The Pharma Innovation Journal 2018; 7(5): 526-529

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(5): 526-529

www.thepharmajournal.com
Received: 11-03-2018
Accepted: 12-04-2018

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Studies on anestrus and their resumption of cyclicity on post-partum anestrus buffaloes

Deepak Suvarn, C Singh and Md. Moin Ansari

Abstract
A present field study in and around the rural area of Danapur situated on western proximity of Patna and only those buffaloes were selected which did not show sign of estrus for one year after parturition and were maintained in small unorganized herd. The investigation was conducted in hot summer months (viz. March to June) season. The enumerated buffaloes were examined per rectally and a total of 38 apparently healthy buffaloes were selected with normal genitalia without having palpable corpus luteum on ovaries and pathological lesion. Selected animals were subjected to deworming prior to this study with broad-spectrum anthelmintic Fenbendazole (3 gms) once. The animals were first treated with PGF2α at the dose rate of (25 mg) intramuscularly on day '0'. The animals, which showed signs of estrus, were discarded from the experiment. The rest of the animals were supplied with mineral mixture in their ration at the rate of 30 gms per animal per day from day '8' today '38'. The buffaloes, which resumed cyclicity during mineral mixture treatment, were also discarded from further investigation. Finally total of 24 buffaloes were selected randomly and divided into four groups (six animals in each group) to observe the effect of Duraprogen at different dosages on resumption of cyclicity. Animals of group C (Control) were treated with normal saline (2 ml) on day 39, 43, 47 and 51 while those under Group T1, T2 and T3 were treated with Duraprogen (Progesterone, 250 mg) intramuscular on day 39. The animals of group T1 were further treated with Duraprogen (250 mg) on day 41, 43, 45, 47 and 49 (i.e. on alternate day). The animal of group T2 were further treated with Duraprogen (250 mg) on day 43, 47 and 51 (i.e. three day interval) while the animals of group T3 were further treated on day 46 (i.e. six day interval). The effect of Duraprogen had significant effect on resumption of cyclicity. The resumption of cyclicity was highly significant in group T1 in which repeated dosages of Duraprogen (250 mg) i.e. on alternate days were administered. The effect of Duraprogen on cyclicity decreases with decrease in frequency of dose i.e. on three day interval and on six day interval. On the basis of investigation, following conclusion were observed that: single dose of prostaglandin (PGF2α) at the dose rate of 25 mg intramascular had no effect on resuming estrus cyclicity in post-partum anestrus buffaloes, mineral mixture feeding at the dose rate of 30 gms per animal per day up to one month had non-significant effect on maintaining reproductive rhythm during summer season.

Keywords: Buffalo, post-partum anestrus, duraprogen, resumption of cyclicity

1. 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 multicausative factors associated problem but its occurrence signals the inadequate nutrition, environmental stress, uterine pathology and improper managemental practices (Roberts, 1971; Kumar *et al.* 2014) [16, 9]. In India delayed puberty, acyclicity after attaining puberty, and post-partum anestrus which lead to prolong inter calving period are major causes of poor reproductive efficiency in cattle and buffaloes.

Real anestrus with inactive, smooth, small and round and flat ovarian condition is a major limiting factor in greater utilization of artificial insemination for rapid improvement of livestock productivity. This also results in loss of production and increased cost of maintenance. The problem of anestrus has been recognized as having moderate to high incidence affecting the fertility of the animal vis-à-vis economy of the farmer (Kurien and Madhavan, 1985; Kumar *et al.* 1986; Sinha *et al.* 1987) [13, 12, 19]. 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) [15] reported an estimated loss from anestrus around Rs. 193.00 per day in cow and Kumar *et al*. (2013) [8] 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. Buffalo has higher incidences of functional anestrus than cattle as the post-partum estrus interval is longer. Besides breed and climate, management and nutrition also play vital role in determining the reproductive disorder in cattle and buffalo. Reproductive failures such as anestrum, repeat breeding and pathological condition of the genital tract suggest the nutritional deficiencies, hormonal imbalance and deranged enzymatic activity affect the normal reproductive behavior of the animal, causing serious morphological and physiological alterations (Roberts, 1971) [16]. 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 (Deshpande *et al.* 2000, Agarwal *et al.* 2001, Kumar *et al.* 2005) [4, 1, 10]. 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 animal (Singh *et al.* 2010; Azawi *et al.* 2012) [17, 2]. Keeping all these reported observations in mind the present investigation was conducted to study the effect of $PGF_{2\alpha}$, mineral mixture and progesterone on resumption of cyclicity on post-partum anestrus buffaloes.

