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Review Article

Anestrus in Cattle and Buffalo: Indian Perspective

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ABSTRACT

Anestrus is a functional disorder of reproductive cycle in cattle and buffalo which is characterized by absence of overt signs of estrus and also affecting the livestock enterprise to a great extent. Incidence of anestrus is more in buffalo than the cattle, and problem is severe during summer. Anestrus is a multifactorial problem but its occurrence signals the inadequate nutrition, environmental stress, uterine pathology and improper managemental practices. It can be classified based on physiological and pathological conditions of the animals. Diagnosis is based on the exploration of the different causative factors involved in. Many therapeutic agents (hormonal and non hormonal) have been used, however, as such there is no single panacea to correct it. Though, recruitment and emergence of follicular wave is usually not affected, the derangement in subsequent growth and development and ovulation of dominant follicle leads to anovulatory anestrus. In order to achieve efficient management strategies, further research is needed for better understanding of its etiology, diagnosis and therapeutics.

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INTRODUCTION

Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo in India, affecting livestock productivity and economics to a great extent. The problem is more severe in sub urban and rural areas of the country. 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. Anestrus is observed in post pubertal heifers, during pregnancy, lactation and in early postpartum period in adult animals. The condition may be associated with uterine pathology such as pyometra, fetal resorption, maceration and mummification. Expression of estrus is also influenced by seasonal changes, stress and aging. In heifers, it poses a herd problem possibly due to low plane of nutrition, stress of seasonal transition or extremes of climatic conditions. Expression of overt signs of estrus is greatly affected by heat stress in buffaloes. Modern feeding and managemental practices also accentuate the problem in commercial dairy farms. Incidence of anestrus though varies in the different managemental system but it is more in buffalo than the cattle, and especially during summer. Anestrus is a multi-causative factors associated problem but its occurrence signals the inadequate nutrition, environmental stress, uterine pathology and improper managemental practices. Diagnosis of the condition is based on the exploration of the different causative factors responsible for it. Though many therapeutic agents (hormonal and non-hormonal) have been used but as such there is no single panacea to correct it. The present review on “Anestrus in cattle and buffalo” has been described in details keeping in mind the Indian perspective.

INCIDENCE

A large variation on incidence of anestrus has been reported in literatures depending upon species, breed, parity, season, level of nutrition, managemental conditions, geographic environment (Table 1 and 2). In general, incidence in India has been reported between 2.13–67.11 and 9.09–82.50 per cent in indigenous cattle (Dessouky and Juma, 1973; Kodagali, 1974; Luktuke and Sharma, 1978; Pargaonkar, 1978; Singh et al., 1981; Rehman et al., 1990; Iyer et al., 1992; Narladkar et al., 1994; Singh, 2003; Yadav et al., 2004; Selvaraju et al., 2005; Thakor and Patel, 2013) and buffaloes (Kodagali, 1968; Luktuke et al., 1973; Singh and Verma, 1994; Nanda et al., 2003; Prajapati et al., 2005; Singh et al. 2006; Modi et al., 2011; Kumar et al., 2013; Thakor and Patel, 2013), respectively. The incidence of anestrus among crossbred cattle has been reported between 2.55–40.4 per cent (Dessouky and Juma, 1973; Narladkar et al., 1994) from different parts of the country. Its incidence in heifers has been reported between 12.37 to 64.66 per cent (Luktuke and Sharma, 1978; Naidu and Rao, 1981; Nayak and Mohanty, 1986; Sinha et al., 1987). Incidence of anestrus is higher in adult cattle and buffalo than the heifers (Bharkad and Markandeya, 2003).

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Table 1: Incidence of Anestrus in cattle and buffalo in India state-wise pattern

States	Cattle Incidence (%)	References	Buffalo Incidence (%)	References
Andhra Pradesh	49.70	Rao, 1993	30.76–50	Rao and Sreemannarayanan, 1982; Naidu, 2004
Gujarat	24.73	Patel et al., 2007	20.84 – 45.97	Kulkarni, 2002; Modi et al., 2011
Madhya Pradesh	53.15	Pandit, 2004	29.12 – 60. 83	Pandit, 2004; Kumar et al., 2013
Maharashtra	2.13–45.97	Narladkar et al., 1994; Kulkarni et al., 2002	29.5–41.40	Bharkad and Markandeya, 2003;
Tamilnadu	16.6	Selvaraju et al., 2005	9.09	Selvaraju et al., 2005
Punjab	43–67.11	Singh et al., 2003	38.98–55.5	Singh et al., 2006
Bihar	39.01	Singh, 1981	–	–
Kerala	65.0	Kutty and Ramachandran 2003	–	–
Kashmir	27.52 & 31.0	Rehman et al., 1990; Bhattacharyya and Buchoo, 2008	–	–
Utter Pradesh	–	–	14.69–45.20	Luktuke et al., 1973
Karnataka	–	–	56.0	Hussain, 1984

Table 2: Incidence of anestrus in India– breeds wise

Breed	Incidence (%)	References
Hariyana cows	0.6–12.13	Khan and Luktuke, 1967
Kankrej cows	11.8–23.8	Agarwal and Buck, 1968
Gir cows	47.89	Kodagali, 1974
Red Kandhari cows	3.0	Pargaonkar and Bakshi, 1987
Deoni cows	2.13	Narladkar et al., 1994
Friesian crossbred	35.96	Sinha et al., 1987
Jersey crossbred	35.5	Sinha et al., 1987
Murrah and upgraded Murrah buffaloes	29.12	Kumar et al., 2013

The period of postpartum anoestrus is usually longer in buffalo than the cattle under similar management conditions (Dobson and Kamonpatana, 1986; Jainudeen and Hafez, 1993). In comparison to cows, buffaloes have lesser number of preantral and antral follicles, smaller sized pre–ovulatory follicle and greater tendency of follicular atresia (Samad and Nasseri, 1979; Danell, 1987; Baruselli et al., 1997) which might be responsible for high incidence of anestrus in buffaloes.

ECONOMIC IMPACT

High milk production and excellent fertility are desirable traits for a profitable dairy enterprise. Infertility due to cyclicity failure or anestrus has great economic impact. Anestrus, leads to economic losses through increased intercalving interval, poor net calf crops, production loss, treatment expenses and cost of replacing mature animal with first calving heifer. There are only few reports from India pertaining to economic impact analysis due to anestrus. Pawshe et al. (2011) reported an estimated loss from anestrus around Rs.193.00 per day in cow and Kumar et al. (2013) reported Rs. 372.90 per day in buffalo. As the incidence of anestrus in India has been reported high, the above figures show great economic impact at country level.

