

## Animal Husbandry

### Artificial Insemination in cattle:

Artificial insemination is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring. In this process, the semen is inseminated into the female by placing a portion of it either in a collected or diluted form into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions. The first scientific research in artificial insemination of domestic animals was performed on dogs in 1780 by the Italian scientist, Lazanno Spalbanzani. His experiments proved that the fertilizing power reside in the spermatozoa and not in the liquid portion of semen. Few further studies under research station conditions helped this technique to be used commercially all over the world including India.

Artificial insemination is not merely a novel method of bringing about impregnation in females. Instead, it is a powerful tool mostly employed for livestock improvement. In artificial insemination the germplasm of the bulls of superior quality can be effectively utilized with the least regard for their location in far away places. By adoption of artificial insemination, there would be considerable reduction in both genital and non-genital diseases in the farm stock.

### **Advantages and disadvantages:**

Artificial insemination (A.I.) is deposition of semen into the female genital tract by means of instruments.

### **ADVANTAGES OF ARTIFICIAL INSEMINATION:**

There are several advantages by artificial insemination over natural mating or servicing.

- There is no need of maintenance of breeding bull for a herd; hence the cost of maintenance of breeding bull is saved.
- It prevents the spread of certain diseases and sterility due to genital diseases.
- Eg: contagious abortion, vibriosis.

- By regular examination of semen after collection and frequent checking on fertility make early detection of inferior males and better breeding efficiency is ensured.
- The progeny testing can be done at an early age.
- The semen of a desired size can be used even after the death of that particular sire.
- The semen collected can be taken to the urban areas or rural areas for insemination.
- It makes possible the mating of animals with great differences in size without injury to either of the animal.
- It is helpful to inseminate the animals that are refuse to stand or accept the male at the time of oestrus.
- It helps in maintaining the accurate breeding and culling records.
- It increases the rate of conception.
- It helps in better record keeping.
- Old, heavy and injured sires can be used.

#### **Disadvantages of A.I:**

- Requires well-trained operators and special equipment.
- Requires more time than natural services.
- Necessitates the knowledge of the structure and function of reproduction on the part of operator.
- Improper cleaning of instruments and in sanitary conditions may lead to lower fertility.
- If the bull is not properly tested, the spreading of genital diseases will be increased.
- Market for bulls will be reduced, while that for superior bull is increased.

#### **SEMEN COLLECTION METHODS AND EVALUATION:**

Various methods of collection of semen have been devised from time to time. The older unsatisfactory methods have gradually replaced by the new modern techniques.

**There are three common methods.**

1. Use of artificial vagina
2. By Electro-stimulation method.
3. By massaging the ampulae of the duct us differences through rectal wall.
4. The ideal method of semen collection is use of artificial vagina which is safe for sire and the collector also.

**Artificial Vagina method**

The artificial vagina has the following parts:

- A heavy hard rubber 2" lose, open at both ends with a nostle for air and water in and outlet.
- Inner sleeve of rubber or rubber liner.
- The semen receiving cone or rubber cone.
- Semen collection tube made of glass or plastic graduate in cc and its fraction correct to 0.1 CC
- Insulating bag Before using for semen collection all the parts are washed thoroughly and sterilized properly, and assembled as artificial vagina, the rubber liner is inserted into the hose; inverting both ends back by folding back from either side opening, and fastening with rubber bands. Now the space between the hard rubber hose and inner rubber liner forms a water tight compartment. The nostle at one end of the hose can be fixed .

**PARTS OF ARTIFICIAL VAGINA**

Turning through the threaded nut up or down. The water jacket of the Artificial - vagina is- filled with hot water at a temperature of 45°C (113°F) by opening the nostle. The graduated semen collection tube is fixed to the narrow end of the artificial vagina hose, and fastened by a rubber band. The inner side of the rubber liner on the anterior side of the artificial vagina is lubricated with sterile jelly to a length of 3 to 4 inches. Air is blown through the nostle into the water jacket, to

create pressure in it, and the same is exerted the rubber linear, to simulate natural vagina.

The temperature of the artificial vagina is to be checked, at each collection, and it should simulate natural vagina at mounting time. If the artificial vagina is to mount later. If it is too cold ejaculate may not be there after a thrust, or even if ejaculate is there; it may be contaminated with urine, and becomes unfit for use.

### **SEMEN COLLECTION METHOD. (A.V.)**

The cow or dummy is secured in service create. The artificial vagina assembled is held at 45° angle from the direction of penis, and the thrust is that angle. The artificial vagina is held with the left hand by a right handed person; and when the bull mounts the cow, the sheath of the bull will be graphed by the operator, directing the gland penis into the artificial vagina, and then the bull gives a thrust to ejaculate.

The operator should evince care so as not to touch the exposed part of the penis. After the bull dismounts, the artificial vagina is taken off from penis and the air vent is opened to release the pressure from the jacket.

The water from the jacket is also drained by opening the nostril. This allows the ejaculate to flow from the cone to the semen collection tube. The semen collection tube is detached from the cone, plugged with cotton wool, and taken to the laboratory for examination. The rubber cone and the semen collection tube can be protected from external contamination or heat or higher, by covering with an insulation bag with zip.

### **SEMEN STORAGE**

The discovery that bull semen could be successfully frozen and stored for indefinite periods has revolutionized AI in cattle. In 1949, British scientists discovered that addition of glycerol to the semen extender improved resistance of sperm to freezing. Glycerol acts to remove water from the sperm cell prior to freezing and prevents the formation of cellular ice crystals which would damage the sperm. There are two methods of freezing and storing semen: dry ice and alcohol (-100 degrees F) and liquid nitrogen (-320 degrees F). Liquid nitrogen is preferred because there is no evidence of fertility deterioration with age. Fertility gradually declines in semen stored in dry ice-alcohol.

