Each of the product contains prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or an analogue of $PGF_{2\alpha}$. The progestin products include the vaginal implant CIDR® and Melengestrol Acetate (MGA), that is consumed orally. The GnRH products are Cystrorelin, Factrel, and Fertagyl (Table 2.5).

There are problems related to estrus synchronization; 1) some cows or heifers were already pregnant and $PGF_{2\alpha}$ will cause abortion when administered to pregnant female; 2) synchronization should never be attempted until there is evidence that the cows have passed the postpartum anestrus period; 3) ensure the heifers are not in prepuberal anestrus (Bearden and Fuquay, 2000).

Table 2.5 Three primary products to synchronize estrus in cows

Product	Commercial Name	Administration	Dose
Prostaglandins:	Lutalyse®	i.m. injection	5 ml
	Estrumatc®	i.m. injection	2 ml
	In-synch®	i.m. injection	5 ml
	Prostamate®	i.m. injection	5 ml
Progestins:	Melengestrol Acetat	Feed	0,5 mg/hd/d
	CIDR	Vagina Implant	1 implant
GnRH	Cystorelin®	i.m. injection	2 ml
	Factrel®	i.m. injection	2 ml
	Fertagyl®	i.m. injection	2 ml
	Ovacyst™	i.m. injection	2 ml

(Source: Lamb, 2005, i.m: intra muscular)

2.5.1 Controlled internal drug release (CIDR)

The CIDR is an intravaginal progesterone insert, used in conjunction with other hormones to synchronize estrus in cows. This device was developed at the Ruakura Agricultural Research Center by the Agricultural Division of the Carter Holt Harvey Plastic Products Group Ltd., which is located in Hamilton, New Zealand in early 1990s. This device has been used for several years to advance the first pubertal estrus in heifer and postpartum estrus in cows. The CIDR also has recently been approved in Canada (Bioniche Animal Health, Belleville, ON, Canada) and United States (Pharmacia Animal Health, Kalamazoo, MI) for synchronization of estrus in beef cattle and dairy heifers **(**Mapletoft *et al*., 2003). The CIDR is a T-shaped vaginal insert containing 1.9 g of progesterone (Canada) or 1.38 g of progesterone (United States) in silicon molded over a nylon spine. The CIDR is inserted into the vagina by a specialized applicator (Macmillan & Petteson, 1993) that collapses the wings for insertion; expulsion of the CIDR causes the wings to straighten, which confers retention by pressure on the vaginal wall. This device is used to induce estrus activity in anestrus cows and prepubertal heifers (Lucy *et al*., 2001). It is also used to synchronize the return to estrus in previously inseminated cattle (Macmillan and Peterson, 1993; Chenault *et al*., 2003).

The CIDR releases progesterone at a controlled rate into the cow's bloodstream. It consists of a plastic core coated with silicon that contains progesterone. The progesterone is absorbed through the vaginal wall and the implant acts as a substitute of corpus luteum. Other hormones may be administered at the time of insertion or on its removal to increase conception rate and estrus response. The use of CIDR devices, along with other hormones that are already on the market (e.g., gonadotropin releasing hormone) has permitted fixed-time artificial insemination with high pregnancy rates in beef cattle (Lamb, 2005).

The major advantages of the CIDR include easier of application and removal of the device. Cows need to be restrained in a squeeze chute, but it is not necessary to catch their heads prior to CIDR insertion. On the other hand, to remove the CIDR, cattle can either be loaded into the alleyway leading into the squeeze chute or restrained in a squeeze chute. The CIDR is removed by pulling the nylon tail that is exposed from the reproductive tract. After CIDR removal, cattle are also injected with $PGF_{2\alpha}$ to initiate regression of any corpus luteum (CL) that may be present in the ovaries. Since there is really no need to catch cattle in the squeeze chute to remove the CIDR, stress associated with handling cattle is considerably reduced. Retention rates of the CIDR are high with less than approximately 1% of the postpartum beef cows losing their inserted/implanted CIDR (Yelich, 2002).

2.6 Factor affecting the successful estrus synchronization in cows

Factors that affect the success of cows to CIDR based estrus synchronization are body condition, parity, and days since calving, facilities, labor and selection of proper methods to estrus synchronization. In farm management, these factors, can reduce the incidence of anestrus or unovulation (Lamb, 2005).

