STUDY THE RESPONSES OF PROGESTERONE ADMINISTRATION ON RESUMPTION OF CYCLICITY ON POST-PARTUM ANESTRUS BUFFALOES

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ABSTRACT

The aim of the present study was to evaluate the response of progesterone administration on resumption of cyclicity on post-partum anestrus buffaloes located in and around Danapur, Patna, Bihar area. 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 grams) once. The animals were first treated with $PGF_{2\alpha}$ at the dose rate of (25 mg) intramuscularly on day '0'. Out of 38 animals 2 buffaloes that had shown signs of estrus have been dropped and they were discarded from further investigation. Finally total of 24 buffaloes were selected randomly and divided into 4 groups (6 animals in each group) to observe the effect of progesterone (Duraprogen, 250 mg) at different dosages on resumption of cyclicity. Animals of Group 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 intramuscular on day 39. The animals of Group T_1 were further treated with Duraprogen on day 41, 43, 45, 47 and 49 (*i.e.* on alternate day). The animals of Group T_2 were further treated with Duraprogen on day 43, 47 and 51 (on three day interval) while the animals of group T_3 were further treated on day 46 (on six day interval). Results showed that in group T_1, animals treated with progesterone (Duraprogen ™250 mg,1ml) 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 progesterone (Duraprogen ™250 mg, 1 ml) I/M on every 4th day (on 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 progesterone (Duraprogen ™250 mg) I/M on every 7th day after administration of 1st injection (on day 39 and 46), one out of 6 animals showed sign of estrus on day 43 and the intensity

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was moderate. Moreover, resumption of cyclicity was highly significant in group T_1 in which repeated dosages of progesterone (Duraprogen ™250 mg, 1 ml) *i.e.* on alternate days, were administered. The effect of progesterone on cyclicity decreases with decrease in frequency of dose *i.e.* on three day interval and on six day interval.

Keywords: postpartum anestrus, cyclicity, buffalo, progesterone

INTRODUCTION

Indian economy is predominantly agrarian and livestock constitutes an integral part of the agrarian economy. The overall output from livestock sector is about 25% of the total output from agriculture sector in which milk alone constitutes around 63% to the total output from the livestock. Dairying provides millions of small marginal farmers and landless labours means for their subsistence. India has the largest holding of bovine population, about one third of the world's population and about half of the Asian population. India is now the largest milk producer in the world producing about 84.6 million tones of milk per annum (India, 2004). Buffalo alone accounts for 53% of total milk production in India. So its importance cannot be ignored in dairy industry. Full genetic potential in terms of milk production from buffalo can be explored only when the reproduction is normal.

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 and Sinha *et al*., 1987).

Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo in India, affecting livestock productivity and enterprise to a great extent. Buffalo has higher incidences of functional anestrus than cattle as the post-partum estrus interval is longer and especially during summer. 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. 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). Nutritional deficiencies and excesses may cause infertility. They may act via the hypothalamus and anterior pituitary thus influencing the production of gonadotropins or directly on the ovaries, thus influencing oogenesis and endocrine function. Considering above mentioned point in view, it is proposed to investigate the effect of progesterone (Duraprogen ™250 mg, 1 ml) administration on resumption of cyclicity on post-partum anestrus buffaloes and as a managemental aid for better reproductive efficiency located in and around Danapur (Patna) area.

MATERIALS AND METHODS

The present study was done in and around the rural area of Danapur situated on western proximity of Patna, Bihar, India. The experiment was based upon small and unorganized dairy units popularly known as 'Khatals'. Danapur is located 25º36' North (latitude) and 85º06' East (longitude) at an altitude of about 60 meters from mean sea level. The total annual rainfall ranges from 100 to 120 cm and the maximum temperature goes above 38º C during May to June.

At first, the private dairy units distributed in the study area consisting of non-descript buffaloes were enumerated through a "door to door" survey method. Only those buffaloes, which did not show estrus up to one year after parturition were selected for this investigation. The selected buffaloes were examined per-rectally for their reproductive status. The animal showing infectious conditions like pyometra, metritis etc. was not selected. Their rectal temperature, respiration rate, pulse rate and ruminal motality rate were also recorded and those within the normal range were selected. Selected buffaloes having normal physique and reproductive status were subjected to deworming prior to this study with broad spectrum anthelmentic Fenbendazole at dose rate of 1.5 gm once and found negative for parasitic infestation were taken for the experiment.