Frozen semen can be stored indefinitely if proper temperature is maintained. A recent report told of a calf born from frozen semen stored for 16 years. Fresh, liquid semen can be successfully stored for 1 to 4 days at 40 degrees F. Semen is

usually stored in glass ampoules. Other methods appear promising, particularly the French-straw. Several AI organizations have gone to this method exclusively. Artificial coloring is frequently added to semen extenders in order to distinguish one breed from another. Complete identification of the bull is required on each individual semen container.

## **INSEMINATION METHODS**

There are different methods of insemination in different species of animals i.e. speculum method, vaginal method and recto vaginal method.

### **RECTO VAGINAL METHOD**

In cattle the safe and best method of insemination is “Recto vaginal method of insemination”. A cow which is in heat is well controlled by placing it in a Travis. The inseminator will get ready by wearing a plastic apron, gumboots and gloves. The semen straw after thawing (keeping the semen straw in warm water for a minute to convert the frozen semen into liquid and the sperms become motile) is loaded in a sterilized A.I. gun and is covered with a plastic sheath. The inseminator will insert the gloved left hand into the rectum after applying the soft soap or other lubricant on the glove and back-racked the animal, and the hand is further inserted and will catch and hold the cervix through the rectal wall. The A.I. gun loaded with semen straw is passed.

#### **Recto-vaginal method of insemination**

Through the vulva to the vagina and cervix and observed with the hand in the rectum that the A. I. gun reaches the cervix, then the semen is deposited by injecting the gun, and after depositing the semen the gun is removed, the empty straw and sheath are discarded.

### **SPECTRUM METHOD**

In this method a speculum is placed in the vagina of the cow, which provides passage outside to the site of insemination, then the inseminating tube is passed through the speculum and semen is deposited at the cervix insemination method.

### **VAGINAL METHOD**

Hand is passed through the vagina and the inseminating tube is guided by hand to the site of insemination and semen is deposited. Here there is a risk of contamination and injury of female genitalia.

## **FROZEN SEMEN AND STORAGE**

Freezing of semen for successful preservation of spermatozoa, for long periods, is of great importance in livestock breeding and farm management. It has made it possible to make available the use of outstanding proven sires for larger number of cows, covering larger area, frozen semen shipment has become possible to different continents in the globe to any place connected with any service. Now a day if farmer wants to use of an outstanding sire for inheritance of high milk yield, he can go in for frozen semen service provided his area is, covered by Artificial insemination, with supply of frozen semen.

At present frozen semen is used in most of the states in India. The technique of semen preservation in straws was developed in France . Freezing of semen is done with a special diluents, which has the following composition.

Sodium citrate dihydrate (angular) 2.4 g. 2.0 gm 8.0 ml 25.0% by volume 50,000 units per 100 ml of semen Fructose Glycerol Egg Yolk Penicillin diluent. Dihydro-streptomycin 50.0 mg per .100ml of semen diluent. Distilled water double glass distilled 100.0ml. The addition of glycerol to the diluent makes the cells more resistant to the rigours of freezing and icy crystals, which form are smaller and smoother thus creating less damage to the spermatozoa. The addition of fructose to the diluent improves sperm resistance to glycerol; and also provides nutrition.

Frozen semen is packed in single dose glass vials or plastic straws at +5°C. The final level of glycerol should be 7.0 to 7.6% during the freezing process. The antibiotics are added to inhibit bacteria and to kill pathogenic organisms. The semen to be diluted in such a way that one ml. of extended semen will contain 20 million motile spermatozoa. The semen must be cooled carefully for spermatozoa to remain with life. The final temperature is lowered to -79°C or still lower. Quick freezing is done for a period of 3 to 5 minutes to -75°C with the help of atmosphere created by liquid nitrogen. In the slow freezing technique cooling is done at the rate of 1 °C per minute from +5°C to -15°C. From -15°C to -31 °C at the rate of 2°C per minute. From -31°C to -75°C at the rate of 4 to 5°C per minute. Thus taking 40 minutes in total, further cooling to -96°C can be done quickly as it is not critical after freezing. Before freezing the diluted semen is equilibrated for 3 to 5 hours or for the best 16 to 20, hours period in refrigerator at 5°C. Frozen semen facilitates the percent use of the semen diluted and frozen, and thus the delivery price is reduced, and it can be supplied with the gaps of months to the A.I technicians as against the supply of fluid semen every days or alternate days. Liquid nitrogen plays a vital role for storing the frozen semen straws, at a temperature of -196°C for longer periods.

### **Artificial insemination (AI) of cattle**

Artificial insemination (AI) is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female. One can cite a number of potential benefits from the use of artificial insemination.

### **Increased efficiency of bull usage**

During natural breeding, a male will deposit much more semen than is theoretically needed to produce a pregnancy. In addition, natural breeding is physically stressful. Both of these factors limit the number of natural matings a male can make. However, collected semen can be diluted and extended to create hundreds of doses from a single ejaculate. Also, semen can be easily transported; allowing multiple females in different geographical locations to be inseminated simultaneously, and semen can be stored for long periods of time, meaning that males can produce offspring long after their natural reproductive lives end.

### **Increased potential for genetic selection**

Because artificial insemination allows males to produce more offspring, fewer males are needed. Therefore, one can choose only the few best males for use as parents, increasing the selection intensity. Furthermore, because males can have more offspring, their offspring can be used in a progeny test program to more accurately evaluate the genetic value of the male. Finally, individual farmers can use artificial insemination to increase the genetic pool with which his or her animals can be mated, potentially decreasing effects of inbreeding.

### **Decreased costs**

Male animals often grow to be larger than females and can consume relatively larger amounts of feed. Also, male animals are often more strong, powerful, and potentially ill-mannered and thus require special housing and handling equipment.