2. Materials and Methods

Only those buffaloes were selected which did not show sign of estrus for one year after parturition and were maintained in small unorganized herd in and around the rural area of Danapur situated on western proximity of Patna. The investigation was conducted in hot summer months (viz. March to June) season. The enumerated buffaloes were examined per rectally and a total of 38 apparently healthy buffaloes were selected with normal genitalia without having palpable corpus luteum on ovaries and pathological lesion. Selected animals were subjected to deworming prior to this study with broad-spectrum anthelmintic Fenbendazole (3 gms) once. The animals were first treated with $PGF_{2\alpha}$ at the dose rate of (25 mg) intramuscularly on day '0'. The animals, which showed signs of estrus, were discarded from the experiment. The rest of the animals were supplied with mineral mixture in their ration at the rate of 30 gms per animal per day from day '8' today '38'. The buffaloes which resumed cyclicity during mineral mixture treatment were also discarded from further investigation. Finally total of 24 buffaloes were selected randomly and divided into four groups (six animals in each group) to observe the effect of Duraprogen at different dosages on resumption of cyclicity. Animals of group C (Control) were treated with normal saline (2 ml) on day 39, 43, 47 and 51 while those under Group $T_{1,}$, T_2 and T_3 were treated with Duraprogen (Progesterone, 250 mg) intramuscular on day 39. The animal of groupT_1 was further treated with Duraprogen (250 mg) on day 41, 43, 45, 47 and 49 (*i.e.* on alternate day). The animal of group T_2 were further treated with Duraprogen (250 mg) on day 43, 47 and 51 (*i.e.* three day interval) while the animals of group T_3 were further treated on day 46 (*i.e.* six day interval). Blood samples from all treated animals were collected on day 39, 43, 47 and 51 and/or the day when animal showed sign of estrus.

3. Statistical analysis

Data were analyzed for averages and standard error as described by Snedecor and Cochran (1967) [20]. Analysis of variance for comparison of various traits between groups was done. The model (Snedecor and Cochran, 1967) [20] was used as follows:

$Y_{ij} = \mu + £_i + e_{ij}$

Y_{ij} is the measurement of jth individual of the ith group.

μ is the overall population mean.

£i is the effect of ith group.

e_{ij} is the random error assumed to be normally and independently distributed with mean "0" and variance σ^2_e i.e. NID (0, σ^2_e).

4. Results and Discussion

All 38 anestrus buffaloes selected for this study and subjected to $PGF_{2\alpha}$ (Lutalyse™) therapy as per schedule, on observation for seven continuous days showed no sign of estrus, confirming absence of any palpable corpus luteum. The findings revealed that administration of prostaglandin did not leave any influence on the reproductive status of the buffaloes. Prostaglandin is well known to have a potent luteolytic effect and intramuscular administration of 25 mg prostaglandin in the animals with palpable corpus luteum cause the degeneration of the CL within 24 to 48 hrs. And return of estrus within 3 to 5 days post treatment (Hafez and Hafez, 2000) [7]. Present investigation findings were not in accordance with the findings of Chauhan *et al.* (1982) [3] and Dhoble and Gupta (1987) [5]. The variation in findings may be attributed to difference in dose rate and difference in frequency of administration. The other possible reason is that all the buffaloes under investigation were in true anestrus as they were not having luteal tissue of the ovary. Since no animal responded to $PGF_{2\alpha}$ treatment hence all 38 treated animals were subjected to mineral mixture treatment from day '8' today '38'. The animals were supplied with mineral mixture (Agrimin™) as per program continuously for 30 days. The animals were kept under close observation for estrus detection. Routine visit and guidance to the owners for proper detection of estrus was adopted. The effect of mineral

