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Induction and Synchronization of Estrus

Prasanna Pal and Mohammad Rayees Dar

Abstract

Estrus cycle is a rhythmic change that occur in the reproductive system of females 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 species which exhibit a single estrus cycle are known as monestrous and 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 estrus inducers can grossly be divided into two parts, that is, non-hormonal and hormonal. Non-hormonal treatments include plant-derived heat inducers, mineral supplementation, uterine and ovarian massage, and use of Lugol's iodine. The hormones that are used in estrus induction are estrogen, progesterone, GnRH, prostaglandin, insulin, and anti-prolactin-based treatment. 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. The combination of GnRH with the prostaglandin F_{2α} (PGF_{2α})- and progesterone-based synchronization program has shown a novel direction in the estrus synchronization of cattle with the follicular development manipulation.

Keywords: estrus, synchronization, GnRH and PGF_{2α}

1. Introduction

From the prehistoric ages, the animals have been an integrated part of human life. With the progression of time, the dependency on domestic animals has only increased. At the present time also the human civilization cannot be imagined without the animal products we are using every day. Currently, an enormous livestock population throughout the world is contributing in achieving the global food security. But, the rapid explosion of human population which is projected to be nearly 9.7 billion within 2050 has increased the demand for animal products. So, researches are being targeted toward getting more production from the animals. The major barriers toward this goal are the different diseases and scarcity of animal feed especially in undeveloped or developing countries. Among the several diseases, problems associated with animal reproduction have always been a matter of great concern for the animal producers. The female animals having a reproductive problem cannot be conceived and produce offspring. Besides, most importantly, it also shuts the door for milk production. The major reproductive issues include

anestrus, repeat breeding, delayed estrus, different infections, etc. Among them the problem of anestrus has an incidence rate of 2.13–67.11% in the bovine population of a country like India which is the largest producer of milk [1]. Anestrus is a condition when there is absence of regular reproductive cyclicity in the female animal. Consequently, the animal becomes unproductive and causes a huge economical loss to the farmers or producers. So, it is very much important to address this problem with much care. Already, there are several established as well as developing methodologies which can induce estrus in anestrus animals. It encompasses practices like administration of hormone to the use of biostimulation. Besides inducing estrus, some technologies can also synchronize it according to the need. Synchronization of estrus can help in simplifying managemental practices as well as in some advanced technologies like embryo transfer, in vitro fertilization, cloning, etc. In this chapter we are going to discuss about the normal estrus cycle, the anestrus problem, and the methodologies developed by the researchers for induction and synchronization of estrus.

2. Estrus cycle of domestic animals

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 kiss-peptin, 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.

3. 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. [1] have divided anestrus into two major parts based on the causes, that is, physiological anestrus and pathological causes of anestrus (Figure 1).