CLASSIFICATION OF ANESTRUS

Based on ovarian activity, anestrus cow has broadly been classified into ovulatory, anovulatory and inactive. Robert (1971) divided the anestrus cow or heifer into two class viz., Class I–cow with a normal functional corpus luteum and Class II–cow with no functional corpus luteum. Wiltbank et al. (2002) classified the anovulatory anestrus into three classes i.e. anovulation with follicular growth up to emergence; anovulation with follicular growth up to deviation but not ovulatory one; anovulation with follicular growth up to ovulatory size. Further, Peter et al. (2009) divided anestrus into four types viz., Type–I, characterized by emergence of follicular wave and growth of follicle up to pre deviation stage; Type–II, characterized by deviation of follicle and follicle grow up to dominance stage followed by atresia; Type–III, characterized by persistent dominant follicle (persistent of large follicle) i.e. follicle grow up to preovulatory or ovulatory size or more but fails to ovulate or regress (follicular and luteal cyst) and Type–IV, characterized by normal ovulation and formation of corpus luteum but corpus luteum persist beyond the expected time of regression (persistent corpus luteum, PCL) resulting into anestrus.

Based on the above informations and for the ease, anestrus has been classified accordingly for better understanding (Figure 1).

PHYSIOLOGICAL ANESTRUS

Animals remain anestrus during certain physiological stages which does not related to infertility viz., before puberty, during pregnancy, lactation and early postpartum period. Accordingly, physiological anestrus has been classified into pre–pubertal, gestational, lactational and post-partum anestrus.

PREPUBERTAL ANESTRUS

The follicular waves in pre–pubertal animals are similar to that of adult but follicles grow in response to FSH secretion only up to the stage where they have a theca interna and then regress. Such heifers remain in anestrus before the onset of puberty. The reasons of pre–pubertal anestrus includes low LH pulse frequency that results in insufficient growth of follicles; inhibitory effect of opioids on LH secretion and high threshold for positive feedback effect of estradiol on LH surge (Noakes et al., 2009).

GESTATIONAL ANESTRUS

The elevated level of progesterone during pregnancy exerts negative feedback effect on GnRH secretion from hypothalamus and reduces LH pulse frequency resulting into anestrus. However, some cattle and buffaloes exhibit estrus during early pregnancy (known as gestational estrus) which is seen most often during first four months of pregnancy. The incidence of gestational estrus has been recorded as 3.33 to 20.3% and 6.05 to 14.40% in Indian cattle (Luktuke et al., 1964; Chauhan et al., 1976; Kaikini and Fasihuddin, 1984) and buffaloes (Luktuke et al., 1964), respectively. Usually cow or buffalo exhibits gestational heat only once during pregnancy, however, few animals show twice or thrice in same gestation.

POSTPARTUM ANESTRUS

Following parturition, all the females undergo through anestrus for a variable but short period of time, known as postpartum anestrus. The period of postpartum anoestrus is usually longer in buffalo than the cattle under similar management conditions (Dobson and Kamonpatana, 1986; Jainudeen and Hafez, 1993), probably due to low LH secretion during early postpartum period (Perera, 2011). Under normal conditions, buffaloes resume cyclicity by 30–90 days (Perera, 2011), however; only about 45% of Indian buffaloes resume cyclicity within 90 days postpartum and rest 55% remain in anestrus for about 150 days (El–Wishy, 2007). Most of the dairy cows resume ovulatory estrus cycle within 15–45 days postpartum (Butler and Smith, 1989; Forde et al., 2011). The physiological postpartum anestrus cannot be avoided and is useful to allow uterine involution prior to first postpartum anestrus.