mixture were noted and analyzed for further investigation. After supplementation of mineral mixture, out of 38 animals under treatment only 2 animals exhibited signs of estrus. Both the animals showed the sign of estrus on day 37 of treatment. The signs of estrus were moderate. Both the animals were put to natural service. Amongst the two, only one buffalo conceived. On day 39th, all the mineral mixture treated animals which did not showed signs of estrus were subjected for further investigation. Out of 38 animals 2 buffaloes that had shown signs of estrus have been dropped and they were discarded from further investigation. Thus out of 36 animals, only 24 animals were selected randomly and divided into four groups having six animals in each group. The animals of Control group were treated with normal saline while the animals under Group T_1, T_2 and T_3 were treated with Duraprogen™ (having 17-α-Hydroxyprogesterone Caproate) as per schedule. The animals of Group T_1 were further given Duraprogen on day 41, 43, 45, 47 and 49 (*i.e.* on alternate day). The animals of Group T_2 were further treated with that on day 43, 47 and 51 (*i.e.* three days interval) while the animals of Group T_3 were further treated on day 46 (*i.e.* six days interval). Blood samples were collected on day 51 and/or the day when animal showed sign of estrus prior to it. The effect of Duraprogen on cyclicity was summarized in table 2 and 3. In control, no animal showed sign of estrus. In group T_1, animals treated with Duraprogen™ 250 mg I/M on alternate day (*i.e.* day 39, 41, 43, 45, 47 and 49), five out of 6 animals showed signs of estrus. Two animals showed signs of estrus on day 44 while other three showed the estrus sign on day 45, 46 and 49. The intensity of estrus was moderate in 2 animals and strong in 3 animals. In group T_2, where animals were treated with Duraprogen™ 250 mg I/M on every 4th day (*i.e.* day 39, 43, 47, 51), three out of 6 animals showed signs of estrus. Two animals showed sign of estrus on day 46 and one on day 50. The intensity of estrus was moderate in 2 animals and strong in 1 animal. In group T_3, where animals were treated with Duraprogen™250 mg I/M on every 7th day after administration of 1st injection (*i.e.* day 39 and 46), one out of 6 animals showed sign of estrus on day 43 and the intensity was moderate. Detection of estrus in 1 out of 6 buffaloes in Group T_3 indicates that the injection of 250 mg progesterone might not have been sufficient to modulate the hypothalamo-hypophyseal-gonadal axis. Thakur, 1989; Kumar *et al.* (2000) [21, 11] reported successful induction of estrus in anestrous buffaloes with administration of 500 mg of Progestrone and Estradiol combination, while Singh (2003) [18] induced estrus in anestrous buffaloes with only Progestrone; supports our observation of induction of estrus in 1 out of 6 buffaloes under group T_3 treated with high dose of Progestrone. However, attempt to induce estrus in buffalo could not achieve height because the experiment was conducted in the months of April to June, the period which is well known to keep buffalo away from breeding. Animals under Group T_2 though received four injections each of 250 mg progesterone at 3rd day interval only three animals were detected in estrus. This might be due to continuous administration of higher doses of progesterone. Detection of estrus in 5 (83.33%) out of 6 buffaloes of Group T_1 receiving 250 mg of progesterone on alternate day, suggests sensitization of hypothalamo-hypophyseal-gonadal axis to release its respective hormones ultimately to trigger the mechanism of folliculogenesis and subsequent fertile estrus. The positive response of consistent administration of even lower dose of progesterone through parentral route release of progesterone through intra-vaginal implant and ear implant, might expert depressing effect on hypothalamo-hypophyseal-gonadal axis; and their withdrawal released the very axis from the negative effect and thereby set to function for release the tropic hormones indirectly or directly responsible for folliculogenesis expression of estrus symptom and ovulation (Hafez and Hafez, 2000) [7]. On day 39th, all the mineral mixture treated animals which did not showed signs of estrus were subjected to further investigation. Out of 38 animals 2 buffaloes showed sign of estrus during mineral mixture treatment were discarded from further investigation. Out of 36 animals, 24 animals were selected randomly and divided into four groups having six animals in each group. The animals of Control group were treated with normal saline on day 39, 43, 47 and 51 while the animals of Group T_1, T_2 and T_3 were treated with Duraprogen™ at the dose rate of 250 mg per ml. intramuscularly on day 39. The animals of Group T_1 were further given Duraprogen on day 41, 43, 45, 47 and 49 (*i.e.