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

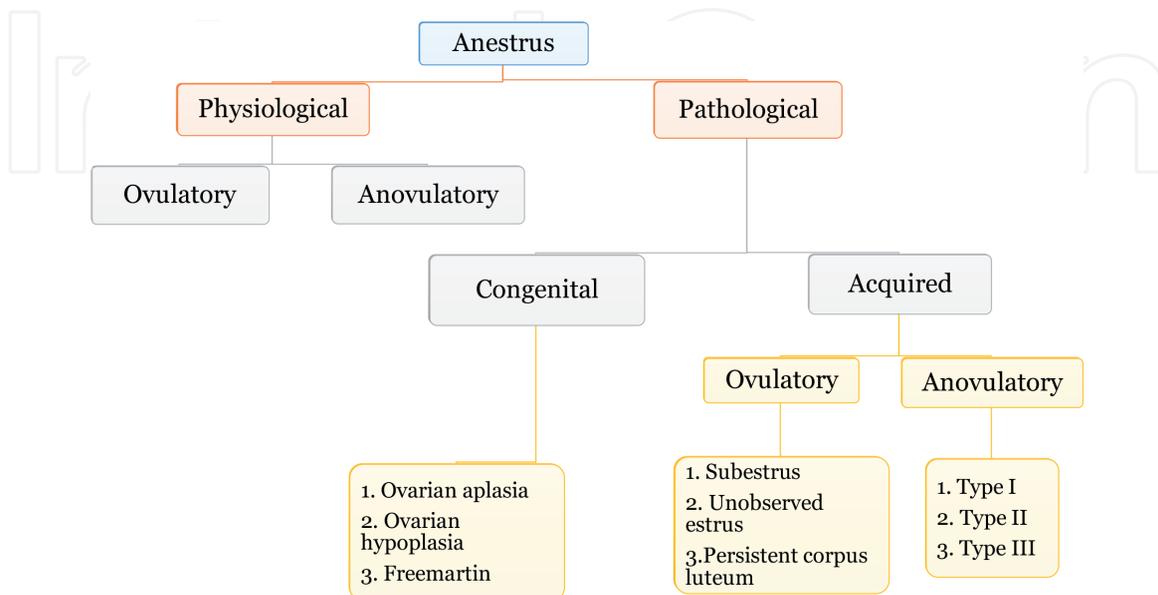


Figure 1.
 Classification of anestrus [1].

show follicular growth, but they cannot mature. Due to the action of FSH, the follicle develops up to the stage of theca interna 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 [1]. 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 [2]. 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 types (I, II, and III) based on the stage of follicular growth [1]. 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.

4. Induction of estrus

The problem of anestrus causes a huge economical loss to the farmers or producers. So, it needs to be solved immediately. Kumar et al. [1] have beautifully classified different ways of estrus induction. The estrus inducers can grossly be divided into two parts, that is, non-hormonal and hormonal. Non-hormonal treatments include plant-derived heat inducers, mineral supplementation, uterine and ovarian massage, and use of Lugol's iodine. The hormones that are used in estrus induction are estrogen, progesterone, GnRH, prostaglandin, insulin, and anti-prolactin-based treatment. All these treatment procedures are described below.

4.1 Non-hormonal treatment

4.1.1 Plant-derived heat inducers

Different plant extracts are being used for the treatment of anestrus traditionally. Several estrus-inducing herbal medicines are available in Indian market. The efficacy of estrus-inducing preparations like Prajana, Janova, Estrona, and Sajani

is well established [3]. Other examples include Aloes, Heat-Up, Fertivet, Heat-raj, etc. These can be applied in delayed puberty, postpartum anestrus, and other problems. Though they can induce estrus in crossbred cows, the conception rate is remained unchanged. *Aegle marmelos* and *Murraya koenigii* are two medicinal plants used for the treatment of reproductive problems in livestock as well as laboratory animals [4–6]. Feeding the leaves of these plants individually or combined can help in starting the cascade of reproductive cycle. It is believed that they act like the gonadotropins. The other possible mechanism behind its efficacy is the antioxidant effect of the plant-derived substances enhancing the luteal function. The demand and usefulness of the plant-derived medicines are increasing day by day. As these are easily available, are economical, and have fewer side effects, these preparations can be successfully utilized especially in village level. Many times, the poor farmers cannot afford the cost of the hormonal estrus inducers which are not always available also. In this situation, herbal mixtures have emerged as a better option. A large comparative study is required to use these drugs as alternative to hormones. It is also recommended that these should be used along with vitamin and mineral supplementation. Kumar and Singh [7] have also reported about the use of the pigeon waste in estrus induction. They fed 100 gm dried pigeon waste for 3 days to anestrus cows and heifers and successfully induced estrus in 40% cows and 44% heifers. This may be due to the high iron, zinc, and other mineral content in the pigeon waste.

4.1.2 Mineral supplementation

Minerals have an important role in the reproduction of domestic animals, and their deficiency can cause several reproductive disorders. Deficiency of calcium is very common in postpartum cattle. Any alteration in Ca:P ration can affect the pituitary secretion and subsequently ovarian function [8]. This can cause delayed puberty, irregular estrus, etc. The optimum ratio of Ca:P should be within 1.5:1–2.5:1. Excess calcium is also harmful as it can disturb the absorption of other minerals. Phosphorus is a very important mineral for the normal reproduction. In the case of phosphorus deficiency, several disorders can be observed like delayed maturity, low conception rate, inactive ovary, etc. [8]. There are reports of other reproductive problems in areas with phosphorus deficiency. It includes silent estrus, delayed puberty, irregular estrus, and long inter-calving period [9]. Sodium and potassium are also necessary for maintaining normal reproductive physiology and energy metabolism, though excess consumption of potassium can cause a problem. Other trace minerals like zinc, selenium, cobalt, iodine, chromium, etc. also have a prominent role in the reproduction of domestic animals. Animals can come into anestrus if proper nutrition is not provided. So, feeding management should be the first approach to prevent the problem of anestrus. Minerals should be supplemented in optimum quantity. The use of area-specific mineral mixture should be encouraged.

