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Jitendra Kumar
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Poonam Yadav
Division of Physiology and Climatology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Shivika Chouksey
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Atul S Rajput
Livestock Production Management Section, Indian Veterinary Research Institute, Bareilly, India

Corresponding Author
Jitendra Kumar
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Anestrous in cattle: A review

Jitendra Kumar, Poonam Yadav, Shivika Chouksey and Atul S Rajput

Abstract
Our country is largest milk producer in the world. Anestrous is one of the most commonly occurring reproductive problems in cattle in India, affecting livestock productivity and economics to a great extent. It is a functional disorder of the reproductive cycle which is characterized by absence of overt signs of estrus manifested either due to lack of expression of estrus or failure of its detection. Anestrous is observed in post pubertal heifers, during pregnancy, lactation and in early postpartum period in adult animals. The main causes of true anoestrus include low plane of nutrition, chronic or debilitating disease, senility, seasonal change and heavy milk yield. Many hormonal preparations like GnRH, eCG, progestogen, $PGF_{2}\alpha$ alone or in combination have been tried on anoestrus animals to induce oestrus and restore the ovarian cyclicity.

Keywords: Anestrous, ovarian activity, Janova

1. Introduction

Anoestrus is the condition where female is unable to express estrus symptoms with in an expected time. It is the most common single cause for infertility in cattle. The anoestrus mainly includes ovarian inactivity (true anoestrus), Silent estrus, unobserved estrus, cystic ovarian disease and persistent CL. Ovarian inactivity is one of the important factors determining the length of calving interval. True anoestrus does not only occur as an over extension of normal post-partum acyclicity but also in other cows which have started to cycle normally after calving may relapse back in to anoestrus. The reason for true anoestrus may be low plane of nutrition, chronic or debilitating disease, senility, seasonal change and heavy lactation (Parkinson, 2001) [44]. Factors influencing the resumption of ovarian activity after calving are milk production, energy imbalance, metabolic diseases, micronutrient, the season of calving, the age or parity of cows, the type of housing, breed differences, the courses of parturition and puerperium (Opsomer, 1996) [41].

It is generally accepted that the occurrence of prolonged periods of ovarian quiescence and anovulation are mainly due to lowered plasma LH level. As it has been proven that a certain threshold frequency of LH secretion is necessary to establish ovarian acyclicity (Grunert, 1981) [22]. Factors that suppress LH pulse frequency are energy deficiency, malnutrition, stress factors, endogenous opioid peptides, suckling and lower insulin concentration (Parkinson, 2001) [44]. True anoestrus is most frequently diagnosed in high yielding dairy cows, first calf heifers which are still growing and beef suckler cows. Interaction between nutrient availability, climatic conditions and establishment of lactation are responsible for enormous fluctuation in the incidence and depth of anoestrus (Parkinson, 2001) [44].

2. Follicular dynamics in anoestrus cow

Follicular development occurred in a wave like pattern as early as 2 weeks of age, with each wave lasting 7-9 days in calves (Evans *et al.*, 1994) [19]. The regular emergence of follicular wave were not altered in early pregnant cows (Thatcher *et al.*, 1991) and also this follicular growth continues in anoestrus cattle (Spicer and Echternkamp, 1986) [68].

Follicular waves resume early (generally 10 days after calving) during the postpartum period (Savio *et al.*, 1990) [61]. It is now clear that anovulatory anoestrus in dairy cows is due to failure of dominant follicle to ovulate rather than to their absence (Roche *et al.*, 1998) [56]. Thus, anoestrus cows have recurrent increase in FSH every 7-10 days shortly after calving (7-14 days). Each new FSH increase is responsible for emergence of a follicular wave, and the decline in FSH results in selection of single dominant follicle. The dominant follicle undergoes atresia rather than ovulation due to its failure to produce sufficient concentration of estradiol to induce a preovulatory gonadotropic (LH) surge (Roche *et al.*, 2000) [55].