### **Increased safety for animals and farmers**

As mentioned, male animals can become large and aggressive. These factors mean that maintaining a bull on a farm may be dangerous. Also, because of the relatively larger size of adult males than females, natural mating is more likely to result in accidents and injury to either the cow or the bull than is artificial insemination.

### **Reduced disease transmission**

Natural mating allows for the transfer of venereal diseases between males and females. Some pathogens can be transmitted in semen through artificial insemination, but the collection process allows for the screening of disease agents. Collected semen is also routinely checked for quality, which can help avoid problems associated with male infertility.

Artificial insemination has some potential drawbacks, however, that must be considered. First, it can be more laborious. Male animals instinctively detect the

females that are in the correct status for conception. With artificial insemination the detection work falls on the responsibility of the farmer. Poor detection results in decreased rates of fertility. Also, increasing the number of offspring per male has selective advantages only if the best males can be accurately determined. Otherwise this process only decreases the genetic variability in a population. Increasing the number of offspring per male always reduces the gene pool. The benefits of more intense selection must be balanced against the negative effects of decreased variation.

## **ARTIFICIAL INSEMINATION TECHNIQUES**

The technique of inseminating a cow is a skill requiring 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 at the best time to obtain acceptable conception rates. Early methods of AI involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Fertility is low and greater numbers of sperm are required. Another method which gained popularity was the "speculum" method. This method is easily learned, but proper cleaning and sterilizing of the equipment is necessary, making it more impractical to inseminate than with the rectovaginal technique which is the most widely used AI method today.

In the recto-vaginal 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 accomplished slowly and deliberately to avoid excessive sperm losses 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, the catheter should not be forced through the cervix since pregnancy is a possibility. Since research data show little variation in conception rates when semen is 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.

The recto-vaginal technique is more difficult to learn and practice is essential for acceptable proficiency but the advantages make this method of 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 from one cow to another.

### **Timing of Insemination for Maximum Conception**

A frequent question concerning AI is: What time during estrus should cows be bred for greatest chance of conception? Since estrus may last from 10 to 25 hours there is considerable latitude in possible time of insemination. Much research work has been conducted on this subject.

Controlled investigations were conducted by Trim Berger and Davis at Nebraska in 1943. These and other studies show that conception rate is lower when cows are bred prior to mid estrus or later than 6 hours after cessation of estrus (standing heat in this case). Maximal conception is obtained when cows are inseminated between mid estrus and the end of standing estrus, with good results up to 6 hours after estrus.

Success in insemination timing is dependent upon a good heat detection program. In large herds, this means assigning individual responsibility for heat detection and a continued education program for labor. A successful heat detection program and subsequent proper timing of insemination will pay dividends in increasing reproductive efficiency.

### **Induction of Early Puberty in cattle:**

Puberty is the period when the sexual organs are functionally developed. Puberty in heifers is characterized by first ovulation and plasma progesterone concentrations above 1 ng/ml (Evans et al., 1995). Sexual maturity is the stage when the animal is able to express its full reproductive potential. Sexual maturity in males is characterized by sperm ejaculate that contains minimum 50 million spermatozoa with minimum of 10% motile sperm (Wolf et al., 1965). The onset of puberty is the result of a series of complex events that occur within the reproductive endocrine system (Foster et al. 1994). A cow maturing at early age will produce more milk in her whole life time. Delayed puberty in cattle and buffalo is a major problem in the dairy industry. Heifers calved at 540 kg were more economical than those at 620 kg (Dawson and Carson, 2004). Rauw et al. (1998) reported that modern reproduction and DNA-techniques in animal breeding programme may result in physiological, behavioural, and immunological problems in animals. Many factors like species, genetic potentiality, plane of nutrition, growth, body weight, role of different hormones, health and other management

conditions have a direct or indirect effect on growth, puberty and sexual maturity in animals (Figure 1). Among all factors, body weight at early age has an important role on life time performance of animals including production and reproduction.

**Age of the animal :** Average age of puberty in cow heifers is between 37 and 34 months but Sahiwal attain puberty at 46 months (Bashir, 2006). In the tropical condition the age at puberty in *Bos indicus* range between 16 and 40 months (Mc Dowell et al., 1976). The Indian buffalo (*Bubalus bubalis*) attain puberty between 16 to 40 month but the average time is over 2.5 years of age (CIRB Annual Report, 1999–2000). Murrah attain puberty as late as an average age of 33 months (NDRI Annual Report, 1995–1996), Nilli Ravi at 32.5 months (Naqvi and Shami, 1999) and Surti at 45.5 months of age (Sule et. al., 2001). Postnatal growth plays an important role in the performance of the dairy animals.

**Genetics and Breed :** Genetic selection is not an effective tool as other environment management approaches are necessary for early puberty. Age of puberty is moderately heritable and in cattle it ranges between 0.16 to 0.57 (Martinez-Velazquez et al., 2003). Nogueira (2004) suggested that selection of heifers should be based on age at first calving and recommended crossbreeding between Zebu and *Bos taurus* cattle for early puberty in zebu cattle. He also suggested for consideration of environmental effect for early puberty in zebu cattle. Zebu cattle are famous for disease resistance and better feed utilization with low management requirement than exotic animals. Naz and Ahmad. (2006) reported a low correlation between maturity age and weight at first conception in Nili-Ravi buffalo. He also reported a genetic correlation for age at maturity with weight at maturity, age at first conception were 0.49 and 0.88, respectively. Thyroid hormones have a great role in the metabolism and cell growth in the body. Fernandez et al. (2014) reported a significant association ( $P < 0.05$ ) between single nucleotide polymorphisms (SNPs) markers and puberty in Angus bulls. So this marker is good indicator for puberty in animals. In case of males scrotal circumference is an important criterion for selection because it is highly heritable and related to reproductive performance (Gressler et al., 2000). During the under nutrition Neuropeptide Y (NPY) is responsible for low secretion of luteinizing hormone (LH). Leptin has an important role in suppression of Neuropeptide Y (NPY). Vaiciunas et al. (2008) reported a lower NPY-Y1 and NPY-Y4 expression had a regulating role for puberty in early-maturing *Bos indicus* heifers. Heifer selection should based on good health condition, structurally large body size and puberty at early age.

