Review Article

ANESTRUS IN BUFFALO

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Abstract

The productivity of buffaloes is lower than that of dairy cows. It is mainly due to lower reproductive efficiency. Delayed sexual maturity, postpartum anestrus, silent estrus and the seasonal breeding pattern are the various reasons for reducing reproductive efficiency in buffaloes. Among these, infertility as manifested by anestrus remains a major economic problem in buffaloes. This review discusses the incidence, classification, factors causing and treatment approaches of anestrus in buffaloes.

Introduction

Buffalo is the most important livestock species in Nepalese agricultural system as it contributes more than two-third of the total milk production and more than 50% of the total meat production in the nation (GoN, 2014). However, the production and productivity of buffaloes in Nepal is very low mainly due to subfertility and infertility (Gautam et al., 2017). In general, the reproductive efficiency of the buffalo is lower than that of cattle (Noakes et al., 2001). Low reproductive efficiency in the buffalo remains a major economic problem globally. Delayed sexual maturity, postpartum anestrus, silent estrus and the seasonal breeding pattern are the various reasons for reducing reproductive efficiency in buffaloes. Field surveys on reproductive disorders from various parts of the world revealed that anestrus was the most common single cause of infertility in buffaloes (Reviewed by El-Wishy, 2007). Anestrus 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 (Kumar et al., 2014). Infertility as manifested by anestrus remained a major problem for reducing reproductive efficiency in Nepalese buffaloes too (Sah and Nakao, 2010; Devkota et al., 2012; Gautam et al., 2017).

Infertility due to cyclicity failure or anestrus has great economic impact. Anestrus leads to economic losses through increased inter-calving interval, poor net calf crops, production loss, treatment expenses and cost of replacing mature animal with first calving heifer (Kumar et al., 2014). There are no reports from Nepal pertaining to economic impact analysis due to anestrus in buffaloes. In India, Kumar et al. (2013) reported an estimated loss of Indian Rs. 372.90 per day from each buffalo due to anestrus. As the incidence of anestrus has been reported high, the above figures show great economic impact at country level.

Classification of anestrus

In buffalo, the anestrus is observed in post-pubertal heifers, during pregnancy, lactation, early postpartum period and during low breeding season. Classification of anestrus in cattle as described by Roberts (1971) can be used also in case of buffalo. He classified the anestrus into two types, i.e. Class I–female with a normal functional corpus luteum and Class II–female with no functional corpus luteum.

Class I anestrus includes (i) Anestrus due to pregnancy, (ii) Anestrus due to persistent corpus luteum (CL), (iii) Anestrus due to ‘weak’ or ‘silent’ estrus, and (iv) Anestrus due to unobserved estrus. Anestrus due to pregnancy is a physiological condition; anestrus due to unobserved estrus is the management problem. However, the anestrus due to persistent CL and silent estrus are the reproductive problems of animals concerned. Persistent CL is usually observed in association with some uterine pathology such as pyometra, fetal maceration, mucometra, mummified fetus and other disease states. Anestrus due to weak estrus or ‘silent estrus’ or ‘silent ovulation’ is the inherent characteristic of buffalo (Zicarelli et al.,1997) that is one of the major problems in buffalo breeding.

Class II anestrus includes anestrus due to small or inactive ovaries with no functional corpus luteum palpable per rectum. These are non-cycling cows. It is termed as ‘true anestrus’.

From the clinical point of view, anestrus can be classified broadly into two categories, viz. **silent estrus** and **true anestrus** (Noakes et al., 2001). In the first form, the animal possesses a palpable CL in one ovary, but has not been detected in estrus due to subestrus or silent estrus, whereas in the second form, the animal has no palpable.