This condition is related to the degree of negative energy balance in early postpartum period. Negative energy balance delays the time of first ovulation through inhibition of LH pulse frequency as well as low level of blood glucose, insulin and IGF-1 that collectively restrain estrogen production from dominant follicles. (Butler, 2000) [11]. The extreme conditions like high temperature and humidity, alter the follicular waves to reduce the diameter of the dominant follicle, limit dominance and hasten the turnover of dominant follicle of the first wave, so that emergence of second wave occurred earlier (Badinga *et al.*, 1993) [8].

3. Postpartum Period in Cow

The postpartum period in cow starts with parturition and ends with complete uterine involution and resumption of cyclic ovarian activity and normal estrus expression. Disturbances during this period due to delay of uterine involution or resumption of estrus activity prolong the calving interval and reduce the lifetime reproductive and productive efficiency. Resumption of normal cyclic activity after calving requires re-establishment of an extremely coordinated hypothalamo-hypophyseal-ovarian axis. Parturition is accompanied by a marked alteration in the pituitary-ovarian relationship. In different species of livestock, estrus cycle gets suppressed during pregnancy and is reported to be restored at varying length of time after parturition. Process of uterine involution includes necrosis of the caruncular stalk, breakdown of the superficial layer of the caruncles and formation of the lochial discharges (Roberts, 1971) [54] and is reported to be generally completed within 12 days postpartum.

In cows, mean interval from parturition to complete involution of uterus reported to be 47 days (Buch *et al.,* 1995) [10]. Complete uterine involution time varies from 25-56 days postpartum as determined by palpation. However, in general, the gross and palpable *involution* of the uterus occurred from 25-30 days postpartum (Roberts, 1971) [54].

4. Role of Minerals and Vitamins in hormone production

Apart from their role in immunity, antioxidant and ionic balance property of minerals and vitamins enhances functional ability of various cells directly and indirectly affecting synthesis of steroid and gonadotroph. Vitamins and minerals are required in cow during advanced gestation and per parturient period to regain normal body function and reproduction during postpartum period.

Manganese plays an important role in synthesis of gonadal steroid (Nocek *et al.,* 2006) [40]. Manganese is required for synthesis of steroids estrogens, progesterone and testosterone (Keen *et al.,* 1990) and pituitary is also a rich source of manganese (Hidiroglou, 1979) [25]. Thus, its deficiency may lead to inhibition of cholesterol synthesis which is a precursor for steroid hormone synthesis. Corpus luteum also contains high concentration of manganese (Brown and Casillas, 1986). Copper facilitates PGE2 action probably by enhancing PGE2 receptor bindings (Georgievskii, 1981) [20]. And its deficiency affects metabolism of thyroxin hormone (Singh *et al.,* 2002) [66]. Iodine is necessary for the synthesis of thyroxin and tri-iodothyronine by thyroid gland which regulates energy metabolism, thus maintaining the basal metabolic rate, normal body development and function of reproductive organs. Hypothyroidism reduces gonadotropin output by the pituitary (Roberts 1971) [54]. Similarly, Zinc regulates FSH and LH activity (Root *et al.,* 1979) [57] and its deficiency thus alters prostaglandin synthesis which may affect luteal function (Graham, 1991) [21]. Calcium dependent mechanism is involved in delivery or utilization of cholesterol by mitochondria or by stimulating the conversion of pregnenolone to progesterone.

5. Diagnosis of anoestrus

One of the factors that increase the calving-conception interval of buffalo during the hot season of the year is poor detection of estrus. The use of teaser bulls, tail head paint, the heat watch system, radio-telemetric pressure transducers and pedometers can improve estrus detection and thus fertility.

5.1 History:

Based on the information *viz*., failure of displaying the overt signs of estrus by the animals after attaining puberty or 60–90 days post-partum; symptoms of estrus shown with cyclicity and which subsequently ceased and revert in to anoestrus. Such cases are diagnosed when presented for pregnancy diagnosis. Many times, owner's complaint that they are not able to detect estrus or have not seen any signs of estrus in that particular animal since long.