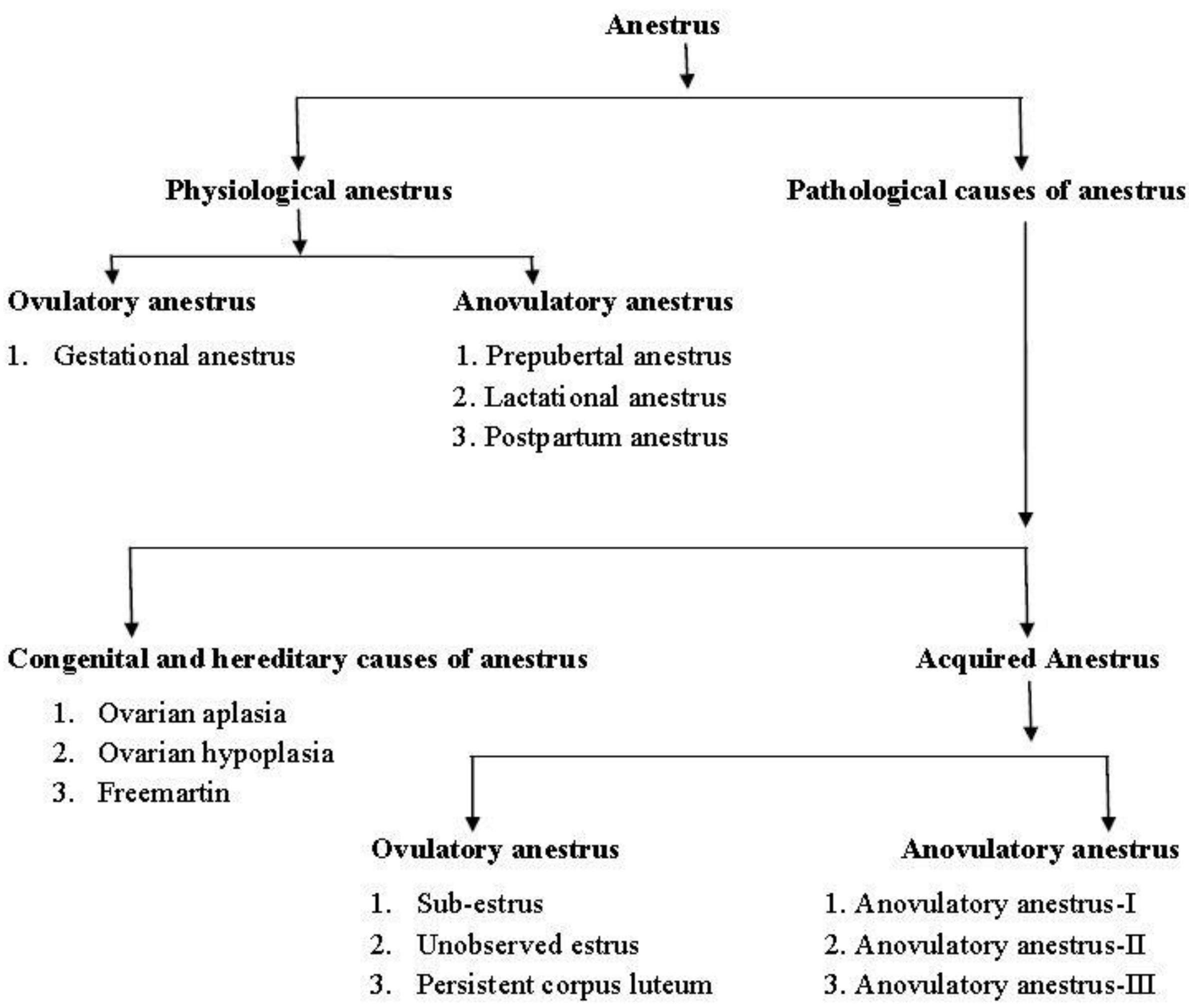

Figure 1: Classification of anestrus in cattle and buffalo

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LACTATIONAL ANESTRUS

High lactation suppresses the fertility in almost all the mammals (Warnick et al., 1950; Baker et al., 1953). Higher level of prolactin in high yielding animals suppresses GnRH secretion and ultimately reduces production of gonadotrophins from pituitary, resulting into anestrus.

PATHOLOGICAL CAUSES OF ANESTRUS

Certain pathological conditions i.e. ovarian agenesis, dysgenesis or derangement of follicular–luteal dynamics leads to anestrus causing infertility and pose a herd problem. Such conditions may be congenital or acquired.

CONGENITAL AND HEREDITARY CAUSES OF ANESTRUS

Congenital and hereditary form of anestrus is upshot of ovarian agenesis or dysgenesis. Ovarian agenesis or aplasia (absence of ovary) is extremely rare condition and probably crop up due to inherited autosomal dominant gene. Bilateral aplastic or gonadless heifers appear normal until breeding age but fail to show estrus and normal development of udder at puberty and are sterile. Such reports from India are meager. Ovarian dysgenesis has been identified as ovarian hypoplasia and freemartin. Ovarian hypoplasia (incomplete development of ovary) is caused by single autosomal recessive gene with incomplete penetration. It may be unilateral or bilateral. The affected ovary is characterized by lack of primordial follicles reserve either partial (partial hypoplasia) or complete (complete hypoplasia) (Settergren, 1964, 1997). Bilateral complete hypoplastic females remain in anestrus whereas partial hypoplastic animals exhibit estrus, conceive and produce viable calves but transmit this undesirable character to the next generation therefore must be avoided. The small size of normal ovary in buffalo heifers often mistaken as hypoplastic ovaries. The incidence of ovarian hypoplasia in Indian cattle has been reported between 0.08–4.3% (Kodagali, 1969; Narasimha Rao and Murthy, 1972; Nair and Raja, 1974; Bonia, 1981; Kumar and Agarwal, 1986) whereas it is less than 1 per cent in Indian buffaloes (Damodaran, 1956; Malik et al., 1960; Narasimha Rao and Sreemannarayana, 1982; Mittal et al., 2010) with a slightly high incidence (1.46%) in Jaffarabadi buffalo (Kodagali and Kerur, 1968). Higher incidence of ovarian hypoplasia (10–23%) has been reported in exotic cattle (Lagerlof and Boyd, 1953). In Free martin (sterile heifer born co–twin with bull calf) the ovaries usually fails to develop and remain hypoplastic resulting into anestrus. The incidence of freemartinism in Indian cattle and buffaloes has been reported low i.e. between 0.10 to 0.20% (Bhagat, 1966; Narasimha Rao and Suryanarayana Murthy 1980, Sharma et al., 2004).

ANOVULATORY ANESTRUS–I

This condition is characterized by growth of follicles only to a stage of follicular wave emergence i.e. up to about 4 mm diameter and then regress (Wiltbank et al., 2002). This type of anestrus is very rare and only 0.003% cases has been reported by Wiltbank et al. (2002), however, expected to occur in about 10% of postpartum dairy cows (Zemjanis et al., 1969; Markusfeld, 1987). Ovaries of such animals are small and smooth with absence of corpus luteum and classified as "inactive ovaries" in earlier literatures. The factors responsible for growth of follicles up to this stage are poorly known, however, to be sure that these events are gonadotrophin independent. On the basis of *in–vitro* studies, various peptide growth factors and cytokines have been implicated as positive (e.g. Kit Ligand, insulin, LIF, FGF2, BMP4, BMP7 and GDF9) and negative (e.g. AMH and CXCL12) regulators of follicular growth at this stage (Kezele et al., 2002; Fortune, 2003; McLaughlin and McIver, 2009). The occurrence of anovulatory anestrus–I is possibly due to deficiency of one or more of these growth regulators presumed to occurs under extreme undernutrition or severe energy deficit.

ANOVULATORY ANESTRUS–II

This type of anestrus animals exhibit normal follicular wave pattern and follicular growth proceeds through emergence but develop only up to deviation or preovulatory stage (not ovulatory size) followed by regression and emergence of new follicular wave 2–3 days later (Roche et al., 1998; Wiltbank et al., 2002; Peter et al., 2009; Ghuman et al., 2010). This process of follicular growth and regression repeated over and again in anestrus animals. It is most common form of anestrus. The ovaries of such animals are small with the absence of a corpus luteum or ovulatory size follicles. Adequate LH pulse frequency is required for growth and development of follicle after emergence of follicular wave. The occurrence of this condition is due to low LH pulse frequency (<1/3–4hrs.).

ANOVULATORY ANESTRUS–III

In this type of anestrus, follicular growth proceeds through emergence and deviation stage and attain dominancy but fails to ovulate and become persistent. Persistent follicle may develop either into follicular cyst or luteal cyst. Follicular cysts are thin walled structure with no evidence of luteinization of granulosa cells (progesterone level <1ng/ml) and characterized by either anestrus or nymphomania (persistent estrus), whereas luteal cysts are thick walled structures with partial luteinization of granulosa cells and characterized by anestrus. This type of anestrus is up shot of absence or attenuation of LH surge (Cook et al., 1990; Hamilton et al., 1995, Peter et al., 2004) due to partial or complete failure of estradiol to bring out normal LH surge (Nanda et al., 1991). This appears to be due to insensitivity of hypothalamic surge centre to the positive feedback of estradiol (Garverick, 2007) and failure of GnRH release (Vanholder et al., 2005). Another probable causes for the development of cysts are occurrence of LH surge at an inappropriate time i.e. when follicle is not capable to ovulate (Vanholder et al., 2005) or reduced population of LH receptors on follicle (Kawate, 2004). In India, the incidence of follicular cysts has been reported 0.5–2.7% in cattle (Kulkarni et al., 2002) and 1.26–3.4% in buffaloes (Bhattacharya et al., 1954; Malik et al, 1960) and 8.0–10.2% in crossbred cattle, whereas incidence of luteal cysts are 1.45–3.0% in cattle (Luktuke and Arora, 1967; Singh, 1981; Kulkarni et al, 2002), 0.66–12.5% in buffaloes (Damodaran, 1956; Rao 1965; Kaikini, 1974; Kutty and Ramachandran, 2003) and 9.0–13.3% in crossbred cattle (Kulkarni et al., 2002). The incidence of follicular and luteal cysts abroad in dairy cattle have been reported 6–19% (Garverick, 1997) and 10–13% (Peter, 2004), respectively, however, these are less frequent in dairy heifers and beef cattle.

