ROLE OF PROGESTERONE SUPPLEMENTATION IN ESTRUS INDUCTION IN MURRAH BUFFALOES UNDER FIELD CONDITIONS IN NON-BREEDING SEASON

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ABSTRACT

The objective of present study was to assess the effect of Modified Co-synch protocol with or without progesterone device on estrus induction and conception rate in Murrah buffaloes under field conditions during summer season. The study was conducted on 30 postpartum anestrous Murrah buffaloes, divided into two groups containing 15 animals in each group. All animals were of parity between 2nd to 5th and body condition score of more than 3. Group 1 animals were treated with Modified Co-synch protocol. In Group 2 animals, in addition to Modified Co-synch protocol progesterone device was supplemented exogenously and kept *in situ* for 7 days. Another objective of study was to estimate plasma progesterone concentration on different days of protocols in both the groups. In Group 1, out of 15 animals that received Modified Co-synch protocol treatment, 12 (80%) animal responded and exhibited signs of heat after the prostaglandin administration and out of which four animal (33.33%) exhibited excellent symptoms, five animals (41.7%) showed medium category estrus and only three animal (25%) responded with poor estrus symptoms. Similarly in Group 2, all 15 animals that received Modified Co-synch protocol along with progesterone device for 7 days responded to treatment and expressed sign of heat and out of which seven buffaloes (46.7%) responded with excellent symptoms, five animals (33.3 %) showed medium estrus symptoms and only three animals (20%) expressed poor estrus signs. In Group 1, out of 12 animals which were inseminated, five animals (33.3%) were found to be pregnant after FTAI at induced estrus and three animals (30%) got pregnant following AI during second estrus. Thus, an overall conception rate (53.53%) with pregnancy of eight buffaloes following induced and subsequent estrous was achieved in this Group. Similarly in Group 2, out of 15 animals, seven buffaloes (46.6%) were found to be pregnant following induced estrous and three animals (37.5%) got pregnant following AI at subsequent estrous. Thus, an overall conception rate (66.67%) with pregnancy of ten buffaloes following induced and subsequent estrous was recorded in this group. Concentration of progesterone in Group 1 was 0.24±0.04, 1.38±0.11, 0.31±0.05, 0.25±0.04 ng/ml (on Day 0, 7, 9, 10) and in Group 2 was 0.26±0.04, 3.75±0.40, 0.24±0.05, 0.25±0.05 ng/ml (on Day 0, 7, 9, 10). Therefore, it was concluded that progesterone

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supplementation in Modified Co-synch protocol treatment during non-breeding season results in very good induction and acceptable conception rate in anestrous buffaloes under field conditions.

Keywords: *Bubalus bubalis*, buffaloes, non-breeding season, anestrous, modified co-synch

INTRODUCTION

Postpartum anestrus is one of the major problems in buffalo reproduction. Under the field condition due to heat stress, photoperiod, negative energy balance due to less availability of green fodder and mineral deficiency during non-breeding season (spring-summer), majority of postpartum buffaloes do not exhibit estrus and suffer from anestrous condition (Baruselli *et al*., 2001; Nanda *et al*., 2003). In India, incidence of anestrous in postpartum buffaloes ranges between 9.18 and 82.50% during summer season (Thakor and Patel, 2013). Various therapeutic agents including hormonal and non–hormonal compounds have been used extensively for the restoration of cyclicity in anestrous cattle and buffalo by several workers with varying degree of success (Kumar *et al*., 2016; Bisen *et al*., 2018). Progesterone enhances the hypothalamic sensitivity to estrogen and is important for the expression of estrus symptoms (McDougall *et al*., 1992) and for better display of estrus behaviour by anestrous buffaloes at induced-estrus priming of hypothalamo-hypophyseal axis with adequate amounts of progesterone is also beneficial (De Rensis *et al*., 2005). Various scientist stated that majority of acyclic/anestrous buffaloes display variable ovarian activity characterized by follicular turn over in one or both ovaries and associated with absence of estrus cycle (Zicarelli, 1997; Ghuman *et al*., 2012). This justifies the need to establish a hormone protocol that can generate an acceptable conception rate in anestrous buffaloes irrespective of their ovarian status (true anestrus or subestrus). Therefore, objective of present study was to evaluate the efficacy of progesterone supplementation alongwith Modified Co-synch protocol in estrus induction and conception rate in anestrous Murrah buffaloes.