5.2 Per Rectal Examination

Pregnancy can be a prominent cause of anoestrus and therefore must be ruled out by careful examination of ovary and uterus, when any animals present for gynecological examinations. On per rectal examination, ovaries are smooth, small and inactive with the absence of corpus luteum in true anoestrus cattle and buffaloes (Agarwal *et al.,* 2004), however, follicles may develop up to prematuration stage and get atretic (Roche *et al.*, 1998; Ghuman *et al.*, 2010) [56]. Functional corpus luteum can be palpated in case of silent estrus/unobserved as well as in anoestrus due to persistent corpus luteum.

5.3 Ultrasonography

Examination of anoestrus buffaloes / cows which are not seen in oestrus for 60 or more days postpartum at 12 days revealed 45% inactive ovaries (true anoestrus ovarian pathology which is not accurately determined by per rectal palpation can be diagnosed by ultrasonography. Different stages of follicular growth and type of anoestrus can easily be detected by ultrasonography. Transrectal ultra sonographic can be used to detect 55% silent ovulation or missing heat (Rahman *et al.,* 2012). It can also differentiate between persistent follicle and persistent CL.

6. Treatment of anoestrus

6.1 Commercial herbal formulation Janova

Feeding of Janova, herbal preparation to 20 postpartum anoestrus cows induced estrus in 75% cows as against 28.6% of control animals (Singal *et al.*, 1995) [64]. Janova (Dabur Ayurvet) formulations are potent combination of herbs formulated scientifically to induce ovarian activity. The proposed mechanism of action is similar to gonadotrophins. Though, a very high success rates for inducing ovulatroy estrus and subsequent conception were reported.

Pugashetti *et al.,* (2009) [47] observed that Janova herbal preparation group of animals showed estrous after 12.8 days of administration. The estrous response was 75% but Ahmed *et al.,* (2003) [4] have reported 43.39% with Janova. The first postpartum, estrus day was 72.8 days from calving. Number of services per conception required was 2.00 and percentages of animals conceived were 62.50%. They also recorded the efficacy of Janova on induction of estrus and subsequent conception rate which was little lower (72.80%), than the finding of Ahmed *et al.*, (2003) [4], Panchal *et al.* (1996) (75.55%) and little lesser (60%) than Singal (1995) [64] and Srivastava, (1997). Animals with Estrona, showed estrus after 13.8 days and estrus signs were well pronounced. First postpartum estrus day was recorded as 73.8 days from calving. Numbers of services per conception were 2.25 and animals conceived were 62.5%. The number of animals observed in estrus were higher (85.5%) compared to all groups.

Deshpande and Sane, (1977) treated 32 anoestrus cows of Gir breed using Prajana capsules and 10 anoestrus cows using Heatrone (5 gm per cow twice daily for 15 days) and found that percentage of induction of estrus were 84.3 and 60% with mean interval of 42.72 and 12.3 days, respectively.

Sawale and Dhoble, (1999) [62] used 25 gm of Heat quick powder as a result dose to induce estrus in 20 anoestrus cows and reported that 85% of treated animals exhibited estrus in an average of 23.64 days with 50% conception rate. They also treated 20 anoestrus cows using one Estrona forte bolus per cow daily for 10 days orally and observed that 70% cows exhibited estrus with mean interval time 14.53 days and conception rate 50%.

Sawale and Dhoble, (1999) [62] again treated 20 anoestrus cows giving 3 Sajani capsules per cow per day for 2 days orally and reported that 70% of cows exhibited esturs in mean interval time of 17.28 days with 72.2% conception rate. They treated 20 anoestrus cows using one Hitali capsule per cow for 2 days along with one spoonful Metrali powder orally for 7 days and observed that 75% cows exhibited estrus with mean interval time 14.53 days and conception rate 50%.

Dhavale *et al.* (1998) treated 20 rural postpartum anoestrus buffaloes using single dose of 50 gm of MAU, a ayurvedic product, orally and found that 60% of treated animals exhibited estrus with mean interval of 14.08 days. These herbal drugs do not interfere with the normal reproductive cycle, have no harmful side effects and mode of administration is simple, practicable and economical.