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ANESTRUS DUE TO PERSISTENT CORPUS LUTEUM (PCL)

In this type of anestrus, the follicular growth proceeds through all the developmental stages and undergo ovulation and CL formation which subsequently turn into anestrus due to failure of luteal regression. This is probably due to absence of estrogenic dominant follicle at the time of luteal regression (Wiltbank et al., 2002) secreting adequate estradiol to induce the formation of uterine oxytocin receptors and consequently resulting in to pulsatile release of PGF2α for luteolysis (Knickerbocker et al., 1986; Thatcher et al., 1989; McCracken et al., 1999). Persistent corpus luteum (PCL) is mostly associated with uterine pathology such as endometritis, pyometra, fetal resorption, maceration, mummification and uterine unicornis (Lynn et al., 1966; Gilbert et al., 1990; Noakes et al., 1990). Retained corpus luteum may also be associated with embryonic death when death of embryo occurs after maternal recognition of pregnancy where corpus luteum persists until resorption of embryo. In uterine unicornis, CL formed on the ovary ipsilateral to the missing uterine horn persists due to a lack of a luteolytic signal from the missing uterine horn. PCL results in anestrus due to inhibitory effect of progesterone secreted by the corpus luteum on hypothalamo–hypophyseal axis for the secretion of gonadotrophins.

SUB–ESTRUS/SILENT ESTRUS/QUITE OVULATION

Sub estrus or silent estrus or quite ovulation is clinically characterized by failure of overt symptoms of estrus, though the animal is surprisingly normal. Under these conditions, follicular development and ovulation occurs normally in animals without the manifestation of overt signs of estrus. Sub estrus is common during the post pubertal period in heifers and early post–partum (30 to 120 days) in high yielding dairy cows. Progesterone secreted from regressing CL of previous cycle potentiates the action of estrogen and seems to favours the manifestation of estrus in next cycle (King et al., 1976; Allrich, 1994). Thus, lack of progesterone priming results in sub–estrus. Such conditions have been frequently reported in dairy buffaloes especially in summer months (Sane et al., 1967; Shah, 1990; Badr, 1993; Singh et al., 2013) and may be the one of the reasons of prolonged calving interval in buffaloes (Singhal et al., 1984; Barkawi et al., 1986). The concentration of estrogen determines intensity of behavioral signs of estrus which is low in high yielding dairy cow (Lopez et al., 2004). Lower concentration of estrogen may be either due to higher metabolism and clearance with a high metabolic load (Sangsritavong et al., 2002) or sub–optimal follicular growth (Awasthi et al., 2007). The probable cause of silent estrus is sub–optimal secretion of estradiol by mature follicles or higher threshold of estrogen in central nervous system to display the symptoms of estrus in that particular individual animal. Silent estrus also appears to be hereditary in predisposition in certain breeds (Lagerlof, 1951). Other causes of sub estrus are heat stress, nutritional deficiencies, overweight, foot lesions, aging and ergotism (fescue toxicity) but most common cause considered for sub estrus is the failure of estrus detection.

ANESTRUS DUE TO FAILURE TO OBSERVE ESTRUS/UNOBSERVED ESTRUS

Estrus detection is critical aspect of dairy herd management where artificial insemination is being practiced. The length of estrous cycle and estrus period varies among breeds and within a breed (Rottensten and Touchberry, 1957; Roxström, et al., 2001). It also varies with season, nutrition, lameness, presence of bull, housing, herd size and production status (King et al., 1976; Purbey and Sane, 1978; Zakari et al., 1981; Lucy, 2003; Lopez et al., 2004; Diskin, 2008; Walker et al., 2008). Earlier, it was reported that intensity and duration of standing estrus is shorter in *Bos indicus* cattle as compared to *Bos taurus* cattle (Anderson, 1936; De Alba et al., 1961; Plasse et al., 1970; Rhodes and Randel, 1978), probably due to small follicular diameter (Lyimo et al., 2000; Bo et al., 2003). However, recent studies indicate that there is no difference in intensity and duration of estrus between *Bos taurus* and *Bos indicus* cows (Bastos et al., 2010). In high yielding cows, many times the estrus cycles become irregular (Bartha, 1971) in terms of its intensity and duration of standing estrus (Lopez et al., 2004), resulting in low estrus detection rates. The condition may be due to low estrogen concentration (Lymio et al., 2000; Lopez et al., 2004), insulin and IGF–I mediated deficiency of follicular growth (Butler and Smith, 1989; Lucy, 2001) or increase metabolism and clearance of estrogen with high metabolic load (Sangsritavong et al., 2002). The short period of estrus often fail to notice by the farmers.

PATHOGENESIS

The concentration of gonadotrophins are almost negligible in late gestation and for a short duration following parturition due to strong inhibition of hypothalamic–pituitary axis through negative feedback effect of high progesterone secreted by corpus luteum and placenta and estrogen from placenta during last trimester of pregnancy. The concentration of FSH rises within 3–5 (ranges 2 to 7) days after parturition (Ginther et al., 1996; Crowe et al., 1998) whereas restoration of LH pulsatility and LH surge mechanism takes rather longer period than the FSH (14–28 days). Emergence of follicular wave occurs within 1 or 2 days of significant rise in FSH concentration (Beam and Butler, 1997; Crowe et al., 1998), however, none of the growing follicles become mature enough to ovulate due to very low LH pulse frequency (Canfield and Butler, 1990; Montiel and Ahuja, 2005; Roche, 2006). Moreover, growth and maturation of follicle also depends upon bioavailability of insulin, insulin like growth factor (IGF)–I and their binding proteins (Butler and Smith, 1989; Lucy, 2001; Fortune et al., 2004). Both insulin and IGF–I are potent stimulators of steroidogenesis and granulosa and theca cells proliferation as well as oocytes growth and maturation (Webb et al., 1999; Lucy et al., 1999; Silva et al., 2009). Hence, inadequate LH pulse frequency, low concentration of insulin and IGF–I impede the follicular growth and reduces the chance of ovulation. These conditions appear to occur in a state of under nutrition/malnutrition (Roche, 2006; Ramoun et al., 2012) and negative energy balance (Richards et al., 1989; Beever et al., 2001). In a state of negative energy balance, the circulating concentration of non–esterified fatty acid (NEFA) due to mobilization of body reserves (Richards et al., 1989; Canfield and Butler, 1990; Grimard et al., 1995) and endogenous opioids increases which in turn decreases the pulsatile secretion of LH. In addition to these, prolactin, oxytocin, cortisol, corticotrophin releasing hormone secreted in response to

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lactation, suckling and stress also reduces the LH pulse frequency and subsequently impair the follicular growth. Ultimately, follicles become atretic and then regress. The process of follicular growth and regression occurs over and again till the above state of affairs persists resulting into anovulatory anestrus.