The selected buffaloes were treated with Lutalyse™ (PGF2α analogue) at the dose rate of 25 mg, intramuscular per animal. The day of administration of the drug was taken as day 0. The animals showing signs of estrus after the treatment were discarded from present investigation and allowed for insemination. 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 2 ml normal saline (N.S.) on day 39, 43, 47 and 51 while the animals of Group T_1, T_2 and T_3 were treated with 1 ml progesterone (Duraprogen™ 250 mg) intramuscularly on day 39. The animals of Group T_1 were further treated with 1 ml progesterone (Duraprogen™ 250 mg) on day 41, 43, 45, 47 and 49 (i.e. on alternate day). While those under Group T_2 were further treated with 1 ml progesterone (Duraprogen™ 250 mg) on day 43, 47 and 51 (i.e. three day interval) and those under Group T_3 were further treated on day 46 (i.e. six days interval).

RESULTS AND DISCUSSION

The effect of progesterone (Duraprogen ™250 mg, 1 ml) on cyclicity was summarized in Table 2 and 3. In control, no animal showed sign of estrus. In group T_1, five out of 6 animals showed signs of estrus. Two animals showed signs of estrus

Table 1. Experimental protocol.

Group	No. of animal	Treatment
Control	6	Injection of normal saline 2 ml I/M on every 4th day (*i. e.* 39 th, 43 th, 47 th and 51 th)
T_1	6	Injection of progesterone (Duraprogen TM250 mg,1ml) I/M on alternate day (*i.e. d*ay 39th, 41st, 43rd, 45th, 47th and 49 th)
T_2	6	Injection of progesterone (Duraprogen TM250 mg,1ml) I/M on every 4th day (*i.e.* day 39th,43rd, 47th and 51st)
T_3	6	Injection of progesterone (Duraprogen TM250 mg,1ml) I/M on every 7th day after administration of 1st injection (*i.e.* day 39th and 46th)

*Duraprogen™ having 17-α-Hydroxyprogesterone Caproate.

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, 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, one out of 6 animals showed sign of estrus on day 43 and the intensity was moderate.

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). 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 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 *et al.* (1989) and Kumar *et al.* (2000) reported successful induction of estrus in anestrous buffaloes with administration of 500 mg of progestrone and estradiol combination, while Singh *et al.* (1983) and Singh (2003) 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.

The buffaloes of Group T_1, Group T_2 and

Table 2. Response of anestrus buffaloes to progesterone administration on resumption of estrus cyclicity.

Treatment	No. of Animal	No. of Animal showing sign of estrus	Intensity of estrus	Insemination	Remark
Group Control-Injection of Normal saline I/M	6	0	-		
Group T_1 -Injection of progesterone (Duraprogen™250 mg,1ml) I/M on alternate day (*i.e.* day 39th, 41st, 43rd, 45th, 47th and 49th)	6	5	Moderate to strong	Natural	Pregnant(3)
Group T_2 -Injection of progesterone (Duraprogen™250 mg,1ml) I/M on every 4th day (*i.e.* day 39th,43rd, 47th and 51st)	6	3	Moderate to strong	Natural	Pregnant(3)
Group T_3 - Injection of progesterone (Duraprogen™250 mg,1ml) I/M on every 7th day after administration of 1st injection (*i.e.* day 39th and 46th)	6	1	Moderate	Natural	Pregnant(1)

Table 3. Effect of progesterone on anestrus buffaloes.

Group	No. of animal	No. of animal in estrus	No. of animal in anestrus	Day on which sign of estrus observed
Control	6	0	6	-
T_1	6	5	1	Day 44, 44, 45, 46 and 49
T_2	6	3	3	Day 46, 46 and 50
T_3	6	1	5	Day 43

Group T_3 that were having higher progesterone concentration on day '40' were detected in estrus between 4 and 9 days in group T_1 and between 6 and 8 days in Group T_2 of last progestrone injection. The buffaloes of group T_1 receiving 1 ml (250 mg) progesterone on alternate days although were having similar higher serum total cholesterol concentration exhibited estrus between day 4 of 2nd progesterone injection and similarly the buffaloes of group T_2 having higher serum cholesterol on day progesterone injection also exhibited the estrus between 6 and 8 days after the two doses of progesterone treatment. 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 to 8 days after first progesterone 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). 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, 1982 and Mc. Donald, 1980). 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.

On critical analysis it may be concluded that repeated dosages of progesterone (Duraprogen 250 mg, 1 ml) on alternate day had better effect than administered at three day interval or at six day interval. It needs further investigation to know the effect of progesterone at different dose and at different frequency on resuming the estrus cyclicity on post-partum anestrus buffaloes.

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