Anoestrus in cows can overcome when they are fed with the fenugreek (*Trigonella foenum-graecum*) powder (Mishra *et al.*, 2002) [35]. Approximately 2-3 kg unripe papaya (*Carica papaya*) fruits were chopped and the pieces were fed to the animal once a day for 4-5 days to bring the animal into heat. Cucumber leaves are fed to the animal to bring it to the regular heat. *Aegele marmelos* (bili leaves) are fed over 6-7 days to animals to overcome anoestrus problem. *Leptadenia reticulate*, *Asparagus racemosus*, *Couroupita guianesis* are for the same purpose.

6.2 Homeopathic therapy

Homeopathy is one of the fastest growing alternative medicines available today. Veterinary homeopathy has a tradition almost as long as the use of homeopathic drugs in humans. Homeopathic drugs such as Sepia have been evaluated in the management of anoestrus cows. Homeopathic combination remedies are also increasingly popular. Recently, encouraging results with a homeopathic combination remedy in management of anoestrus have been reported in dairy cows and buffalo.

A pilot study using sepia for anoestrus in 101 cows, gave encouraging results (Williamson *et al.*, 1991) [81]. Upadhyay (1995) [78] reported that sepia was effective in the treatment of pre or post vaginal prolapse in 23 cows and 16 buffaloes as a single dose treatment. Dutta (2002) [16] treated 10 postpartum crossbred cows using 0.5 ml Sepia 1M, sprayed into buccal mucosa on days 1, 2, 3 postpartum and observed that 77.78% of treated cows showed complete uterine involution compared to 66.67% of the untreated group on day 30 postpartum. Sepia was also found to be effective in enhancing regeneration of endometrium in postpartum cows.

Enbergs, (2000) [18] studied the effect of homeopathic preparations on prevention of postpartum disorders in 150 cows by administering Traumeel, on day 1 postpartum and further treatment using *Lachesis compositum* and *Carduus compositum* in trial 1, *Ovarian compositum* and *Hormeel* in trial 2 given on days 7 and 14 postpartum and found that in treated groups cycle began earlier, the luteal phase was less prolonged and there were fewer silent heater.

Hummelchen (2002) [26] treated periparturient dairy cows with two homeopathic preparations *Cardus compositum* and *Coenzyme compositum* and reported that there were improvement in the following: expulsion of afterbirth, endometritis, parturient paresis, ketosis, artificial insemination results and culling rate in treated groups. Mata (1994) treated 16 buffaloes with history of vaginal prolapse using *Podophyllum 200*, 10-15 sugar granules soaked with this drug were given orally thrice daily till recovery and reported that 12 animals (75%), recovered successfully.

6.3 GnRH-PG-GnRH Regimen

The GPG protocol are now commonly used which improves conception rates as more cows are synchronized by the GPG programme (Moreira *et al.*, 2000; Navaukraw *et al.*, 2004; Bello *et al.*, 2006) [37, 39, 9]. The response to GPG programme in cyclic cows is affected by stage of the cycle at initiation and presence of a functional corpus luteum at the time of the PG injection (Vasconcelos *et al.*, 1999; Moreira *et al.*, 2000) [37]. 'GPG' programmes involving injection of GnRH, followed 7 days later with injection of $PGF_2\alpha$ and then two days later with another GnRH treatment, and with or without set time artificial insemination 12-24 h after the final GnRH treatment have been used to synchronise oestrus in dairy and beef cattle (Stevenson *et al.*, 1996; 1999; Lamb *et al.*, 2001) [71, 72, 29]. The response to these programmes in anovulatory cows and heifers is variable (Stevenson *et al.*, 2006) [73]. Only 50 to 57% of anovulatory cows have elevated progesterone 7 days later, indicating either a failure of ovulation to the initial GnRH injection or a shortened luteal phase (Lamb *et al.*, 2001; Moreira *et al.*, 2001; Stevenson *et al.*, 2006) [29, 38, 73] and similarly only 40 to 55% of mixed ovulatory status cows ovulated after the first GnRH in other studies (Navanukraw *et al.*, 2004; Bello *et al.*, 2006) [39, 9]. Fewer anovulatory cows ovulated after the final GnRH in a GPG programme than cycling cows (84% vs. 91%; Stevenson *et al.*, 2006) [73] and only 50% of cows not ovulating to the first GnRH injection ovulated following the second (Moreira *et al.*, 2001) [38]. However, those previously anovulatory cows that ovulate to both the first and second GnRH injections of a GPG programme have similar fertility to cycling cows (Moreira *et al.*, 2001) [38]. Only 53% of previously anovulatory cows treated with GPG had elevated P4 concentrations 14 to 17 days after the completion of the programme again indicating either failure of ovulation or a short luteal phase (McDougall *et al.*, 2001) [34]. In comparison 89% of anovulatory cows treated with progesterone and oestradiol benzoate (OBD) had elevated P4 concentrations 14 days after treatment (Rhodes *et al.*, 1999). In another study 5 vs. 11% of cows had