**Growth and body weight:** Maturity of the heifer depends on the body weight rather than age. Lower growth rate occurs due to underfeeding or imbalanced feed

composition. Birth weight also affects growth rate and age at puberty. Many genetic and non-genetic factors are responsible for body growth in animals. Genetic factors along with nutrition, hormones, animals's individuality and many other factors determine the growth of animals. Feeding high energy or high concentrate diets not only reduce the age of sexual maturity but also lowers the time period for attaining the age of first calving. Buffalo heifers reared on seasonal green forages and crop residues resulting in poor growth rates and delayed onset of puberty (Bhatti et al., 2007). The body weight gain may have a greater influence on onset of puberty (Wiltbank et al., 1966). About 60 to 65% of mature body weight may be a standard during the starting breeding season in heifers (Endecott et al., 2013). Da Luz et al. (2012) reported that Murrah buffalo reached sexual maturity at 2 year of age and at this time sperm production is 13 million sperm per gram of testis. The monthly weight gain was faster upto 3 month of age and slower from 3 to 6 month of age in case of swamp buffalo (Das, 2004) and Murrah buffalo. A particular body weight has a role in attainment of puberty (Lemond, 1970) and a low body weight causes delay in onset of puberty (Maquivar et al., 2006). During the early phase of life, body's cells and parenchyma cells of the mammary gland develop very fast than the late phase of life. However, over conditioned animals presently facings many health related problems resulted in poor productive and reproductive performance.

**Nutritional management:** Balanced feeding and improved management can be helpful in better growth and early sexual maturity (Heinrichs et al., 2005). The first important factor that affects age of maturity is the plan of nutrition (Poy and Panday, 1971). Delay in puberty also occurs due to inadequate supply of feed and essential nutrition during the early growing period. But some authors reported that a predetermined body size at which puberty will occur within each specific breed (Oyedipe et al., 1982). The onset of puberty at the early stage (between 4 and 6.5 months of age) occurs due to high plan of feeding (Gasser et al., 2006). Colostrum is essential at early age of life to provide the natural immunity to the newborn calves. At the starting stage colostrum feeding through the bottle leads to higher intake rate than the pail feeding (Smijisha and Kamboj, 2012). A good quality calf starter should contain 18% crude protein and 3.0 Mcal/Kg metabolizable energy (NRC, 2001). Khan et al. (1992) worked on the Sahiwal calves and reported that the restricted suckling calves gained live weight 49% faster with 80% improvement in efficiency of converting milk into live weight. Similar observations were reported by the Bwire et al. (1996) in Zebu heifers, they reported that restricted suckling tended to gain more body weight (g/day) than the than those on artificial rearing system. Contrary to this report Crabtree. (1967) reported that pre-weaning management system had no significant effect on growth

performance. Nanda et al. (2003) observed that better nutrition reduces the age of maturity in buffalo heifers. Shatavari (*Asparagus racemosus*) can be used as a feed supplement for growth and puberty in dairy animals. It has anti-stress properties (Kumar et al., 2008) and causes early puberty and increase in weight of ovaries, uterus and teats in female (Sharma, 2011). Feeding of Shatavari @150mg/kg BW/day has significant effect on attaining higher average daily gain, early attainment of puberty and age at first service in Sahiwal heifers (Jamara et al., 2014) (Table 1). Proteins and energy are most critical nutrients influencing the growth of calves. High level of nutrition is essential during the growth period in the buffalo calves but the cattle calves require low level of protein than the buffalo calves (Basra et al., 2003). Contrary to this report Fluharty and Loerch. (1995) reported that higher protein concentrate mixture supplementation did not increase the growth rate. According to NRC (2001), heifers fed with dietary level of ME 124% gained higher growth rate than on other diets. Anjum et al. (2014) worked on the Sahiwal heifers with StairStep Feeding (consists of two rations having 20% below or 20% above of NRC energy levels) and reported that 100 % of animal came in puberty at 22 months of age than control (83%). Fiaz et al. (2012) reported that high dietary energy level (ME 124% of NRC) enhanced the growth parameter but adequate performance of Sahiwal heifers in terms of age of puberty was achieved even at low lower dietary energy level (ME 88% of NRC). The feed conversion efficiency was higher when the Sahiwal heifers were fed with the extra dietary energy than the recommendations of NRC (2001). He also reported that age of sexual maturity, age at conception and serum progesterone level were not influenced by the different dietary energy level. The supplementation of different energy levels in the diet of heifers is an effective key for the optimum growth rate from 13 to 18 month of age. The diet containing 16% protein and 3.0Mcal/kg energy is sufficient for the growth of red Sindhi calves (Javaid et al., 2014). The diet with proper concentration of nutrients reduces the age of maturity in buffalo heifers. The animals fed with green fodder along with of 2.0 Kg concentrate ration reach maturity earlier ( $727.77 \pm 44.17$  days) than the control group ( $993.33 \pm 68.78$  days) (Rafiq et al., 2008). Animals require specific minerals for the growth and skeleton development. The supplementation of minerals (Chaudary et al., 1991) and UMMB (urea molasses mineral blocks) (Garg et al., 1990) may be associated with early maturity. Phosphorus is involved in the many metabolic process and cellular metabolism in the body (Rasby et al., 1998), VFAs concentrations and bacterial population in the rumen (Zain et al., 2010). Previous studies revealed that optimal growth rates can be achieved when diets containing P level from 0.20% to 0.22% of DM (Tillman et al., 1959), 0.33% to 0.40% of dietary P concentration (Ferguson and Sklan, 2005) and Ca to P from 1:1 to 7:1 (Wise et al., 1963). Anjum et al. (1996) reported that the ration containing 0.75% Ca and 0.31% P on dry