2.6.1 Body condition score (BCS)

Body condition score (BCS) is a reflection of the immediate past and current nutritional status of both female cow and calf performances (Lamb, 2005). Poor body condition is associated with increase post-partum interval, weak calves at birth, low quality and quantity of colostrum, reduce milk production, increase dystocia and lower weaning weights. Increasing post-partum interval will result in a younger and smaller calf at weaning which will give lower incomes if the calf was sold at weaning. Weak calves at birth may not get adequate colostrum and more susceptible to disease, reduce weaning weights, reduce feedlot performance and less desirable carcass traits. Research clearly shows that cows in moderate body condition will have a shorter interval from calving to first estrus than cows in thin condition. This supports the conclusion that BCS is one of the most important factors in determining subsequent reproductive performance (Lamb, 2005). To properly evaluate body condition for cattle, an observer must be familiar with its skeletal structures, muscle and fat positioning. Although there are several methods available to determine body composition, many cattle producers use a scoring system based on body weight chage. Richards *et al*. (1986) used a scale of 1 to 9, with 1 being emaciated and 9 being obese (Table 2.6). A linear relationship exists between body weight change and body condition score (using a 1 to 9 scale), where approximately 90/lb weights change is associated with one unit change in BCS.

Table 2.6 Description of body condition scores (BCS)

Body Condition Score (BCS)	Detailed Description
1	Clearly defined bone structure of shoulder, ribs, back, hooks and pins are easily visible. Little muscle tissue or fat is present (Emaciated).
2	Small amount of muscle in the hindquarters. Fat is present, but not abundant. Space between spinous process is easily seen (poor).
3	Fat begins to cover loin, back and fore ribs. Upper skeletal structures are visible. Spinous process is easily identified (Thin).
4	Fore ribs becoming less noticeable. The transverse spinous process can be identified by palpation. Fat and muscle tissue are not abundant, but increasing in fullness (Borderline).
5	Ribs are visible only when the animal has been shrunk. Processes not visible. Each side of the tail head is filled, but not mounded (Moderate).
6	Ribs are not noticeable to the eye. Muscle in hindquarters is plump and full. Fat around tail head and covering the foreribs (Optimum).
7	Spinous process can only be felt with firm pressure. Fat cover is abundance on either side of tail head (Optimum).
8	Animal is smooth and blocky appearance; bone structure is difficult to identify. Fat cover is abundant (Fat).
9	Structures are difficult to identify. Fat cover is excessive and mobility may be impaired (Extremely fat).

(Source: Richards *et al*., 1986)

2.6.2 Record keeping

Maintaining a good record keeping system is a key to success in any reproductive management system ((Lamb, 2005). For estrus synchronization to success, producers need to know when the cows calved, whether the cow had a difficult birth, and birth weight of all calves. The best protocol to start synchronization is when cows are more than 45 days after calving. Without accurate records, these decisions can be extremely subjective.

2.6.3 Parity

Two years old (primiparous) cows require more time to initiate cycling activity than older (multiparous) cows, even if they calved before multiporous cows. This is due to their greater energy needs, burden to sustain lactation and their own growth, which have greater energy priority than the onset of reproductive estrus cycle. The cow's first priority is body function maintenance to preserve life. Once that maintenance requirement is met, remaining nutrients will accommodate her own growth. Finally, lactation and the initiation of estrus cycles are supported. Older cows have no growth requirement, thus nutrients are more likely prioritized for milk synthesis and initiation of estrus cycles. Due to this priority system, young, growing cows generally produce less milk (Lamb, 2005).

2.6.4 Days of postpartum

Stevenson *et al*. (2003) stated that cows began their estrus cycles when the interval between calving and the onset of estrus synchronization were longer. The proportion of cows cycling increases in a curvilinear fashion across the day of postpartum (Figure 2.4). The proportion of cows initiating estrus cycles increased by approximately 7 % for every 10 days interval from calving. Thus, more cows had initiated estrus cycles when they had longer periods of time between calving and the breeding season (Anderson, 2010).