progesterone concentrations <1 ng/ml at 14 days after treatment in GPG and progesterone vs. GPG groups (Melendez *et al.*, 2006). In mixed groups of cycling and anovulatory cows GPG programmes have not resulted in improved fertility compared to insemination on oestrus detection alone in a number of studies (Cordoba and Fricke, 2002; Gumen *et al.*, 2003). Specifically, in anovulatory cows, GPG did not improve any measure of fertility compared to insemination on detection of estrus (Gumen *et al.*, 2003).
Additionally, GPG was not found to be to be superior to double PG treatments in one meta-analysis (Rabiee *et al.*, 2005) [49]. Treatment of anovulatory cows using progesterone and eCG or oestradiol benzoate (OBD) were not found to be different from no treatment in another meta-analysis (Rabiee *et al.*, 2004) [48]. However, a subsequent study found improved 4 week in-calf rate and a shorter interval from start of breeding to conception following progesterone and ODB treatment of anovulatory cows compared to no treatment (McDougall and Compton, 2005) [33].
Commencement of the GPG protocol are now commonly used which improves conception rates as more cows are synchronised by the GPG programme (Moreira *et al.*, 2000; Navanukraw *et al.*, 2004; Bello *et al.*, 2006) [37, 39, 9]. The likely mechanism that the pre-synchrony ensures that the GPG programme commences when more cows have a first wave dominant follicle of sufficient size to be responsive to the GnRH and hence will ovulate (Vasconcelos *et al.*, 1999) [80]. It should be noted that in true anovulatory cows no corpus luteum is present before the commencement of the GPG programme (by definition) and hence PG pre-synchrony is unlikely to improve the efficacy of GPG in these cows (Moreira *et al.*, 2001) [38].

7. Biochemical Profile

Like any other system of the body the reproductive system is also dependent on the biochemical constituents for maintaining normal functioning and thus an abnormal change in the level of any such biochemical constituents is liable to impair the reproductive performance and health. Thus, investigation and evaluation of such biochemical constituents could be helpful in assessing reproductive performance in postpartum animals.

7.1 Total Protein

In cow, Hamana and Usui, (1972) [24] reported non-significant change in total serum protein in early and mild pregnancy but a drop of 14% by 8th month post parturition, while Pestevsek *et al.*, (1980) [49] in cows, also showed non-significant change in serum protein before and after parturition. Ranjhan and Pathak (1979) [53] showed the importance of protein for growth and development of fetus, fetal membrane and mammary gland, and also its action as a reserve for milk production in early lactation in buffalo. Bond and Wiltbank (1970) showed that protein levels do not affect birth weight of calves however, showed effect on milk production. Patil and Deshpande (1979) [45] reported the role of protein in expression of early postpartum estrus in Gir cows. Osman *et al.*, (1988) [42] reported that higher protein level adversely affect reproduction in Friesian cows.
Agrawal *et al.*, (2015) observed that the serum total protein concentration was higher in the normal cyclic animals than in the anoestrus, but the difference was non-significant (P>0.05) whereas, Sabasthin *et al.*, (2012) observed that the total protein values were significantly lower in the repeat breeding animals compared to the regularly cycling cows.