CL and does not exhibit estrus because she is acyclical (true anestrus). Silent estrus or quiet ovulation is clinically characterized by failure of overt signs of estrus, though the follicular development and ovulation occurs normally. Sub estrus is common during the post pubertal period in heifers and early post–partum (30 to 120 days) in high yielding animals. Progesterone secreted from regressing CL of previous cycle potentiates the action of estrogen and seems to favour 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 the early postpartum period, during the humid and hot season, in malnutritioned and suckled buffaloes (Shah, 1990; Badr, 1993; El-Wishy 2007; 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). Due to the high (20–80%) incidence of sub estrus, large numbers of buffaloes are left unbred and substantially contribute to more days open (Honparkhe et al. 2008).

Incidence of anestrus in buffalo

In the few studies available on breeding and institutional herds, the frequency of postpartum and postservice anestrus was 64–76 and 24–36%, respectively (Schmidt et al., 1963; Chauhan and Singh, 1979). In India, a large variation on incidence of anestrus has been reported in literatures depending upon breed, parity, season, level of nutrition, management conditions and geographic environment; the incidence of anestrus in buffaloes ranged from 9.1% to 82.5%; (Reviewed by Kumar et al., 2014; Nanda et al., 2003). In a clinical survey among cases of reported anestrus, 58.4% were true anestrus, 33.3% silent estrus and 8.3% of buffaloes had infantile genitalia (Samad et al., 1984).

There are limited studies regarding the incidence of anestrus in Nepalese buffaloes. In a recent study by Gautam et al. (2017), out of 68 infertile buffaloes, majority of cases (91.2%) were anestrus followed repeat breeding (8.8%). Within anestrous group, 54.8% buffaloes were in true anestrus characterized by inactive ovaries, and 45.2% were in silent estrus or missing estrus. Among silent estrus or missing estrous group, 64% had dominant follicle and 36% had corpus luteum as the major structure in the ovary. In another study by Sah and Nakao (2010), out of 135 anestrous buffalo cows, 61.4% had true anestrus with ovarian dysfunction and 33.3% had silent ovulation, while among 111 buffalo heifers, 76.6% were in true anestrus and 18.9% had silent ovulation. Similarly in another study, among anestrous buffaloes, the incidence of true anestrus with inactive ovaries was >70% from March to June that peaked (>80%) in April/May and remained around 50% in August and October-December (Devkota et al., 2012).

Factors causing anestrus in buffalo

Anestrus in buffalo is a multifactorial problem; its occurrence signals the inadequate nutrition, environmental stress, uterine pathology and improper management practices. Nutrition, season of calving, parity, management practices and abnormal parturition were found to influence the frequency of postpartum anestrus in buffaloes (Reviewed by El-Wishy, 2007).

Genotype

The period of postpartum anestrus 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 follicles 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.

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 causes of anestrus in heifers. 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; 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 affects the reproductive performance of females (Bearden et al., 2004).

Body condition score (BCS) 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). 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). Devkota et al. (2012) reported that there were greater proportion of poor BCS (<2.5) buffaloes during low breeding months (January to June) as compared to good breeding months, and also, >75% of anestrus buffaloes with poor BCS showed true anestrus round-the-year. From their findings, they speculated that the higher incidence of anestrus during low breeding season was mainly due to low BCS that was attributed to shortage of feed and fodder availability during such months.

Season/Environment

As reported by different authors (Shah et al., 1989; Singh and Lal, 1994; Zicarelli, 1997), buffalo cows exhibit a distinct seasonal change in displaying estrus, conception rate and calving rate. Although buffaloes are well adopted in hot and humid climate, the ovarian activity is greatly reduced by heat stress and manifested in the form of anestrus (Singh et al., 2000). Ovarian inactivity is more frequent in summer (41–46%) than in other seasons (7–33%) calvers (Rao and Sreemannarayana, 1982; Chauhan et al., 1984). With the onset of summer (April), breeding efficiency in both young and adult buffalo is reduced, reaching a nadir in the dry hot months of May and June when approximately 80% of non-pregnant buffalo have quiescent ovaries. Silent estrus is also more common in the hotter months than from August to January and it takes longer for ovarian cyclicity to resume after calving in the summer (80 additional days) (Singh et al., 2000). Devkota and Bohora, (2009) also reported that there was a distinct seasonal variation of anestrus condition in Nepalese buffaloes with spring and early summer (January to June) as the low breeding season and July to December as the active breeding season. This phase coincides with higher ambient temperature, longer day length, and the scarcity of green fodder. Therefore, it can be speculated that, such seasonal anestrus during dry and hot months might be due to various causes namely- heat stress, shortage of feed/fodder and the photoperiod.