MATERIAL AND METHODS

The study was conducted on 30 postpartum anestrous Murrah buffaloes under field conditions in Hisar and Jhajjar districts of Haryana during the summer season (May to July). In present study efficacy of Modified Co-synch protocol has been evaluated in inducing estrus and conception rate in postpartum anestrous buffaloes. In addition to the above protocol effect of supplementation of progesterone had also been evaluated along with the Modified Co-synch on estrus induction and conception rate in Murrah buffaloes in field conditions. Ovarian screening was carried out twice at an interval of 12 days to confirm absence of any palpable cyclic structure (follicle or corpus luteum) on the ovaries by per rectal examination of genitalia. In addition, blood samples were also analyzed at 12 days apart for progesterone concentration. The buffaloes had <0.6 ng/ml plasma progesterone concentration on both sampling days were considered as acyclic. Group 1 animals were given 10 µg GnRH on day 0, 500 µg Cloprosetnol on day 7 and 2000 I.U. hCG on day 9 followed by artificial insemination on same day and 24 h later. In Group 2 progesterone device (TRIU-B, 0.96 g progesterone) was administered per vaginally on day 0 along with 10 µg GnRH injection given intramuscularly and kept *in situ* for 7 days. On

day 7 after removal of progesterone device 500 µg Cloprosetnol was injected intramuscularly. On day 9, 2000 I.U. hCG was given followed by artificial insemination on same day and 24 h later. Furthermore, blood samples were collected on day 0, 7, 9 and 10 of the treatment days for estimation of plasma progesterone concentrations. The blood samples were collected in heparinized vacutainers and transported to laboratory immediately in cool box. The blood samples were centrifuged at 3000 rpm for 10 minutes and supernatant plasma was stored at -20°C until analysis. Plasma progesterone concentrations were analyzed using ELISA kit (Calbiotech). The sensitivity of the progesterone assay was 0.22 ng/ml. The intra- and inter-assay coefficient of variation was 5.36 and 9.68%, respectively.

RESULTS AND DISCUSSION

In the present study, efficacy of two estrus induction protocols were evaluated for inducing estrus in anestrous buffaloes during non-breeding season under field conditions. In Group 1 (Table 1), out of 15 animals that received Modified Co-synch protocol treatment, 12 (80%) animal responded and exhibited sign of heat after the prostaglandin administration and out of which four animal (33.3%) exhibited excellent symptoms, five animal (41.7%) showed medium category estrus and only three animal (25%) responded with poor estrus symptoms. With the same Co-synch protocol, others also reported 80 to 90% estrus induction rate in anestrous and cyclic buffaloes during breeding season (Carvalho *et al*., 2004; Biradar *et al*., 2016; Buhecha *et al*., 2016). Kumar *et al.* (2016) also reported 81.3% of estrus induction rate in postpartum anestrous Murrah buffaloes under field conditions during breeding season. In contrast with the above findings, Tiwari *et al*. (2014) reported slightly lower estrus response of 66.67% in postpartum anestrous buffaloes during summer season. This suggests that season of the year affects the efficacy of treatment protocol, however, our findings does not support this. In our first Group animals, mean progesterone values were on basal level at the time of start of experiment and they increased significantly to 1.38 ng/ml. It indicates that at the start of experiment some of the animals were having follicular activity and these follicles were luteinized by the GnRH and acted as source of progesterone on day 7.