matter basis with Ca: P ratio 2.5:1 is more suitable for better weight gain in Nili-Ravi buffalo. Niacin plays a critical role in mitochondria respiration and the metabolism of carbohydrate, lipids and amino acids. Oral administration of niacin has resulted in increased microbial protein synthesis and higher weight gain in growing animals (Flachowsky, 1993). Contrary to this observation, Kumar et al. (2006) reported that supplementation of niacin at 100 and 200 ppm in the diet of buffalo calves had no significant effect on their growth and nutrient utilization. Use of higher levels of vitamin E in the diet improves the growth and skeleton development in the calves.

**Hormones: Key factor for puberty:** The fundamental requirement for the onset of puberty is the secretion of a gonadotropin releasing hormone (GnRH) from the hypothalamus which stimulates release of gonadotropin hormone i.e. luteinizing hormone (LH). GnRH plays an important role in the regulating secretion of LH, follicular development, and secretion of steroid hormones (Figure 1). Madgwick et al. (1995) worked to know the effect of GnRH on sexual puberty in heifer calves from 4 to 8 weeks of age and concluded that GnRH treated heifers reached puberty earlier than control heifers ( $56.8 \pm 1.7$  vs.  $62.8 \pm 2.4$  weeks) with high level of LH hormone ( $0.58 \pm 0.06$  ng/ml vs.  $0.41 \pm 0.02$  ng/ml) and greater number of LH pulses ( $2.0 \pm 0.19$  vs.  $1.32 \pm 0.12$  pulses per 10 h.) than the control. The changes in the metabolic status cause changes in metabolic hormones leading to the onset of puberty. In case of bull calves increased level of LH causes early age of puberty (Evans et al., 1995), testicular development (Chandolia et al., 1997) and increased spermatogenesis cycle (Rawlings and Evans, 1995). Growth hormone (GH) has important role in growth and development during postnatal life. Growth hormone releasing factor (GRF) has an important role for activity of hypothalamus–pituitary–gonadal axis. Haldar and Prakash. (2006) worked on the Murrah buffalo with administration of  $10 \mu\text{g}/100 \text{ kg}$  body bGRF (Bovine gonadotropin releasing factor) to each animal and reported that GRF has a significant effect on the body weight, plasma progesterone concentrations and onset of puberty. Buffalo heifers treated with bovine growth hormonereleasing factor (bGRF) showed puberty onset at an age of  $887.5 \pm 17.5$  days (Mondal and Prakash, 2004). Plasma Insulin like growth factor I (IGF-I) has important role in regulation of cell growth, cell differentiation, cell function and immune function. Many authors reported about the role of IGF- I in growth of the cattle (Ortega et al., 2008; Lancaster et al., 2008). Laxmi et al. (2014) used fermented yeast culture (*Saccharomyces cerevisiae*) which stimulated IGF- I for growth of ruminal bacteria in low body weight Murrah buffalo calves. This is due to increasing FCE (feed conversion efficiency) and digestibility of essential nutrients. Long term administration of GRF causes faster growth in buffalo calves resulted in higher body weight due to

increase plasma LH level (Mondal and Prakash, 2004). Progestogens have a role in initiation of oestrus and ovulation in prepubertal heifer. It is due to enhanced luteal function and stimulation of endocrine system. Polat. (2009) reported that PRID (progesterone releasing intravaginal device) 1.55 g of progesterone and 10 mg of oestradiol benzoate is effective on delayed pubertal heifer. Gulia et al. (2010) reported that the animals secreted high level of testosterone during the early growth period for attaining the early sexual maturity. So maintenance of testosterone during early phase of life is important. Leptin hormone produced mainly by adipose tissue has a role on onset of puberty, energy balance and feed intake. Vaiciunas. (2008) reported a higher leptin level regulates puberty in early-maturing *Bos indicus* heifers. Hormonal supplementation is helpful to reduce the age of sexual maturity in the Indigenous cattle and buffalo.

**Photoperiod :** Photoperiod can alter long-term physiological processes, particularly reproduction and production. Longday photoperiod hasten puberty and accelerates lean growth in dairy heifers. Perera et al. (1989) reported that light has no effect on growth (16.2 kg. vs. 20.8 kg.) but higher progesterone (0.41ng/ml vs. 0.19) and high prolactin level was observed in case of Surti buffalo. Long day photoperiod (LDPP) treated calves tended to have higher mean concentrations of PRL relative to SDPP (short day photoperiod) animals (11 ng/ml vs 5 ng/ml) (Rius et al., 2005). LDPP causes decline in the levels of melatonin which is important for the reproductive performance in the animals (Walker et al., 1996). Kassim et al. (2008) worked on the buffalo heifers, divided animals into two groups as G1 for long photoperiod in which heifers were exposed daily 16 hours of light and 8 hours darkness per day. The second group (G2) consisted of natural photoperiod of 8 hours light and 16 hours darkness daily. They reported that animals in G1 had higher values in live body weight than G2 at puberty and first ovulation.