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). Heat stress reduces the luteinizing hormone (LH) pulse frequency and therefore follicle maturation and estradiol production in buffaloes (Palta et al. 1997; De Rensis and Scaramuzzi, 2003). Higher environmental temperature increases oxidative stress (Bernabucci et al., 2002) and reproductive disorders and oxidant/antioxidant imbalance in buffaloes are related to each other (Anmed et al., 2008). There is critical shortage of feed and fodder during winter, spring and early summer in tropical and sub-tropical regions, and the buffalo maintained on lower levels of nutrition have sluggish ovarian activity and lower circulatory progesterone concentrations than well-fed buffalo (Kaur and Arora, 1984). Photoperiod (by melatonin secretion) plays a pivotal role in seasonal breeding characteristic in buffalo. The proportion of buffaloes exhibiting estrus during the period of short-day length is significantly greater than during the period of long day length, indicating that decreasing day light is a stronger determinant of the resumption of ovarian activity (Barile, 2005). Fewer buffalo (26-31%) exhibit estrus under long day length and artificial short photoperiods can be imposed to reduce this problem (Singh et al., 2000). Decreasing day length is thus a strong determinant of onset of postpartum ovarian activity. Melatonin production is stimulated by decreasing photoperiod in buffalo and in summer anestrous buffalo injected with 18 mg melatonin once or twice each day come into estrus (Hassan et al., 2000). Besides these, high environmental temperature causes hyper–prolactinaemia and suppressing the secretion of gonadotrophin which leads to alteration in ovarian folliculogenesis and steroidogenesis (Sheth et al., 1978; Verma et al., 1992).

Suckling

Postpartum anestrus is prevalent in dairy (Shah et al., 1990) and suckled swamp buffaloes (Jainudeen, 1988). Suckling suppress the postpartum ovarian activity in buffalo (El–Fadaly, 1980; Honnapagol et al., 1993), resulting into extended postpartum anestrus period. 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 causing anestrus.

Level of milk production

High milk yielders show significantly longer postpartum anestrus period (Harrison et al., 1989) 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).

Parasitic Infestations

Parasitism 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) was widely prevalent among dairy herds and had significant association with anestrus (Bruhn et al., 2013).

Periparturient Diseases

Periparturient diseases such as abnormal calvings, metritis, mastitis and ketosis also influence onset of postpartum cyclicity (Fonseca et al., 1983). Postpartum uterine infection suppress GnRH release and possibly LH secretion (Peter et al., 1990; Mateus et al., 2002), probably due to inflammatory response (Sheldon and Dobson, 2004; Williams et al., 2007) and thus, ovarian activity remains suppressed in uterine infections.

Diagnosis

Anestrus is diagnosed based on history and clinical examination. Transrectal palpation is the most practical and inexpensive tool to diagnose the ovarian cyclicity and pregnancy status of the buffalo. In case of true anestrus, ovaries are smooth, small and inactive with the absence of corpus luteum (Agarwal et al., 2004) while the uterus is flaccid (Noakes et al., 2001); however, follicles may develop up to prematuration stage and get atretic (Roche et al., 1998; Ghuman et al., 2010). In case of silent estrus or unobserved estrus, functional corpus luteum or dominant follicle can be palpated. Although transrectal palpation is an inexpensive technique that has long been accepted as the most important examination tool, its accuracy is limited compared to more recent diagnostic aids, namely progesterone profile (Metwelly, 2001; Zakaria et al., 2001), laparoscopy (Ambrose et al., 1993) and ultrsasonography (Abuzeid et al., 2000). Securing more information on reproductive history, level of milk production, BCS, palpation of the uterine horns, comparing the size of the two ovaries and vaginal examination may improve the results of manual palpation of the ovaries. Progesterone determination in these cases can be highly valuable (El-Wishy, 2007). 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 (Kumar et al., 2014).