7.2 Albumin

Serum albumin concentration showed changes at parturition in high yielding dairy cows. Treacher *et al.*, (1976) noted about 10% decrease in its concentration in periparturient dairy cow, while Stout *et al.*, (1976) [74] found higher concentration of serum albumin at calving between day 1 and 90 post calving in Holstein cows. Little (1974) [30] and Rowlands *et al.*, (1975) [58] showed that serum albumin concentration fall shortly after calving and then increased gradually during the first few months of lactation in dairy cows. Mahour *et al.*, (2011) [31] recorded that the level of serum albumin was significantly higher (P< 0.05) in anoestrus cow as compared to induced cows.

7.3 Calcium

The altered dietary calcium: phosphorus ratio affects the reproductive performance of animals. Calcium dependent mechanism is involved in steroid biosynthesis in ovaries (Shemash *et al.*, 1984). GnRH stimulation of LH release from pituitary cells involved in calcium dependent manner and the LH was not released in the absence of certain calcium concentration (Hurley and Doane, 1989) [27]. Joe Arosh *et al.*, (1998) reported that the concentration of serum calcium was significantly lower (P< 0.01) in anoestrus cows (8.98 + 0.38 mg%) than that of normal cyclical Jersey crossbred cows (10.71±0.36 mg%). Kalita *et al.*, (1999) observed the mean concentration of calcium in normal cycling, repeat breeder and anoestrus cows to be 11.23± 0.36, 10.02±0.28 and 9.75±0.3 mg% respectively.
Tandle *et al.*, (1977) [76] did not observe any significant difference in level of serum calcium between cyclical (10.07±0.31 mg%) and anoestrus non-descript cows (9.33±0.01 mg%). Similarly, Ramakrishna (1997) [51] also reported that there was no significant difference between cyclical (9.58±2.27 mg%) and anoestrus crossbred jersey cows (9.40 ±2.72 mg%). Samad *et al.*, (1980) [59] estimated serum calcium level in anoestrus heifers with genital hypoplasia and postpartum anoestrus cows with non-functional ovary and observed that there were no significant difference in level of serum calcium in anoestrus heifers (9.10 mg%), cows (9.25 mg%) and normal cycling cows (9.60 mg%). Kumar *et al.*, (1986) studied the level of serum calcium level in anoestrus cows and heifers and reported that calcium level were markedly less in anoestrus cows and heifers (7.44±0.26mg% and 7.05±0 30mg%) than normal cyclical cows and heifers (8.98±0.22mg% and 9.20±0.34mg%). Vadnere and Surendra Singh (1989) [79] also reported that serum calcium level in anoestrus crossbred cows was 9.667±0.28mg/dl, which was significantly lower than the level in normal cycling crossbred cows.
Agrawal *et al.*, (2015) observed that the calcium concentration was significantly higher (P< 0.01) in normal cyclic than anoestrus. EI-Shahata and Maatyb (2010) [17] found that calcium play a key part in improving the number and size of ovarian preovulatory follicles as well as the ovulation rate. Low calcium level in acyclic animals might be due to failure to maintain normal calcium level as a result of some metabolic disturbances or due to an increased calcium excretion.

7.4 Total Cholesterol

Zaman *et al.*, (1985) [83] and Butler (2000) [11] reported that the urea and cholesterol levels decreased as the animals approached towards cyclicity. Increased urea concentration leads to impaired fertility in cows as higher plasma urea concentrations interfere with normal inductive actions of progesterone on the microenvironment of the uterus thereby causing suboptimal conditions for the support of embryo development (Butler, 2000) [11]. The utilization of cholesterol for optimum steroid hormone biosynthesis to maintain the cyclicity may be correlated with lower level of cholesterol in cyclic animals (Zaman *et al.,* 1985) [83].

Dutta *et al*., (1988) [15] suggested that high cholesterol content in normal cyclical animals compared to anestrus cattle could be an indicator of enhanced steroid secretion. Pereek and Dean (1985) also pointed out that hypo-cholesterolemia might lead to improper output of steroids. The reduced level of total cholesterol during anestrus state in the present study suggested that it might have had a role in normal reproduction in buffaloes. Mahour *et al.,* (2011) [31] observed the mean total cholesterol level was significantly higher in anoestrus cows in comparison to normal cyclic cows. Whereas Ramakrishna (1997) [51] observed that cholesterol level was significantly higher in normal cycling crossbred cows as compared to anoestrus cows.