**Climatic effect:** Variation of the season has an important role in the body weight of the animals. A positive relationship was observed between season and onset of puberty. Winter season is favorable for early puberty in dairy animals. The non-significant effect of season was observed in swamp buffalo at 6 month of age but significant effect was observed from 7 to 12 month of age (Das, 2004). Zaman (1996) also found a non significant effect of season on the body weight. During initial six month of life autumn and winter season has a positive effect for early weight gain and puberty. Effect of season is related with managemental and nutritional practices. Penchev et al. (2014) reported lower calving age in the heifers born in summer and autumn in Bulgarian Murrah heifers.

**Exposure to male:** Biostimulation or male effect is the stimulus provoked by the presence of males, which induces estrus and ovulation through genital stimulation,

pheromones or other external cues (Tiwari et al., 2014). Biostimulation is helpful for early puberty in males and females. Heifers exposed to bulls attained puberty at an earlier age than heifers that were not exposed to bulls. Presence of a vasectomised bull has been reported to hasten the onset of puberty in heifers (Rekwot et al., 2001). Roberson et al. (1991) reported that more number of heifers exposed to bulls came in puberty earlier than the non exposed heifers (61.8 % vs. 45.4%). Izard et al. (1982) reported that the urine treated heifers came in puberty earlier than the control group ( $222 \pm 4.9$  days vs.  $277 \pm 5.0$  days). Biostimulation may be effective and economic tool to boost sexual maturity in animals. The knowledge of effectiveness, conditions and procedure are important for successful implementation.

Age at puberty and sexual maturity are important economic traits. Many factors are directly or indirectly related to age at puberty in dairy animals. Huge economic losses have been related to delayed puberty. Among the all factors affecting age at puberty, nutritional is most important. Higher correlations have been observed between age at puberty and body weight. This can be achieved by proper nutrition than other factors. For early puberty, heifer must be fed properly to get a sufficient weight for puberty at recommended time. So nutritional management should be given priority for reducing age at puberty. Use of green fodders along with mineral mixture reduces the age of puberty and maintains the proper growth. The Ca to P should be supplemented at the ratio of 1:1 to 2.5. Long-day photoperiod hastens growth and sexual maturity in the dairy animals. Diet containing balanced energy and protein concentration helps in proper growth and puberty. The use of herbal preparation like Shatavari @ 150mg/kg BW/day has significant effect on growth and sexual maturity. Application of new knowledge and modern technologies are necessary for growth and reproductive management for dairy animals.

### **Synchronization of Estrus in cattle:**

Estrus cycle can be defined as the rhythmic changes that occur in the reproductive system of a female animal starting from one estrus phase to another. The normal duration of estrus cycle is 21 days in cow, sow, and mare, 17 days in ewe, and 20 days in doe. The domestic animals can exhibit a single estrus cycle or more than one estrus cycle in a year. The canine species show only one cycle in its breeding season; hence they can be called the monestrous. Other species which come into estrus twice or more are termed polyestrous animals. Among them some species have estrus cycles in a particular season and defined as seasonal polyestrous. It

includes goats, sheep, and horses. On the other hand, cattle undergo estrus throughout the year. The seasonal polyestrous animals are greatly regulated by the photoperiod of the season for their reproductive activity.

The estrus cycle can be grossly divided into two phases, that is, follicular phase and luteal phase. The main event occurring in follicular phase is the development of the ovarian follicles, whereas in luteal phase there is formation and growth of the corpus luteum (CL). The follicular phase is again consisting of proestrus and estrus. The proestrus lasts for 3–4 days and the estrus phase only for 12–18 hours. FSH (follicle-stimulating hormone) is the principal hormone controlling the follicular phase. It causes enlargement of the follicles, increase in estrogen secretion from the granulosa cells of the ovary, and increase in the vascularity of the female reproductive tract. After the proestrus phase, there is a rapid increase in the luteinizing hormone (LH) level known as LH surge. This surge is responsible for the ovulation of the matured graafian follicle. In cattle, ovulation generally occurs 12 hours after the end of the estrus. At estrus phase, the animal shows the signs of estrus or heat. It includes mucous discharge from the vagina, restlessness, frequent micturition, bellowing, swelling of the vulva, etc. The animal tries to mount other animals and also stands to be mounted by other animals called as standing heat. After the estrus phase, the ruptured follicle starts to convert into corpus luteum and the animal enters into luteal phase. This phase is also divided into metestrus and diestrus. The duration of metestrus is 3–4 days, whereas diestrus can last from 10 to 14 days. In metestrus the estrogen level starts decreasing and progesterone increases. Though ovulation occurs in metestrus phase in cattle, it happens in the last portion of estrus phase in other domestic species like sheep, goat, horse, etc. The uterine contraction subsides and endometrial glands start growing in metestrus. The progesterone level continues increasing in diestrus and achieves a peak on 13–14 days after estrus phase. Afterward the size of the corpus luteum also starts decreasing, and the follicle grows if the animal is not pregnant. In the case of pregnant animals, the CL does not regress and secrete progesterone throughout the gestation period. If the animal is not conceived, the CL is destroyed after the end of this phase, and the animal enters into the follicular phase.

FSH and LH are the two gonadotropins majorly responsible for the events in estrus cycle. These are secreted from the anterior pituitary upon the stimulation of gonadotropin-releasing hormone (GnRH). GnRH that resides on the top of hypothalamo-pituitary-gonadal (HPG) axis controls the reproductive activities of the animals. The FSH and LH eventually act on the gonads and secrete sex steroids like estrogen and progesterone in female and testosterone in male. Estrogen and testosterone help in the development of secondary sexual characters in females and males, respectively. The secretion of GnRH depends upon different internal and

external signals. For example, leptin secreted from the adipose tissue and melatonin from the pineal gland have a clear effect on the GnRH release. It is also stimulated by kisspeptin, a neuropeptide secreted from preoptic and arcuate nucleus of hypothalamus. So, any physiological or pathological condition which disturbs the release of GnRH can affect the normal reproductive behavior of the animals. The overall hormonal balance is very much essential for maintaining estrus cyclicity.