The influence of time on estrus cyclicity on the first day of the breeding season differed between primiparous and multiparous cows. For primiparous cows, the percentage of cows cycling at the onset of the breeding season did not differ among the first three of 21-day calving intervals (Figure 4) but was lower for those very late-calving primiparous females. In contrast, estrus cyclicity was higher for multiparous cows calving in the first 6 weeks of the calving season (> 50%) compared with those that calved after 6 weeks (< 40%) (Stevenson *et al*, 2003).

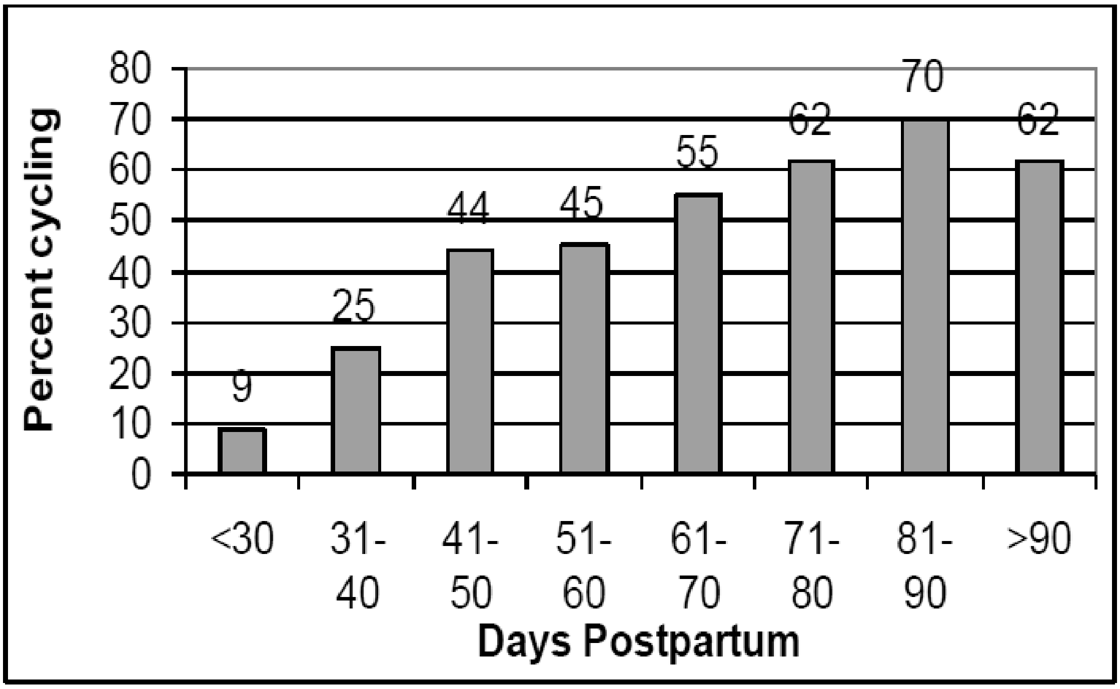

Figure 2.4 Proportion of suckled cows that were cycling on first day of breeding season (Source: Stevenson *et al*., 2003)

2.6.5 Facilities

Availability of good facilities is one of the factors to ensure the successful estrus synchronization program. After estrus synchronization more cows were expected to come on heat at a single time than without synchronization. Cows need to be pushed through the chute for injection. Therefore, working facilities need to be able to accommodate the extra work. It not only should have reliable holding and sorting pens, but also a good alley and chute system (Lamb, 2005).

2.6.6 Labour

Reliable labour is an issue that many people neglect to consider when planning a synchronization program. Heat detection is important for the success of synchronization program. Therefore any labour associated with this process needs to know exactly how cows act when they are on heat. In many cases, this is one of the reasons why many programs failed.

2.6.7 Other Factors

Many more factors need to be considered, such as employing a proficient AI technician. Regardless of the system, the producers must follow the right procedures, or example the drugs to be administered other examples.

2.7 Artificial insemination (AI)

Artificial insemination is a process by which sperm are collected from the male, processed, stored and artificially introduced into the female reproductive tract for the purpose of conception (Bearden and Fuquay, 2000). Artificial insemination has become one of the most important technique ever devised for the genetic improvement of farm animals. It has been most widely used for breeding cattle and provide bulls of high genetic merit available to all (Webb, 2003).