* on alternate day). The animals of Group T_2 were further treated with that on day 43, 47 and 51(*i.e.* three day interval) while the animals of Group T_3 were further treated on day 46 (*i.e.* six day interval). Blood samples were collected on day 43, 47, and 51 and/or the day when animal showed sign of estrus. The buffaloes of Group T1, Group T2 and Group T3 that were having higher progesterone concentration on day '40' were detected in estrus between 4 and 9 days in Group T1 and between 6 and 8 days in Group T2 of last Duraprogen injection. The reproductive behaviour of these two groups revealed clearly that these buffaloes were although having developing follicle on the ovary might be secreting elevated level of estradiol but could not exhibit the overt sign of estrus and it took 6-8 days after first Duraprogen injection for exhibition of estrus. It might be due to suppression of the mechanism responsible for the final development of graffian follicles, secretion of higher concentration of estradiol by the developed follicle and changes in female genitalia and behaviour of the buffaloes due to the decrease in estradiol-progesterone ratio in the circulation and imbalance in the co-ordination in the functioning of hypothalamo-hypophyseal-gonadal system required for bringing the animal in estrus (Hafez, 1982) [6]. The observation suggest that in both the groups the mechanism responsible for steroid metabolism and its disintegration might have taken 5 to 9 days to reduce the concentration of progesterone in circulation increasing the estradiol-progesterone ratio to bring the animal to estrus. The dose related response of progesterone administration has also been observed during present experimentation in terms of synchronization of estrus by progesterone treatment in anestrus buffaloes. It has been observed through present experimentation, that the administration of 250 mg progesterone to buffaloes as a single injection did not have any influence on estrus cyclicity. It may be presumed that administration of single injection of 250 mg progesterone might not be sufficient to sensitize the hypothalamo-hypophyseal-gonadal system to establish co-ordination in the organs to integrate the functional activity therein. As minimum threshold level of any hormone is required to activate its target organ (Hafez, 2000 and Mc. Donald, 1980) [7, 14]. The injection of 500 mg progesterone twice have influenced the system similar to the single dose responsible to bring the animals to estrus cyclicity even then the excess higher dose of progesterone does not have positive response to bring the animal to estrus. The observation of present experimentation suggest that either 250 mg progesterone

followed with 500 mg of progesterone or 500 mg progesterone either as single or double injection in anestrus buffaloes are sufficient to set the possible mechanism to bring the animal to estrus. However, before drawing any conclusion on the efficacy of the synchronization of estrus in buffaloes by using prostaglandin and progesterone combination various trial of progesterone at different doses and frequency on large number of animals are required. The abruptly higher concentration of serum cholesterol detected in cycling animals than in the noncycling ones have suggested that intrinsic mechanism like implication of hypophyseal and gonadal hormones in conjunction with thyroxin and corticoids (Zala *et al*. 1972) [22].

5. Conclusion

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. On critical analysis, among infertility cases in post-partum buffaloes nearly 50% cases are of true anestrus and single dose of prostaglandin ($PGF_{2\alpha}$) at the dose rate of 25 mg intramascular had no effect on resuming estrus cyclicity in post-partum anestrus buffaloes. Mineral mixture feeding at the dose rate of 30 gms per animal per day up to one month had non-significant effect on maintaining reproductive rhythm during summer season. Duraprogen had significant effect in resuming estrus cyclicity in post-partum anestrus buffaloes. Repeated dosages of Duraprogen *i.e.* 250 mg (1 ml) on alternate day had better effect than administered at three day interval or at six day interval.

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