7.5 Blood Glucose

Glucose is one of the most important metabolic substrates required for proper function of reproductive processes in beef cows. It has been hypothesized that low blood glucose, as a result of underfeeding and low energy diets, may be linked to reduced progesterone concentrations and lower fertility (McClure, 1970) [32]. Low blood glucose may be detected by the hypothalamus; such that if glucose is not available in proper concentrations, GnRH secretion will be impaired. Therefore, it is advantageous to ensure proper nutrition, in order to increase gluconeogenesis and potentially stimulate GnRH secretion.

The plasma glucose levels of this study are in agreement with Anita *et al.,* (2004) for normal cyclic and anestrus buffaloes. On the other hand, Singh *et al.,* (2010) [65] observed that plasma glucose was not a metabolic regulator responsible for initiation of ovarian cyclicity. The hypoglycemic state in buffaloes reduced the hypothalamic-hypophyseal-ovarian axis signal transmission leading to anestrous condition.

Agrawal *et al*., (2015) observed that serum glucose level of normal cyclic cow was significantly ($P< 0.05$) higher than post-partum anoestrus animals. The serum glucose was reported to be an important factor which modulates reproduction and the same at lower level is postulates as the cause for decreased fertility rate as well as for non-cyclic (Yadav *et al.,* 1995) [82].

7.6 Phosphorus

Phosphorus is often associated with reproductive abnormalities in cattle and the marginal deficiency is found to be sufficient to cause disturbance in pituitary-ovarian axis without manifestation of deficiency symptoms and might a cause for inducing infertility (Joe Arosh *et al.,* 1981).

Anoestrus, subestrus, irregular estrus and delayed sexual maturity are the consequence of phosphorus deficiency (Blood *et al.,* 1989). Blood level below 3 mg % usually indicates phosphorus deficiency. In heifers and cows that are in fair state of nutrition, but are exhibiting anoestrus, the condition may be due to simple phosphorus deficiency or phosphorus and protein or carbohydrate deficiencies (Roberts, 1971) [54]. Eltohamy *et al.,* (1980) pointed out that low phosphorus level in serum is responsible for infertility.

Samad *et al.,* (1980) [59] estimated serum inorganic phosphorus level in anoestrus cattle due to genital hypoplasia, nonfunctional ovary and normal cyclical cows. He found that mean level were lower in anoestrus cows (3.31±0.37 and 3.42+0.62 mg/100ml, respectively) than cyclical cows (4.05+0.43 mg/100ml). Purohit *et al.* (1993) found that serum phosphorus level were lower in anoestrus rathi cows (5.24+0.89 mg/100ml) than cows at their induced estrus by using fertivet 5.65+0.66 mg/100ml).

Sangeeta Nair *et al.,* (1988) [60] stated that in normal cyclic crossbred cows, serum inorganic phosphorus is at its peak during estrus (4.84±0.23mg %) which may be due to elevated levels of estrogen. Among the abnormal cycling group, the highest level was observed in the nymphomanic cows (4.81±0.08 mg %) and the lowest level in the anoestrus cows (3.56+0.26 mg %).

Kumar *et al.,* (1986) observed that lower serum phosphorus concentration (4.23±0.81 and 3.98±0.23 mg %) was observed in anoestrus cows and heifers an that in normal cyclical cows and heifers (4.98±0.06 and 4.95±0.10 mg %). Joe Arosh *et al.,* (1998) also reported also reported that the concentration of serum calcium and phosphorus were significantly lower in anoestrus cows than that of normal cyclical jursery crossbred cows.

Tandle *et al.,* (1997) [76] recorded highest higher level of serum phosphorus in oestrus cows (4.71±0.44 mg%) than in estrus non-descript cows (3.09±0.09 mg%). Similarly, Das *et al.,* (2002a) measured significantly lower level of serum inorganic phosphorus in anoestrus cows (3.823±0.093 mg/100 ml) as compared to normal cyclic crossbred cows (5.513±0.265 mg/100 ml).