FACTORS AFFECTING ANESTRUS

Nutrition

Nutritional status of animals affects the follicular growth, maturation and ovulation (Diskin et al., 2003). Under nutrition is the one of the most prevalent cause of anestrus in heifers. Extended postpartum period of anestrus (>150 days) are usually observed in cattle of tropical area under free range rearing system, probably due to shortage of feed and good quality fodder. Reduced feed intake during late gestation or/and early postpartum period or negative energy balance (NEB) due to very high metabolic load following parturition especially in high yielders delays postpartum restoration of LH pulsatility, resulting into prolonged postpartum anestrus (Wiltbank et al., 1962; Rutter and Randel, 1984; Easdon et al., 1985; Nolan et al., 1988; Connor et al., 1990; Hegazi et al., 1994). Under high metabolic load, nutrients are utilized for production rather than reproduction (Ferguson, 2001). In addition to NEB, the deficiency of minerals like calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn) and manganese (Mn) are also associated with anestrus (Hidiroglou, 1979; Campbell et al., 1999). It is well established that minerals play an intermediate role in the action of hormones and enzymes at cellular level and its deficiency ultimately affect the reproductive performance of females (Bearden et al., 2004).

Occurrence of anestrus is not only due to underfeeding or malnutrition but also occurs owing to high feed intake which promotes high metabolism and clearance of ovarian steroids (estrogen and progesterone) from body by enhancing the hepatic perfusion (Sangsritavong et al., 2002), especially in high yielders. Low estrogen in high yielding animals result in sub estrus as intensity of estrus behaviors is directly related to its concentration (Lymio et al., 2000: Lopez et al., 2004). Excessive clearance of ovarian steroids also leads to anovulation (Walsh et al., 2007) and delayed luteal regression (Opsomer et al., 2000; Petersson et al., 2006).

Body Condition Score (BCS)

Body condition score is the measures of nutritional status of animals and is an important factor influencing the reproductive performance (Baruselli et al., 2001). Extremes of BCS (very low and very high) at pre–calving, calving and early postpartum period delay onset of cyclicity (Butler and Smith, 1989; Markusfeld et al., 1997; Pryce et al., 2000). However, BCS at calving is a better indicator of resumption of postpartum cyclicity than prepartum BCS (Whitman, 1975; Lalman et al., 1997). For optimum reproductive performance, BCS of 3.5 (on five point scale) is required at calving (Bhalaru et al., 1987; Ribeiro et al., 1997). Restricted feed intake during late gestation and early postpartum period result in low BCS, consequently, leads to prolonged postpartum anestrus (Dziuk and Bellows, 1983; Robinson, 1990).

Environmental Stress

Environmental stress (extreme cold and heat) affect the development of follicles and manifestation of estrus both in cattle and buffaloes. Although buffaloes are well adopted in hot and humid climate, however, ovarian activity is greatly reduced by heat stress and manifested in the form of anestrus (Singh et al., 2000). Heat stress affects folliculogenesis, follicular fluid micro environment and oocyte quality. In buffaloes, decline in feed intake during summer results in reduced secretion of gonadotrophins (El–Sawaf et al., 1979). Besides this, high environmental temperature causes hyper–prolactinaemia and suppressing the secretion of gonadotrophin which leads to alteration in ovarian folliculogenesis and steroidogenesis. Among cattle, reproductive functions are suppressed more in *Bos indicus* than *Bos taurus* during winter (Randel, 2005), however, such effect of extreme cold has not been reported in buffaloes.

Lactation

High yielding cattle and buffalo shows significantly longer postpartum anestrus period (Harrison et al., 1989; El–Azab et al., 1984) or weaker signs of estrus (Harrison et al., 1990). El–Fadaly (1980) reported that buffalo producing >8 liters milk per day had longer postpartum anestrus (107±36 days) than those producing <8 liters per day (77±30 days). However, others have reported no significant correlation between milk yield and postpartum anestrus period (El–Keraby et al., 1981; Kawthar et al., 1985).

Suckling

Suckling suppress the postpartum ovarian activity both in cattle (Wiltbank and Cook, 1958; Oxenreider and Wagner, 1971; Stagg et al., 1998; Quintansa et al., 2009) and buffalo (El–Fadaly, 1980; Honnapagol et al., 1993), resulting into extended postpartum anestrus period. Moreover, postpartum anestrus is longer in continuously suckled than restricted or partial suckled cow and buffaloes (Bastidas et al., 1984; Nordin and Jainudeen, 1991). Postpartum anestrus is longer in suckled beef cow than milked dairy cow. Suckling stimulates prolactin, cortisol and oxytocin secretion that have negative effect upon GnRH–LH axis. Higher level of these hormones suppresses the GnRH secretion and increases the concentration of endogenous opioid peptides; β–endorphin (Malven et al., 1986) thus, ultimately reduces the LH pulse frequency (William, 1990) which delays resumption of postpartum cyclicity.

Parasitic Infestations

Heavy parasitism is one of the stressful conditions and is more common in growing than in adult cattle. It affects the future productive and reproductive efficiency in infested animals (Heath et al., 1997). Parasitic infection like fascioliasis, theleriosis schistosomiasis and trypanosomiasis infection in animals cause anemia and weight loss and ultimately results into anestrus. Recently, it has been found that *Neospora caninum* infection (Neosporosis) is widely prevalent among dairy herds and has significant association with anestrus (Bruhn et al., 2013).

Genotype

The resumption of postpartum cyclicity depends upon species as well as breeds. The postpartum anestrus period is shorter in milked dairy cows as compared to suckled beef cows but suckled dairy cows have longer postpartum anestrus period than beef cows (Short et al., 1990). The period of postpartum anoestrus is usually longer in buffalo than the cattle (Dobson and Kamonpatana, 1986; Jainudeen and Hafez, 1993). How genotype affect the resumption of postpartum cyclicity is not fully understood, however, it may be due to physiological differences among breeds and

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species, difference in milk production and feed intake (Short et al., 1990).