This progesterone perhaps increased the sensitivity of hypothalamus to oestradiol (McDougall *et al*., 1992; De Rensis *et al*., 2005) secreted by growing follicles following luteolysis by PG injection on day 7 and animals exhibited heat symptoms in this group. Looking at progesterone profiles of individual animals on day 7 also supported this view since buffaloes having higher progesterone values exhibited more pronounced intensity of heat. Similarly in Group 2, all 15 animals (100%) that received Modified Co-synch protocol along with progesterone device for 7 days responded to treatment and expressed signs of heat, and out of which seven buffaloes (46.7%) responded with excellent symptoms, five animal (33.3%) showed medium estrus symptoms and only three animal (20%) expressed poor estrus. When data between the groups was compared, the results indicates that though, the values are not statistically different but estrus induction rate (100% vs 80%) and number of animals exhibited excellent estrus response (46.7% vs 33.3 %) still higher in Group 2 compared to Group 1. This suggests that as we expected the progesterone supplementation in Group 2 increased the rate

and intensity of estrus induction response as progesterone concentration were significantly higher in Group 2 compared to Group 1 (3.75±0.40 vs 1.38±0.11 ng/ml) on day 7 of treatment. Similar to our findings, the 100% estrus induction rate was also achieved by other scientists in buffaloes where Cosynch protocol was supplemented with progesterone (Nakrani *et al*., 2014; Patel *et al*., 2013; Pinheiro *et al*., 1998). Compared to Cosynch alone addition of progesterone source in Cosynch protocol also increased the estrus induction rate in anestrous buffaloes (Busch *et al*., 2008; Lamb *et al*., 2001; Lima *et al*., 2011).

Conception rate (Table 2) was elucidated at 90-100 days post-insemination by per rectal palpation. In Group 1, out of 12 animals which were inseminated, five animals (33.3%) were found pregnant after FTAI at induced estrus and three animals (30%) got pregnant following AI during subsequent estrus. Thus, an overall conception rate 53.53% was recorded with following induced and subsequent estrus. However, others recorded only 7 to 30% conception rate in anestrous buffalo during low-breeding season (Baruselli *et al*., 2001; Ghuman *et al*., 2014) which is much lower than our findings. This indicates that as per our expectations, our Modified Co-synch protocol proved to be beneficial during summer season. In Group 2, out of 15 animals, seven buffaloes (46.6%) were found to be pregnant following induced estrous and three animals (37.5%) got pregnant following AI at subsequent estrous. Thus, 66.67% overall conception rate was recorded following induced and subsequent estrous in Group 2. These findings suggested that prolonged exogenous progesterone administration through TRIU-B device led to enhanced folliculogenesis, follicular growth and ovulation and primed the endometrium for better conception rate (Rhodes *et al*., 2002). Similarly, progesterone priming by addition of progesterone device to Cosynch protocol resumed cyclicty in acyclic buffaloes and resulted improvement in pregnancy rate from 48 to59% (Lamb *et al*., 2001). De Rensis *et al*. (2005) also reported that progesterone supplementation to the Ovsynch protocol in buffaloes increased conception rate in noncyclic buffaloes from 4.7 to 30%.

In Group 1, at the start of treatment i.e. on the day of first GnRH injection, plasma progesterone concentration (Figure 1) as anticipated were recorded at basal level i.e. 0.24±0.04 ng/ml. These levels rose to maximum on day 7 (1.38±0.11 ng/ml; Figure 1) which was significantly higher ($P<0.01$) than day of first GnRH injection.

These values again came down significantly to basal level on day of insemination i.e. day 9 (0.31±0.05 ng/ml) and on day 10 (0.25±0.04 ng/ml). This suggests that there was a follicular activity on the ovaries of some animals and these follicles were lutinized by the GnRH injection resulting in formation of CL and source of progesterone on day 7 which was followed by decreased progesterone concentration on day 9 indicating luteolysis of corpus luteum by PG injection. However, it can be confirmed by ultrasound which was not feasible in present study under field conditions. Above facts are in agreement with hypothesis that majority of acyclic/anestrous buffaloes display variable ovarian activity characterized by follicular turn over in one or both ovaries and associated with absence of estrus cycle (Zicarelli, 1997; Ghuman *et al*., 2012). In anestrous buffaloes and cattle, Buhecha *et al*. (2015); Buhecha *et al*. (2016) also suggested the presence of pre-ovulatory dominant follicle of large size at first GnRH injection of Ovsynch as indicated by elevated progesterone concentration on day 7 (4.55±0.28 ng/ml and 3.03±1.02 ng/ml, respectively). Bhoraniya *et al*. (2012) also showed

Table 1. Estrus induction rate (%) along with varying intensity of estrus in buffaloes following synchronized estrous cycle through Modified Co-synch protocol with (Group 2) and without (Group 1) progesterone supplementation.