### **Anestrus and its types**

Anestrus is the lack of estrus or heat syndromes in female animals. It can be observed in heifers as well as cow. A good number of post-parturient cows show anestrus. Anestrus can be caused by different reasons and can be classified into different ways. Kumar et al. have divided anestrus into two major parts based on the causes, that is, physiological anestrus and pathological causes of anestrus. Physiological anestrus can be either ovulatory or anovulatory. Ovulatory anestrus is seen during gestation period of the animal. Anovulatory anestrus can be prepubertal, lactational, or postpartum. The animals before coming into puberty show follicular growth, but they cannot mature. Due to the action of FSH, the follicle develops up to the stage of theca internal but thereafter starts degrading. The LH pulse frequency is also low, and the threshold for the positive feedback of estradiol on LH surge is also very high. So, there is no ovulation and no estrus. Gestational anestrus is common in all the animals. As there is a persistent corpus luteum present in the ovary throughout the gestation period, there is always an elevated level of progesterone. Progesterone has a negative effect on GnRH secretion and cyclicity stops. Though sometimes, cattle and buffalo can show estrus in the first few months of gestation. It is called gestational anestrus. The signs of estrus are indifferent from the nonpregnant animals in estrus, but the duration is shorter. These animals also exhibit standing heat. The estrus should be carefully differentiated from true estrus to avoid undesirable effect on pregnant animals. At the end of gestation period, there is a decrease in progesterone level. Still the animals are unable to come into estrus cycle, known as postpartum anestrus. This anestrus provides some time for involution of the uterus so that animals can come into estrus subsequently. But, this duration should not be prolonged. Many times, due to lack of proper nutrition and several postpartum diseases, the animals do not show estrus. Proper care and management in the periparturient period can solve this issue. It is ideal to conceive the animal within 2 months of parturition to get one calf each year. When the animals are in lactation also, the estrus cycle can be disturbed especially in high yielders. A high level of prolactin hormone required for the milk synthesis can suppress the GnRH level. This is termed as lactational anestrus.

Pathological causes of anestrus can again be of two types, that is, congenital and hereditary causes of anestrus and acquired anestrus. Congenital and hereditary causes are observed in ovarian aplasia, ovarian hypoplasia, and freemartin. Acquired anestrus can be ovulatory or anovulatory. Examples of ovulatory acquired anestrus are subestrus, unobserved estrus, and persistent corpus luteum. Acquired anovulatory anestrus has been classified into three type (I, II, and III) based on the stage of follicular growth. In the case of type I, the follicles grow up to four millimeters and start regressing. In type II, the follicles grow further up to deviation and preovulatory stage but regress thereafter, and the next follicular wave starts. In type III, the follicle reaches up to the dominant stage but fails to ovulate and converts into persistent follicle.

### **Synchronization of estrus**

The manipulation of the estrous cycle or induction of estrus brings a large percentage of a group of females into estrus at a short, predetermined time . One of the advanced managemental processes through which the humane errors and managemental costs could be minimized is synchronization of estrus. It is predominantly useful in sheep, where timely heat detection is difficult due to exhibitions of less external heat symptoms and also in large herd of cattle. It helps in fixing the breeding time within a short predefined period and thereby scheduling the parturition time at the most favorable season in which newborns can be reared in suitable environment with ample food for augmenting their survivability. As timely breeding of the animals is possible with this technique, fertility in farm animals may be expected toward the upper side. By improving the production efficiency of animals, estrus synchronization provides more economic returns to the owner.

Synchronization can shorten the breeding period to less than 5 days, instead of females being bred over a 21-day period, depending on the treatment regimen. Production of a uniform group of calves for the future replacement in the animal farm is another important benefit of this program. The current and future aspect of estrous synchronization is to focus on combining traditional methods of controlling cycle length with the follicular development manipulation. The combination of GnRH with the prostaglandin F<sub>2α</sub> [20]- and progesterone [29]-based synchronization program has shown a novel direction in the estrus synchronization of cattle with the follicular development manipulation.

### **What is the basic approach for estrus synchronization?**

To control the timing of the onset of estrus by controlling the length of the estrous cycle is the basic approach for the estrus synchronization. Various approaches for controlling cycle length are as follows:

Prostaglandin administration to regress the corpus luteum of the animal before the time of natural luteolysis

Progesterone or synthetic progestin administration to suppress ovarian activity temporarily

Creating estrous synchrony by using gonadotropin-releasing hormone or an analogue, which causes ovulation of a large follicle, helps in synchronizing estrous cycle in anestrus female.

### **Methods of estrus synchronization:**

#### **Prostaglandin treatment**

Luteolytic agent such as prostaglandin  $F2\alpha$ , or an analogue, which causes the regression of the corpus luteum can be used to synchronize estrus [30, 31]. Administration of  $PGF2\alpha$  is only effective from 8 to 17 days of the estrous cycle when functional corpus luteum is available in one of the ovaries. Fertility is high after prostaglandin synchronization. Synchronization of estrus and fertility with this product are good in cyclic females but not in non-cycling cows.

**One-shot prostaglandin:** In this method a single injection of prostaglandin is given to cyclic females, and then these females are bred as they express estrus.

**Two-shot prostaglandin:** In this method two injections of prostaglandins are given at an interval of 10–14 days [32] once the stage of estrous cycle in the cows is unknown and detection of estrus is not required before or between injections.