Parity

A longer postpartum anestrus period have been reported in primiparous than pluriparous buffaloes (Ali and El–Sheikh, 1983; Barkawi, 1984). Moreover, as the parity increases, the postpartum anestrus period decreases (El–Sheikh and Mohamed; 1965; El–Wishy and El–Sawaf, 1971; Shah et al., 1989; Mahdy et al., 2001). However, others have reported there is no correlation between parity and postpartum anestrus period (El–Fouly et al., 1976; Borghese et al., 1993).

Periparturient Diseases

Periparturient Diseases such as abnormal calvings, metritis, mastitis and ketosis also influence onset of postpartum cyclicity (Fonseca et al., 1983; Opsomer et al., 2000). Delayed uterine involution also holdup resumption of ovarian activity. Postpartum uterine infection (clinical or sub clinical) suppress GnRH release and possibly LH secretion (Peter and Bosu, 1988; Peter et al., 1990; Mateus et al., 2002), probably due to inflammatory response (Sheldon and Dobson, 2004; Herath et al., 2006; Williams et al., 2007) and thus, ovarian activity remains suppressed in uterine infections.

DIAGNOSIS OF ANESTRUS

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 which subsequently ceased and revert in to anestrus. Such cases are diagnosed when presented for pregnancy diagnosis. Many times, owners complaint that they are not able to detect estrus or have not seen any signs of estrus in that particular animal since long.

Progesterone Estimation

True anestrus is usually characterized by a lack of ovarian progesterone production (Peter et al., 2009). Presence of basal level (0.5–1 ng/ml) of progesterone in the blood samples at an interval of 8–10 days further confirms the diagnosis. If the concentration of progesterone is more than 1ng/ml, it is suggestive of presence of corpus luteum and anestrus in such situation might be due to unobserved estrus/silent estrus/persistent corpus luteum.

Per Rectal Examination

Pregnancy can be a prominent cause of anestrus 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 anestrus 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). Functional corpus luteum can be palpated in case of silent estrus/unobserved as well as in anestrus due to persistent corpus luteum.

Ultrasonography

Ovarian pathology which is not accurately determined by per rectal palpation can be visualized by ultrasonography. Different stages of follicular growth and type of anestrus can easily be detected by ultrasonography. Transrectal ultrasonographic examination of anestrus buffaloes cows which are not seen in oestrus for 60 or more days postpartum at 12 days revealed 45% inactive ovaries (true anestrus), 55% silent ovulation or missing heat (Rahman et al., 2012). It can also differentiate between persistent follicle and persistent CL.

TREATMENT

Anestrus can be treated according to their cause, however; there is no single panacea to correct it. Various therapeutic agents including hormonal and non–hormonal compounds have been used extensively for the restoration of cyclicity in anestrus cattle and buffalo by several workers with varying degree of success (Glotra et al., 1970; Deshpande et al., 2000, Agarwal et al., 2001, Kumar et al., 2005). In order to ensure effective treatment, the health and nutritional status of the animals must be in good conditions. Besides deworming, the supplementation of vitamins, minerals and antioxidants in feed are useful to improve health status of the animals.

NON HORMONAL TREATMENTS

Plant Based Heat Inducers

Plants have been used for the treatment of animals since long back. Plants synthesize varieties of phytochemicals such as alkaloids, glycosides, terpenes and tannins (secondary metabolites) as a part of their normal metabolic activity and many of these have therapeutic actions when consumed by animals. Many plants are rich source of vitamins and minerals whereas some have estrogenic property which is useful in restoration of cyclicity in anestrus animals. Almost all the parts of plant such as seeds, berries, roots, leaves, bark and flowers have been used as therapeutic agents either directly (crude drugs) or their active principles, after separation though various chemical process. Many plants such as *Murraya koenigii* (curry leaves), *Nigella sativa* (kalonji), *Abroma augusta* (Ulatkambal), *Saraca asoca* (Ashoka), *Trigonella foenum–graecum* (Methi), *Bambusa aruninacea, Carica papaya, Asparagus recemosus, Leptadenia reticulate, Courupita guianesis, Pergulacia daemia, Semecarpus anacardium cucumber, and jute plants* either alone or in combinations have been fed to treat the anestrus animals with variable response on induction of estrus (Kabir et al., 2001; Das et al., 2002; Mehrotra, 2002; Mishra et al., 2002; Rajkumar et al., 2008; Kumar and Punniamurthy, 2009). Kabir et al. (2001) reported 50% estrus induction in anestrus buffaloes using mixture of *Abroma augusta* (root) and *Nigella sativa* (seed) in 2:1 ratio. Rajkumar et al. (2008) reported higher success rate in anestrus cattle i.e. 83.33 and 66.66% using Methi seed (@ 200g/day/cow for 20 days) and bark of Ashoka tree (@ 50g/day/cow for 20 days), respectively.

Indigenous herbal preparations such as Prajana HS (Indian Herbs), Janova (Dabur), Sajani (Sarabhai), Heat up (Century) Heat raj (Ranjan), Fertivet (Ar Ex Labs) and Aloes compounds (Alarsar) are commercially available and effective in restoration cyclicity with good success rates (Deshpande and Sane, 1977; Patil et al., 1983; Dutta et al., 1988; Sudhir Chandra Reddy et al., 1990; Hussain et al., 2009). These formulations are potent combinations of herbs, formulated to induce ovarian activity.

Utero–Ovarian Massage

Utero–ovarian massage is the oldest, simplest, cheapest and effective method to induce estrus in anestrus cattle and buffaloes (Romaniuk, 1973; Zduńczyk et al., 1992; Rahawy, 2009; Mwaanga et al., 2010). Estrus induction in cattle and

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buffalo varies between 40 to 80% following utero–ovarian massage daily/on alternate day/weekly for 3–4 weeks (Mwaanga et al., 2004; Naidu et al., 2009; Gupta et al., 2011). The mechanism by which ovarian massage induces cyclicity is not clearly understood, however, probable mechanism includes: activation of intrinsic intra–ovarian factors; enhancement of blood circulation to the ovaries and uterus that increases the availability of hormones and growth factors; stimulation of local oxytocin production by the ovaries which consequently influence local blood circulation and luteolysis, if CL is present (Romaniuk, 1973; Lobb and Dorrington, 1992; Monget and Monniaux, 1995; Mwaanga et al., 2010).