Treatment approach

Most of the true anestrous cases are due to nutritional deficiencies. In most cases, the disorder resolves spontaneously with the arrival of more favourable climatic conditions and adequate feeding (Noakes et al., 2001). Treatment of anestrus due to debility, cachexia or a lack of total digestible nutrient (TDN)/energy, protein, or other minerals should follow a careful examination and diagnosis. If the cause is due to chronic debiliting diseases, these must be corrected or alleviated so the female returns to good condition with near normal body weight. Additional levels of TDN and other nutrients need to be fed to those animals. Therapy may require a number of months. Estrus will not occur in debilitated animals until they receive feed over and above the requirements for maintaining life and are in a positive energy balance. In heifers and parous animals in a fair to good nutritive state but failing to show estrum because of phosphorus deficiency, steamed bone meal or diclacium phosphate should be fed free of choice. Complex mineral supplements or specific mineral supplements may be necessary in anestrous, mineral-deficient animals with a low blood hemoglobin level (Roberts, 1971).

Once the animals are in good nutritional state, progesterone based estrous synchronization protocols would be effective to induce estrus. 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. The priming of hypothalamo-hypophysial-gonadal (HPG) axis with adequate amounts of progesterone is beneficial for the recovery of HPG axis function, and hence, a better display of estrous behavior by anestrous buffaloes at induced estrus (De Renis et al 2005; Ghuman et al., 2012). Furthermore, the sufficient priming of endometrium with progesterone may be necessary to enhance the conception rate (Stevenson et al., 2006). 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 (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). 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 (Singh et al., 2010; Azawi et al., 2012). Sometimes GnRH or FSH injections would resume the ovarian cyclicity and induce estrus. Theoretically to stimulate cows in anestrum with nonfunctional or 'smooth' ovaries, FSH is indicated.

Doses of gonadotropes to treat anestrum in cattle are 750 to 1500 iu PMSG or 40 to 100 iu FSH. Theoretically this FSH treatment might be followed in 3 to 5 days by an injection of LH (Roberts, 1971).
Some literatures claim that herbal preparations such as Prajana HS (Indian Herbs), Janova (Dabur), Sajani (Sarabhai) etc. are effective in restoration of cyclicity with good success rates (Reddy et al., 1994; Hussain et al., 2009). It has been also stated that utero–ovarian massage is the oldest, simplest, cheapest and effective method to induce estrus in anestrous cattle and buffaloes (Rahawy, 2009; Mwaanga et al., 2010).
Treatment of silent estrus often requires careful, frequent and accurately recorded examination of the genital tract of affected cows, careful assessment of herd management practices, and diplomatic education and instructions for the herdsman and cattleman. Prostaglandin (PGF2α) is the treatment of choice for persistent corpus luteum and sub estrus having responsive CL in the ovary. Natural or synthetic analogue of PGF2α 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 PGF2α is effective only during days 6–16 of the cycle and in the presence of active corpus luteum. Lugol's iodine treatment is cheaper and effective means of management of anestrus particulary in case of persistent CL, but response has been variable (Tapas et al., 2000; Tomar, 2004; Gupta et al., 2011).
Anestrous animals with cystic ovaries should be treated by removal of cysts, injection of LH or GnRH. Heifers with freemartinism, absence of gonads, ovarian tumors etc should be culled out as there is no satisfactory treatment.

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