#### **Progesterone treatment**

High levels of progesterone in the female's system are maintained with the help of progestogens [33], even after the regression of the corpus luteum. After the progestin removal, synchrony of estrus occurs up to 2–5 days. Melengestrol acetate (MGA) (oral feeding), Syncro-Mate-B (SMB) (ear implant), and CIDR (intravaginal device) are the commercial products which fall into this category. The longer the progestin was administered to cattle, the higher the rate of estrous

synchronization, but the fertility of the synchronized animals was lower. Kaltenbach et al. [34] and Wiltbank [35] reported that the estradiol was luteolytic when administered early in the bovine estrous cycle. Combining progestin treatment and estradiol administration at the initiation enabled the period of progestin to be shortened (9–14 days) without reducing the percentage of animals exhibiting a synchronized estrus. This treatment regimen was the basis for the commercial products Syncro-Mate-B, PRID, and CIDR. Administration of progestin at “sub-luteal” levels demonstrated that it inhibits estrus and ovulation and synchronizes estrus in cattle, but that a persistent, estrogen-secreting follicle develops when progestin treatment extends the estrous cycle.

### **GnRH-based treatment**

Estrus synchronization and fertility with a combination of GnRH and prostaglandin  $F2\alpha$  are good for cyclic females, and this combination may induce cyclicity in cows experiencing postpartum anestrus. The new methods of estrus synchronization more precisely and control the time of ovulation more exactly in order to allow a single, timed insemination without the need for detection of behavioral estrus. Administration of GnRH during the estrous cycle in bovines causes regression or ovulation of the dominant follicle and initiates the emergence of a new wave of follicular growth. Ovsynch, CO-Synch, Select-Synch, and Hybrid-Synch are the four systems for synchronization of estrus with GnRH-PG combinations.

At day 1 GnRH injection is used to program follicle growth in cyclic females and to induce ovulation in anestrus females, and  $PGF2\alpha$  on day 8 induces regression of CL that is present to cause a decline in progesterone. Then on days 10–11, the second GnRH is given which induces ovulation of dominant follicles that have been preprogrammed by the first GnRH treatment. The major GnRH programs that do not involve use of the CIDR are described as follows:

**GnRH-PGF system:** This represents the simplest GnRH-based system. A common name for this system is “Select-Synch.” In this system a single dose of GnRH and prostaglandin was injected on day 1 and day 8, respectively. Some cows (8%) exhibit estrus up to 48 hours before PGF (day 6). The early estrous are fertile and cows can be inseminated 12 hours after detection. The peak estrous response occurs 2–3 days after PGF with a range of 1–5 days. With this system, a minimum of 5 days of estrous detection after PGF and 2 days prior PGF is required to detect most heats.

GnRH-PGF + GnRH system: This system is a GnRH-PGF system in which second GnRH injection is given to all or some cows between 48 and 72 hours after PGF (days 2–3), with timed AI on all or a portion of the herd.

In Ovsynch program, an injection of GnRH on day 1, an injection of prostaglandin on day 8, a second injection of GnRH on day 10, and then a timed insemination on day 11 are given [20]. The first GnRH injection alters follicular growth by inducing ovulation of the dominant follicle in the ovaries after the GnRH injection to form a new or additional CL [20]. Thus, estrus usually does not occur until a PGF<sub>2</sub> $\alpha$  injection regresses the natural CL and the secondary CL which is formed from the follicle induced to ovulate by the first GnRH injection. Based on transrectal ultrasonographic evidence, a new group of follicles appear in the ovaries, within 1–2 days after the first injection of GnRH [38]. From those follicles, a newly developed dominant follicle emerges, matures, and can ovulate after estrus is induced by PGF<sub>2</sub> $\alpha$ , or it can be induced to ovulate after a second GnRH injection. This GnRH release luteinizing hormone, the natural ovulation-inducing hormone of the estrous cycle. The stage of the estrous cycle when Ovsynch was initiated also affects synchronization and conception rate [38]. Ravi Kumar and Asokan [39] reported higher conception rate in subestrus buffaloes initiating the treatment with Ovsynch during the later stages of estrous cycle, but conception rate was nil in anestrus buffaloes though incidence of cyclicity was observed due to the treatment. Benefits of this program are as follows: there is tight synchronization of estrus, most females respond to the program, and it boosts estrus in non-cycling cows that are at least 30 days postpartum.

In CO-Synch program, an injection of GnRH on day 1, an injection of prostaglandin on day 8, and then a second injection of GnRH with breeding on day 10 are given. The benefits are as follows: there is tight synchronization of estrus, most females respond to the program, and it boosts estrus in non-cycling cows that are at least 30 days postpartum.

The Hybrid-Synch program is applied with an injection of GnRH on day 1, an injection of prostaglandin on day 8, and then estrous detection and breeding from day 8 to 11. Second injection of GnRH was given to the females which were not observed in estrus from day 8 to 11 and were bred on day 11. Hybrid-Synch program has a lower cost and less handling compared with Ovsynch and CO-Synch but more than Select-Synch. The program appears to have the highest conception rates among all GnRH-prostaglandin programs.

## **Managerial interference**

In general management has a tremendous role in the reproduction of animals. Proper nutritional management of the herd is essential for successful implementation of several synchronization programs in both cows and heifers. Managerial procedures like timed insemination and calf removal have been reported to be useful for synchronization of estrus and may also be applied in most of the synchronization programs for better results. Usually conception rates on timed insemination are lower than for visual observation. However, this lower conception rate may be counterbalanced by the reduction in management from timed insemination. Suckling frequency of calves causes a hormonal response which inhibits return to estrus, which is evident in beef cows. Short-term calf removal combined with other forms of synchronization increases estrus synchrony and conception rates in cows. Even a 48-hour calf removal alone has been shown to cause synchrony and cyclicity in some cows. This procedure is suitable, but requires better management and good facilities to prevent separated cows and calves from rejoining with each other.