4.1.3 Uterine and ovarian massage

It is the most economical method for the treatment of anestrus. In this method, gentle massage of the uterus and ovary is done perrectally. There are reports which state about its utilization in estrus induction. There is no clear mechanism of action of this method. Possibly, it can be attributed to increased blood circulation on the surface of the ovary and stimulation of ovarian intrinsic factors [10, 11]. Application of this method needs experts who have a good idea about the anatomy of female reproductive system.

4.1.4 Lugol's iodine

Intrauterine application of Lugol's iodine can effectively induce estrus in cattle, buffalo, etc. [12, 13]. A dose of 20–30 ml is sufficient for treatment. It also shows a good conception rate with cost-effectiveness. It actually acts as uterine irritant and increases blood supply there. It can also stimulate the hypothalamus for the secretion of GnRH, and thus the reproductive cycle is regained [13].

4.2 Hormonal treatment

4.2.1 Estrogen

Estrogen is a very important hormone for the reproductive cycle of the animals. Administration of estrogen can help the animal to come into estrus [1], though it may be ovulatory or anovulatory. If a dominant follicle is present in the ovary, there will be ovulation. If no dominant follicle is present, it can be anovulatory. Estrogen promotes the ovulation through LH surge as estrogen shows a positive feedback effect toward the pituitary at this time. Use of estrogen is limited nowadays due to its side effects. Prolonged administration of estrogen can cause cystic ovary, peristalsis of the oviduct, etc. [1]. These can also lead to several infections like ovaritis, adhesion, etc.

4.2.2 Progesterone

Progesterone is secreted from the corpus luteum in a normal estrus cycle. With the decrease in the progesterone level, the follicles start growing. The same situation can be mimicked externally. Progesterone can be administered externally for a certain duration, and its withdrawal can cause induction of estrus. Several intravaginal progesterone-releasing devices are available. It includes CIDR (controlled internal drug release), PRID (progesterone-releasing intravaginal device), etc. Ear implant of progesterone is also available. These devices are generally used for 7–9 days and can be combined with other hormones like GnRH, prostaglandin F₂ α (PGF₂ α), etc. [1]. Other ways to use progesterone are oral progesterone compound and intramuscular injection.

4.2.3 GnRH

GnRH and its analogues can be successfully used to induce estrus in animals. It induces ovulation, if mature follicle is present by inducing the LH surge. GnRH can improve conception at the timed artificial insemination (AI) after estrous synchronization with prostaglandin F₂ α [14]. GnRH given after PGF may enhance fertility through its direct or indirect (via LH secretion) action on the ovulatory follicle, and it may act in a similar fashion at insemination after spontaneous estrus [15]. Gonadotropin-releasing hormone improved fertility at first postpartum inseminations in some studies [15], but not all investigations [16]. Increasing progesterone after insemination may be one way to improve fertility in cattle. It is possible that LH released by GnRH could enhance fertility through its effects on luteal function [17].

4.2.4 Prostaglandin

For persistent corpus luteum and subestrus, PGF₂ α is the treatment of choice. Successful management of silent estrus in cattle and buffaloes can be done by the

natural or synthetic analogue of PGF₂ α as a single dose with a reasonable degree [18, 19]. PGF₂ α is only effective between days 6 and 16 of the cycle and in the presence of active corpus luteum. Administration of 25 mg of natural PGF₂ α intramuscular or 250–500 μ g of synthetic ones is required to regress the CL in both cattle and buffaloes [1]. Pursley et al. [20] described Ovsynch protocol may be used to treat subestrus or unobserved estrus.

4.2.5 *Insulin*

Encouraging results have been found in the use of insulin for induction of estrus in animals either alone or in combination [21–23]. The recommended dose is 0.25 IU/kg body weight subcutaneously for 3–5 days. Promising results for management of anestrus in cattle have been observed with the use of GnRH or eCG pretreated with insulin [22, 23] and buffaloes [21, 24]. Insulin enhances the follicular growth in true anestrus buffalo which is a prerequisite for GnRH to be effective [24].