Lugol's Iodine

Lugol's iodine treatment is cheaper and effective means of management of anestrus but response has been variable (45 to 91.7%) among cattle and buffaloes (Porwal et al., 1976; Ryot et al., 1990; Agarwal and Pandit, 1991; Reddy et al., 1994; Megahed et al., 1995; Kendre and Bhosker, 1996; Singh and Thakur, 1999; Tapas et al., 2000; Tomar, 2004; Gupta et al., 2011). Lugol's iodine solution (5%) has traditionally been used as a cervical paint. It is presumed that painting of Lugol's iodine on posterior part of the cervix causes local irritation and brings about reflux stimulation at anterior pituitary for secretion of gonadotrophins and consequently cyclicity. Lugol's iodine is an irritating solution and intrauterine infusion of Lugol's solution (0.5 to 1.0%) causes hyperemia (enhanced circulation) of uterine mucosa resulting into degree of iodine absorption from uterus. The absorbed iodine probably increases the metabolic rate of body through stimulating the thyroid hormone secretion (Sanchez, 1995). Increased metabolic rate trigger the ovarian functions by enhancing the energy utilization (El–Shahat and Badr, 2011). Injectable Lugol's iodine has also been used with the same assumption (Sarkar, 2005). Another probable mechanism of intrauterine use of Lugol's iodine is that it acts as chemical curator (due to its irritating nature) and replaces the uterine mucosa with new tissue. The newly formed tissues of endometrium release luteolytic factors ($PG_{F2\alpha}$) that reaches to the corpus luteum via utero–ovarian pathway and causes luteolysis (Gupta et al., 2011). Thus, it initiate the estrus cycle, if anestrus is due to PCL. Now–a–days, the use of Lugol's solution is not being recommended for treatment of anestrus due to its irritating nature and damaging effect on endometrium.

HORMONAL TREATMENTS

Estrogens Based Treatment

Exogenous administration of estrogen produce estrus signs in anestrus animals with or without concurrent ovulation. In presence of dominant follicle, estrogen administration results in expression of estrus and ovulation because of its positive feedback effect over pituitary for LH surge. For this reason, it has been used to induce ovulation (Saiduddin et al., 1968; Peters, 1984; Garcia–Winder et al., 1988; McDougall et al., 1994) and to reduce postpartum anestrus period. Conversely, estrogen induces anovulatory estrus in absence of dominant follicle. Estrogens have also been shown to cause luteolysis in ruminants (Wiltbank, 1966; Brunner et al., 1969) probably through stimulating the prostaglandin secretion from endometrium as well decreasing the level of circulating LH. One or two doses of intramuscular injections estradiol (3–10mg) or estrone (5–15mg) at three days interval can be used to regresses the retained corpus luteum associated with pyometra, mummification and mucometra. However, it should be kept in mind that high and prolonged dose of estrogens leads to cystic ovaries, abnormal motility and peristalsis of oviduct that may results in introduction of infections to the ovarian bursa through oviduct, causing ovaritis and adhesions. These side effects of estrogens limit its use for induction of estrus in anestrus animals.

Progesterone Based Treatment

Exogenous administration of progesterone mimics the luteal phase of the estrus cycle by exerting negative feedback effect over hypothalamus and pituitary for LH release. Upon withdrawal of progesterone, the normal follicular phase of the cycle is stimulated. However, for such treatment seem to be effective, abrupt decrease in progesterone level is required at the end of treatment. Intravaginal progesterone releasing devices such as PRID (progesterone–releasing intravaginal device), CIDR (controlled internal drug release) and CueMate are effective in restoration of cyclicity in anestrus animals (Ramamohana Rao, 1981; Singh et al., 1988; Singh et al., 2010; Azawi et al., 2012). Upon withdrawal, the concentration of progesterone decline abruptly and onset of estrus and ovulation occurs within 2–8 days after the end of treatment (Agarwal and Tomar, 2003; Hafez and Hafez, 2008). Ear implants (Crestar and Synchromate–B) also produce required abrupt decrease in progesterone concentration at the end of treatment. Progesterone therapy alone is not particularly effective for the treatment of anovulatory anestrus; hence other hormones have been incorporated in most of the progesterone based therapy. To achieve better responses, the intravaginal devices or ear implants are generally used for 7 to 9 days, combined with other hormones (prostaglandins, GnRH, PMSG/eCG and estradiol) towards the end of progesterone treatment and estrus induction rate has been reported between 80 to 100% by most of the workers (Lakra et al., 2003; Rhodes et al., 2003; Kumar and Mandape, 2004; Nayak et al., 2009; Singh et al., 2010; Azawi et al., 2012).

Feeding of oral progestational compounds (10–14 days) such as MAP (6–methyl–17–acetoxyprogesterone), CAP (6–chloro–6–dihydro–17–acetoxyprogesterone), MGA (Melengestrol acetate) and DHPA (16α–17–Dihydroxyprogesterone acetophenide) (Hansel and Malven, 1960; Hansel et al., 1966; Wiltbank et al., 1967; Shanker et al., 1988) and long term (4–14 days) intramuscular injections of progesterone @ 50–100 mg either alone or in combination with other hormones (Willet et al., 1950; Ulberg and Lindlay, 1960; Agarwal., 1983) have been used for induction/synchronization of estrus, however, both oral and parenteral routes are impractical for inducing the cyclicity as concentration of progesterone does not decline abruptly at the end of treatment.

The estrus response is better with progesterone based treatment, however, conception rates are usually low at induced estrus especially after long term progesterone therapy probably due to altered follicular growth, high rate of follicular atresia, poor sperm transport, failure of fertilization, low cleavage rate, poor transport of fertilized ova and early embryonic death (Jainudeen and Hafez, 1966; Hawk, 1971; Lamond et al., 1971; Wordinger et al., 1976).

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Gonadotropic Releasing Hormone (GnRH) Based Treatment

The single intramuscular injection of GnRH analogue (10 to 20µg Buserelin) has been used effective in induction of estrus and concurrent ovulation with variable response (45.5 to 87.5%) within 4–22 days (Dhoble and Gupta, 1986; Pattabiraman et al., 1986; Sonwane et al., 1994; Narasimha Rao, 1997; Nautiyal et al., 1997; Markandeya and Patil, 2003; Prahalad et al., 2010). The variable response may be due to differential action of GnRH on different stages of follicular development. It induces ovulation, if mature follicle is present at the time of administration by inducing the LH surge. However, single injection of GnRH is not always effective in deep anestrus animals. On the contrary, it stimulate emergence of new follicular wave through enhanced secretion of FSH, thus effective in long term. Pulsatile/intermittent injections of small dose of GnRH (at every 2 hours, intravenously) has been tried in order to induce LH pulses (Edwards et al., 1983; Peters et al., 1985; Spicer et al., 1986), however, intermittent injection make this technique impractical.