2.7.1 Advantages and disadvantages of artificial insemination (AI)

The greatest advantage of artificial insemination (AI) is that it makes possible maximum use of superior sires. Natural service would probably limit the use of one bull to less than 100 mating per year. In 1968, AI usage enabled one dairy sire to provide semen for more than 60,000 services. Exposure of sires to infectious genital diseases is prevented through artificial insemination which reduces the danger of spreading such diseases. Time required to establish a reliable proof on young fertility bulls is reduced through the use of artificial insemination. Other advantages include early detection of infertile bulls, avoid the use of old or crippled bulls and eliminate the danger of wild bulls handling (Webb, 2003).

There are a few disadvantages of artificial insemination, which can be overcome through proper management. A human detection of heat is required. Success or failure of AI depends on how well this task is performed. Artificial insemination requires more labor, some special facilities for corralling and insemination are required as well as managerial skill (Bearden and Fuquay, 2000).

Proper implementation of AI requires special training, skill and practice. The AI industry and dairy cattle breeders should make every effort to sample as many young sires as possible (Webb, 2003).

2.7.2 Artificial insemination techniques

The technique of inseminating a cow is a skill that requires adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Semen must be deposited within the tract of the cow at the best location and time to obtain acceptable conception rates (Webb, 2003).

Early method of AI involved deposition of the semen in the vagina, as would occur in natural mating. This method was not satisfactory because resulted in low. Fertility required greater numbers of sperm (Salisbury *et al.*, 1978). Another method which gained popularity was the "speculum" method or cervical insemination (Bearden and Fuquay 2000). This method was easily learned, but proper cleaning and sterilizing of the equipment was necessary, making it more impractical to inseminate than the rectovaginal technique which recently is the most widely used AI method (Webb, 2003).

In the rectovaginal technique, a sterile disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix into the uterus. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is withdrawn. Expulsion of the semen should be slowly accomplished and deliberately to avoid excessive sperm loss in the catheter. The body of the uterus is short; therefore, care should be taken not to penetrate too deeply which might cause physical injury. In animals previously inseminated animals, the catheter should not be forced through the cervix because possibility of pregnancy. Since research data showed little variation in conception rates when semen was placed in the cervix, uterine body or uterine horns, some people recommend incomplete penetration of the cervical canal and deposition of semen in the cervix (Bearden and Fuquay, 2000).

The rectovaginal technique is more difficult to learn and practice is essential for acceptable proficiency. The advantages of this method make insemination more desirable than other known methods. With practice, the skillful technician soon learns to thread the cervix over the catheter with ease. If disposable catheters are used and proper sanitation measures are followed, there is little chance of infection being carried between the cows (Webb, 2003).

2.8 Pregnancy diagnosis

The most popular methods currently employed for early pregnancy diagnosis in cattle are rectal palpation, ultrasonography and hormone or pregnancy-specific antigen analysis (mainly progesterone, pregnancy-specific antigens, and oestrone sulphate).

However, all these pregnancy diagnostic techniques have their own advantages and disadvantages with regard to the time interval of diagnosis after service, diagnosis accuracy and efficiency, and effect on embryonic/foetal losses (Jainudeen and Hafez, 2000).

Jainudeen and Hafez, (2000) suggested that an early diagnosis of pregnancy is required for the following purposes:

a) To identify non pregnant animal as soon as possible after mating or artificial insemination so that production time lost from infertility may be reduced by appropriate treatment or culling.
b) To certify animal for sale or insurance purpose.
c) To reduce waste in breeding program using expensive hormonal technique.
d) To assist in economic management of livestock.

2.8.1 Rectal palpation

Transrectal palpation of the uterus for pregnancy diagnosis in cattle was first described in the 1800's (Cowie, 1948). This is the oldest and most widely used method for early pregnancy diagnosis in dairy cattle today. Manual transrectal palpation of the reproductive tract approximately 35 days post service is commonly used to determine pregnancy status (Franco *et al.*, 1987; Pieterse *et al.*, 1990).

The accuracy of this method depends on the experience of the operator and the criteria that are used: fluctuation of the uterus as a result of the presence of foetal fluids, identification of the amniotic vesicle, and slipping of the chorio–allantoic membrane. However, it has been suggested that a positive diagnosis of pregnancy can be made as early as 27 days post service in some animals by palpating and identifying the amniotic vesicle. Pregnancies of greater than 45 days' duration can be determined by detecting the foetal membrane “slip”, cotyledons, or the foetus itself (Zemjanis, 1970; Paisley *et al*., 1978).