4.2.6 *Anti-prolactin*

Summer anestrus in buffaloes could be due to hyperprolactinemia, with this assumption bromocriptine, an anti-prolactin drug [25], has been used. Melatonin is also known to suppress prolactin secretion [26]; however, melatonin has been reported as stimulator of both GnRH and gonadotrophin secretions in buffaloes. As the plasma concentration of melatonin is low during summer, induction of estrus and ovulation by using melatonin implants have been reported by Ghuman et al. [27] in all treated summer anestrus buffalo heifers; however, the time taken to induce estrus and ovulation was highly variable (4–36 days).

5. 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 [28]. 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 F₂ α [20]- and progesterone [29]-based synchronization program has shown a novel direction in the estrus synchronization of cattle with the follicular development manipulation.

5.1 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:

1. Prostaglandin administration to regress the corpus luteum of the animal before the time of natural luteolysis
2. Progesterone or synthetic progestin administration to suppress ovarian activity temporarily
3. 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.

5.2 Methods of estrus synchronization

5.2.1 Prostaglandin treatment

Luteolytic agent such as prostaglandin F₂ α , or an analogue, which causes the regression of the corpus luteum can be used to synchronize estrus [30, 31]. Administration of PGF₂ α 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.

- a. 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.
- b. 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.

5.2.2 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 [36].

5.2.2.1 Techniques of progesterone treatment

- a. MGA feeding: MGA was added to feed such that females received 0.5 mg/head/day for 14 days and if MGA was administered, cyclic females begin to show estrus. This estrus was subfertile, and it was recommended that females should be bred on the second estrus following MGA removal [37].
- b. Syncro-Mate-B (ear implant) treatment late in the estrous cycle (>14 days) in cow gives lower conception rates. The ideal time for SMB treatment to begin is between the 8th and 12th day of the estrous cycle to maximize estrus response.
- c. Application of CIDR.

CIDR insert for cattle is made by molding a thin layer of silicon and progesterone mixture (10% w/w) around a nylon spine under high temperature. It contains 1.38 g progesterone and is designed to maintain higher blood concentrations of progesterone to at least 2 ng/ml for up to 10 days. The CIDR is easily inserted into the vagina and has good retention capacity (2.5% loss rate is normal); a flexible nylon tail is attached to it for easy removal. The CIDR provides an exogenous source of the progesterone, and its removal on treatment day 7 results in a rapid fall in plasma progesterone levels, which results in estrus synchronization in those animals responding to treatment.

5.2.3 GnRH-based treatment

Estrus synchronization and fertility with a combination of GnRH and prostaglandin F₂ α are good for cyclic females, and this combination may induce cyclicity in cows experiencing postpartum anestrus [20]. 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 [20]. 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 α 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:

- a. 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.
- b. 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 PGF₂ α 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 PGF₂ α , 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 anestrous 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.

c. Progesterone in combination with GnRH-PG:

Oral administration of MGA to the cows for 14 days is performed, and 10 days after the withdrawal of MGA, GnRH injection was given. PGF₂ α is given after 7 days of GnRH injection. Patterson et al. [29] reported that 80% of the cows showed estrus within 48–96 hours after PGF₂ α injection.

5.2.4 CIDR to GnRH-based protocol

Failure to synchronize cyclic animals appropriately or to induce fertile ovulation potentially in anestrous females can have major effects on the success of a synchronization program. This CIDR to GnRH-based program has the potential to decrease losses in each of these areas. The most common use of this system comprises insertion of the CIDR on day 1 and its withdrawal on day 8. GnRH injection is given on the day of CIDR insertion and CIDR is kept in situ for 7 days. Injection of prostaglandin is given on the day of CIDR withdrawal, and then the second GnRH injection is given after 2 days of prostaglandin injection.

The primary advantage of inclusion of the CIDR in this program is that it guarantees that females will be exposed to progesterone during the period between day 1 and day 8. This progesterone exposure will result in normal (21 days) rather

than short (10 days) cycles in earlier anestrous cows. Moreover, the withdrawal of a progestin has been demonstrated to induce onset of cycles in some anestrous females; the likelihood of an ovulation is enhanced. A second advantage to inclusion of the CIDR in this program is that the early heats (day 6 to day 9) that are inherent to these systems are prevented. The progesterone released by the CIDR will prevent estrus and ovulation between days 1 and 9.

5.2.5 Managemental 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. Managemental 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.

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