Group	Estrus induction rate%	Excellent estrus	Medium estrus	Poor estrus
Group 1	12/15 (80)	4/12 (33.3)	5/12 (41.7)	3/12 (25)
Group 2	15/15 (100)	7/15 (46.7)	5/15 (33.3)	3/15 (20)

Table 2. Conception rate (%) recorded at FTAI and subsequent AI in estrus in buffaloes following synchronized estrous cycle through Modified Co-synch protocol with (Group 2) and without (Group 1) progesterone supplementation during summer season.

Group	CR after FTAI (%)	CR after subsequent AI (%)	Overall CR (%)
Group 1	5/15 (33.3)	3/10 (30)	8/15 (53.33)
Group 2	7/15 (46.6)	3/8 (37.5)	10/15 (66.67)

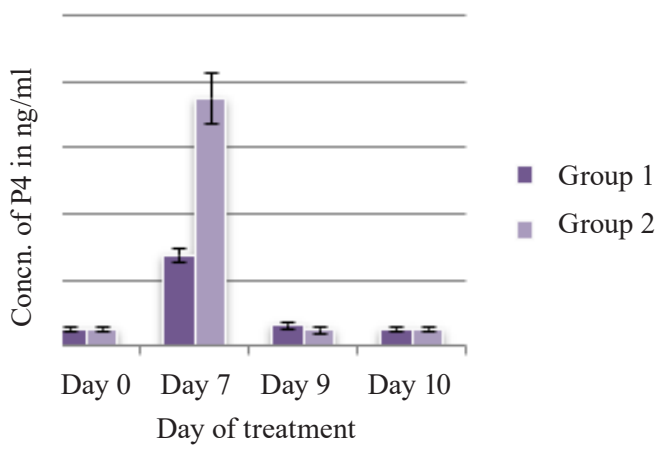

Figure 1. Graph showing variation in progesterone concentration on day 7 of both the groups.

increase in progesterone concentration on day 7 up to 5.73±1.26 ng/ml suggesting presence of follicle on day 0 of the Ovsynch protocol in post-partum anestrous Kankrej cows. Similarly in Group 2, at the start of treatment i.e. on the day of GnRH injection, plasma concentrations were at basal level i.e. 0.26±0.04 ng/ml, which attained highest level on day 7 (3.75±0.40 ng/ml) and again came down to basal level on day 9 and 10 (0.24±0.05 and 0.25±0.05 ng/ml) when animals were inseminated. There was no significant difference between the progesterone concentration on day 0 i.e. at the time of first GnRH and on the day of insemination within the groups. When the progesterone concentrations were compared between the groups, there was no difference in values on day of GnRH injection and on day of insemination, however, values on day 7 were significantly higher ($P<0.05$) in Group 2 (3.75±0.40 ng/ml) compared to Group 1 (1.38±0.11 ng/ml) suggesting supplementation of progesterone by the intra-vaginal device in Group 2 animals. In similar study, Bhoraniya *et al*. (2012) reported elevated progesterone concentration on day 7 (3.55±0.34 ng/ml) when administered progesterone device and synchronized with Ovsynch in post-partum anestrous cows. Ghuman *et al*. (2012) also achieved the same results when similar protocol was used for synchronization in lactating anestrous buffaloes. Thus, our study concluded that supplementation of progesterone in Modified Co-synch protocol treatment during non-breeding season improved estrus induction and conception rate in anestrus Murrah buffaloes.

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