Ramakrishna (1997) [51] reported that serum inorganic phosphorus concentration was lower in anoestrus crossbred Jersey cows (4.29±0.15 mg/100 ml) than cyclic cows (5.30±0.117 mg/100 ml), whereas Sivaiah *et al.,* (1986) reported that there was no significant difference in serum phosphorus level between anoestrus cows (6.21±1.24 mg%) and estrus Ongole crossbred cows (6.36±1.50 mg%). Raj *et al.,* (2006) also studied the plasma mineral profile of anoestrus and normal cyclic Sahiwal heifers and could not find significant difference in phosphorus concentration between cyclic and anoestrus heifers, respectively.

8. Hematological profile

8.1 Hemoglobin and packed cell volume

Response to histamine release and due to antigen antibody reaction or due to increased parasitic load. Gradual return of eosinophils in blood suggests continuous stress of disease. Normal physiological value is 5-10% of total leukocyte count, optimum levels of hemoglobin (11.71±0.12 gm/dl) and PCV (33.40%) are required for the efficient transport of oxygen and they are essential for normal health and reproduction in cows. Lack of sufficient quantities of hemoglobin in blood is responsible for reduced oxygen transport to vital tissue, it causes reduced oxidation of nutrients, which in turn affect the whole cellular metabolism in gonadal cells which is metabolically more active (Swenson and Reece, 1993). If the normal level of hemoglobin and PCV are not maintained there is every possibility of upsetting the reproductive cycle and postpartum reproductive performance. Lower PCV, Hb value in cow might be due to anemic condition (Kumar *et al.,* 2006) [28]

8.2 Total Leukocyte count (TLC)

In cow, Straub (1959) related the change in leukocyte picture at parturition as typical mild to moderate in response to stress. However, in cow Moberg (1955) observed leucocytosis to be due to increase in the number of neutrophils by 50%, while lymphocyte level reaches to low at parturition. In cow, Kerr *et al.*, (1951) showed a distinct drop of lymphocyte on the day of parturition and subsequently showed gradual return to normal level by day 5 postpartum. Moberg (1955) reported TLC value to be 17,700 (11800-26700) at parturition and 10,000 (5700-18000) per micro liter of blood by 24 hours postpartum.

8.3 Differential leukocyte count (DLC)

A major function of the polymorph nuclear leucocyte (PMN) is the phagocytosis and destruction of invading microorganisms thus protecting the animal from infection. A congenital or acquired defect in PMN function results in an enhanced susceptibility to infection with bacterial or fungal infection (Baehner, 1972). However, factor affecting the activities of PMN cells during infection is a complex process.

Polymorphonuclear leucocytes have been considered as the agents responsible for the uterine defense mechanisms (Broome *et al.*, 1959). In mare, Asbury (1986) reported the significant number of neutrophils in the endometrial smear indicates inflammatory changes. Neutrophils remain active in the initial stage of inflammation. In cows, the ratio of neutrophil to lymphocyte is reported to be 2:5. Neutrophil with shift to left and persistent eosinophilia suggests mild infection while neutrophilic with relative lymphopenia and absolute eosinopenia indicate moderate to severe infection. The normal range of neutrophils in cow is 23-25% of total leukocyte. Lymphocytes show a relative or absolute decrease during the initial phase of infection and produces antibodies in acute infection with lymphocytosis. In cattle physiologically normal level ranges between 57-72% of total leukocyte count which is also the predominant leukocyte. Monocyte or macrophages have a special enzyme system that helps in counteracting pathogens prevalent in chronic disease, in cow it ranges between 3.2-4.6% of the total leukocyte count. Eosinophilia occurs in response to histamine release and due to antigen antibody reaction or due to increased parasitic load. Gradual return of eosinophils in blood suggests continuous stress of disease. Normal physiological value is 5-10% of total leukocyte count. Basophils on the other hand, indicate the inflammatory reactions by causing dilation and increased permeability of local blood vessels. In cows, basophils are rarely seen in peripheral circulation with 0.6 to 0.7 basophile of the total leukocyte count.

9. References

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