Rectal palpation has also been performed very early to predict the outcomes of pregnancy. Paisley *et al*. (1978) suggested that upon rectal palpation, finding a quiescent uterus and a fully developed corpus luteum in an animal serviced 20–22 days previously is highly suggested that the animal has conceived. It was suggested that this method could be 85 to 90% accurate at predicting the outcome of breeding. The more criteria included in the pregnancy diagnosis, the greater the accuracy of predicting the breeding outcome. However, such accuracy is accompanied by a significant increase in embryo/foetal mortality (Franco *et al*., 1987; Pieterse *et al*., 1990). Based on breeding and calving records, palpation per rectum 35 days after post insemination proved to be 99% accurate in diagnosing pregnancy and non-pregnancy (Badtram *et al*., 1991).

One of the commonly indicated disadvantages of rectal palpation is the risk of inducing embryo/foetal losses. However, the effect of manual rectal palpation on the incidence rates of induced embryo/foetal losses varies according to different reports. For example, the rate of induced foetal death due to pregnancy diagnosis by rectal palpation per rectum between day 42 and 46 was estimated to be 11.8% based on milk progesterone content at day 63, or 9.5% at day 90 (Franco *et al*., 1987). Moreover, it was observed that there were significant differences between palpators in the induction of foetal attrition (Abbitt *et al*., 1978; Franco *et al*., 1987). On the other hand, the incidence of prenatal mortality following pregnancy diagnosis by rectal palpation (done by clinicians and senior veterinary students) was 5.8%, 6.0%, and 0.8% or 802 pregnancies diagnosed at less than 35 days, between 35–45 days and over 45 days, respectively (Paisley *et al.*, 1978). It was concluded that multiple palpations may contribute to foetal losses, but the incidence was not high enough to justify changing technique or avoid the rectal palpation by veterinary students.

2.8.2 Ultrasonography

Ultrasonic imaging technology allows non-invasive visualization of the reproductive tract and has allowed the study of dynamic interactions (Pierson and Ginther, 1988). Accurate pregnancy diagnosis could be achieved based on the recognition of a proper embryo with a beating heart, at between 26 and 34 days, by using ultrasonography (Pierson and Ginther, 1984; Pieterse *et al*., 1990). Twenty Under controlled condition in heifers, the conceptus (embryonic vesicle) was first detected as a discrete nonechogenic (black) oblong structure in the uterine horn pisilateral to the corpus luteum between 12 to 14 days post ovulation using a high quality diagnostic ultrasound (Pierson and Ginther, 1984). Kastelic *et al*. (1991) reported that the first detection of the embryo and heartbeat was possible as of 21.4 days, on average (range 19–27 days), post ovulation in 58 heifers. A considerable difference was noted on the reliability of ultrasound examinations performed under field conditions between an early stage (days 21 to 25) and of those performed at a later stage (days 26–33). The sensitivity and specificity of the ultrasound examinations between day 21 and 25 were 44.8% and 82.3%, respectively. However, between day 26 and 33, the results were 97.7% and 87.8%, respectively (Pieterse *et al*., 1990). On the other hand, Badtram *et al*. (1991) reported that the diagnostic accuracy and sensitivity of ultrasound examinations carried out by two operators were 50% and 25% between day 16 and 22 post insemination, versus 70.2% and 68.8% between day 23 and 31, respectively.

Pieterse *et al.* (1990) concluded that pregnancy diagnosis in the cow before 30 days post service using a real-time B-mode ultrasound with a 5-MHz probe was not highly accurate under field conditions. On the other hand, one of the most profound clinical and research applications of ultrasonography in cattle involves the detection of embryonic death and monitoring the internal physical condition (Pierson and Ginther, 1984; Kastelic *et al*., 1991). Kastelic *et al*. (1991) observed an apparent reduction in embryonic heart rate prior to embryonic death in heifers. They suggested that indications of embryonic distress or of impending death would be obtained by monitoring embryonic heart beat. For research purposes this would allow, determination of the precise time of embryo death following a given treatment. Thus, diagnostic ultrasound is a powerful tool for monitoring embryo/foetal viability in cattle.