To achieve better response, GnRH has been combined with other drugs such as phosphorus injection (Tonophosphan), prostaglandin, estradiol and progesterone (Shams et al., 1991; Rhodes et al., 2003; Sirmour et al., 2006). The Ovsynch protocol or GPG regimen (GnRH–PG–GnRH), developed by Pursley et al. (1995) to synchronize ovulations in dairy cows has been widely used to treat anestrus cattle and buffaloes and results are also promising. Under this protocol first injection of GnRH (at day 0) induces ovulation, if dominant follicle is present and if not luteinizes with emergence of new follicular wave 1 to 2 days later, $PGF_{2\alpha}$ injection given on day 7 regress the CL formed in response to first injection of GnRH and second injection GnRH on day 9 induces ovulation of new dominant follicle subsequently, all the treated animals are inseminated within 16–20 hours of second injection of GnRH.

Gonadotrophins Based Treatment

Pregnant mare serum gonadotrophin (PMSG) or equine chorionic gonadotrophin (eCG) is strong stimulator of ovarian activity because of its predominant FSH like activity. Therefore, it has been used extensively for superovulation. However, single intramuscular injection in low doses either alone or in combinations with others has been used successfully to treat anestrus cattle and buffaloes (Echternkamp, 1978; Wettemann, 1982; Dabas and Bardhan, 2006; Muneer et al., 2009; Prahalad et al., 2010; Kumar, 2012). PMSG prevent and reverse the process of atresia in small follicles (Moor et al., 1984), hence its use for management of anestrus in buffaloes in low doses could be used satisfactory as follicular atresia is very common in buffaloes. Human chorionic gonadotrophin (hCG) has also been used for management of anestrus with fair degree of success (Dabas and Bardhan, 2006).

Prostaglandin Based Treatment

Prostaglandin ($PGF_{2\alpha}$) is the treatment of choice for persistent corpus luteum and sub estrus. Natural or synthetic analogue of $PGF_{2\alpha}$ as a single dose has been used with a reasonable degree of success for management of silent estrus in cattle and buffaloes (Nautiyal et al., 1998; Singh et al., 2001). It should be born in mind that $PGF_{2\alpha}$ is only effective between days 6–16 of the cycle and in the presence of active corpus luteum. An intramuscular injection of 25mg (total dose) of natural $PGF_{2\alpha}$ or 250 to 500 micrograms of synthetic ones is required to regress the CL in both cattle and buffaloes. However, a lower dose of $PGF_{2\alpha}$ (5mg) are also effective to regress the CL through intra–vulvo–submucosal (IVSM) (Dhaliwal et al., 1988; Narasimha Rao and Venkatramaiah, 1990). Alternatively, Ovsynch protocol as describe by Pursley et al. (1995) may be used to treat sub estrus or unobserved estrus.

Insulin Based Treatment

Use of insulin for induction of estrus in animals either alone or in combination is a fairly recent development and results are very encouraging (Shukla et al., 2005a&b; Ramoun et al., 2007; Gupta et. al., 2010). The recommended dose is 0.25 IU/kg body weight subcutaneously for 3–5 days. Use of GnRH or eCG pretreated with insulin has shown promising results for management of anestrus cattle (Shukla et al., 2005 a&b) and buffaloes (Gupta et al., 2010; Kumar et al., 2012; Ramoun et al., 2012). It has also been shown that insulin enhances the follicular growth in true anestrus buffalo which is prerequisite of GnRH to be effective (Ramoun et al., 2012). The treatment of true anestrus buffaloes under field condition was reported satisfactory when single intramuscular injection of PMSG (500 IU) was combined with subcutaneous injections of insulin @ 0.25IU/Kg body weight for five consecutive days (Kumar, 2012).

Anti–Prolactin Based Treatment

Hyper–prolactinaemia has been reported during summer in buffaloes that could be one of the reasons for summer anestrus in buffaloes. With this assumption anti–prolactin drug such as bromocriptine (Verma et al., 1992) has been tried. Melatonin is also known to suppress prolactin secretion (Wuliji et al., 2003). Moreover, melatonin has been reported as stimulator of both GnRH and gonadotrophin secretion in buffaloes. As the plasma concentration of melatonin is low during summer, Ghuman et al. (2010) has reported estrus induction and ovulation in all treated summer anestrus buffalo heifers using melatonin implants, however, time taken to induce estrus and ovulation was highly variable (4–36 days).

PREVENTION

Prevention of anestrus is preferable over treatment and can be achieved by maintaining the healthy status of the animals by adopting efficient farm managemental practices. Nutrition is probably most important factors, affecting ovarian activity. Special attention must be given to prevent the negative energy balance in high yielders. It can be achieved by providing adequate ration during pre– and postpartum period. The supplementation of vitamins, minerals and antioxidants in feed appeared to be promising in restoration of cyclicity (Baldi et al., 2000; Anita et al., 2003; Koley and Biswas, 2004; Selvaraju 2009; Sah and Nakao, 2010; Kumar, 2012).

As suckling decreases the LH pulsatility and prolongs the postpartum anestrus period, weaning could be one of the effective managemental tools to reduce postpartum anestrus period following restoration of pulsatile secretion of LH in postpartum animals. It has been shown that weaning (complete, temporary and partial) is associated with increased GnRH secretion, LH pulse frequency and more LH receptors on follicular cells followed by ovulation within few days. The level of opioid may also decrease due

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to alleviation of physiological effects of suckling consequently, initiate normal ovarian cycles (Williams and Griffith, 1995) in cattle and buffaloes.

Biostimulation (induction of cyclicity through introduction of males into a group of females) activates LH secretion followed by ovulation (Gelez and Fabre–Nys, 2004) in females through olfactory and sensory cues. Exposure of postpartum cows (Tauck et al., 2010) and buffalo (Gokuldas et al., 2010) to a bull decreases the postpartum anestrus interval and advances the puberty in heifers. It can be elicited with vasectomized bulls, andronized females or steers. Though, it is a relatively inexpensive managemental tool, the response is inconsistent due to variability in parity, season, cow to bull ratio, body condition score at parturition and postpartum stages at which bulls are exposed. In cattle and buffaloes, the satisfactory response to biostimulation is achieved only when females are exposed to bulls immediately and continuously after parturition.

In addition to above preventive measures, certain managemental practices such as efficient detection of estrus either visually or by bull parading and other techniques, routine pregnancy diagnosis (40–60 days post breeding), prevention of occurrence of post-partum uterine infections and periparturient diseases (ketosis and mastitis), regular deworming and synchronization of estrus especially in buffaloes where heat detection is more difficult, could be advantageous in preventing the occurrence of anestrus.

CONCLUSION

Anestrus is a multi-causative factors associated problem affecting livestock enterprise to a great extent. Diagnosis of the condition needs to be prompt and at the earliest to prevent its occurrence for effective treatment. As such there is no single panacea to correct it. Further, research is needed especially at cellular and molecular level for better understanding of its etiology, diagnosis and therapeutics.

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