EFFICACY OF MODIFIED CO-SYNCH PLUS PROTOCOL WITH OR WITHOUT PROGESTERONE DEVICE FOR ESTRUS INDUCTION AND CONCEPTION RATE IN MURRAH BUFFALOES UNDER FIELD CONDITIONS DURING SUMMER SEASON

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ABSTRACT

The aim of present study was to evaluate efficacy of Modified Co-Synch Plus Protocol with or without progesterone device for estrus induction, progesterone concentration and conception rate in Murrah buffaloes under field conditions during summer season. The study was conducted using 30 postpartum anestrus Murrah buffaloes divided in two groups. In Group 1 (n=15), the buffaloes were administered with PMSG 400 IU on -3 day, Buserelin acetate 10 µg, on day 0, cloprostenol 500 µg, on day 7, and on day 9 hCG 2000 IU. Thereafter, timed artificial insemination (TAI) was done at the time of hCG injection and 24 h later once again. In Group 2 (n=15), the buffaloes were treated with same protocol as Group 1, in addition to progesterone device, which was placed in vagina on day 0 and was removed on day 7 of the protocol. Thereafter, TAI was done as in Group 1. Plasma Progesterone concentration was estimated during the different days (-3, 0, 7, 9 and 10 day) of protocol. The estrus induction rate was 86.6% and 100% in Group 1 and Group 2 respectively showing very good estrus (46%), good estrus (30.7%) and poor estrus (23.07%) in Group 1 while very good estrus (46.6%), good estrus (40%) and poor estrus (13.4%) in Group 2. The conception rates in Group 1 and 2, at FTAI and overall including subsequent estrus were 46.2% and 61.53% and 53.33% and 73.33% respectively. The plasma progesterone concentrations were higher ($P<0.05$) on day 7 in Group 2 than Group 1 (4.16±0.32 *vs* 2.30±0.24 ng/ml). Both two protocol treatment during non-breeding season resulted in very good estrus induction and acceptable conception rate in anestrous buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, anestrous, modified co-synch plus, estrus induction, progesterone supplementation

INTRODUCTION

Buffaloes is an important livestock resource for the rural economy due to the fundamental role played by this species in many climatically disadvantaged agricultural systems (Gasparrini, 2013), hence considered

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as a pillar of dairy industry in South and South-East Asia including India. As production profile is flipside of pregnancy; the productivity of female buffaloes is essentially affected by the animal's reproductive efficiency, which is impaired by the late maturity of females, poor estrus expression, longer intercalving intervals and reduced ovarian activity during summer (Singh *et al*., 2000). Estrus synchronization protocols dramatically improve reproductive success by allowing producers to breed more females in less time by reducing the length of breeding and calving seasons. Therefore, by intervention in the reproductive events by using various hormonal protocols with fixed time artificial insemination (FTAI) can make reproduction in these animals more advantageous and practical, especially during the seasonal anestrus (Baruselli and Carvalho, 2005). In Literature, an array of studies are available using various combination of GnRH based protocol along with inclusion of either progesterone releasing devices or PMSG or HCG with variable success in estrus induction and conception rate in anestrus Murrah buffaloes under farm conditions (Carvalho *et al*., 2004; Bisen *et al*., 2018; Sharma *et al*., 2017; Kumar *et al*., 2016) but field studies lacks substantial evidence specially during summer season. Therefore, present study was planned to evaluate efficacy of Modified Co-Synch Plus Protocol with or without progesterone device for estrus induction, progesterone concentration and conception rate in Murrah buffaloes under field conditions during summer season.

MATERIALS AND METHODS

The study was conducted using 30 postpartum anestrus Murrah buffaloes under field conditions in Hisar and Jhajjar districts of Haryana during the summer season (May to July 2017) when the environmental temperature ranges between 42 to 45^{o}C. The buffaloes were selected randomly on the basis of the history of anestrous provided by the owner. All the buffaloes were between 2nd and 6th parity, with body condition score ranged between 3 and 4 of 5 points scale. All the buffaloes were free from any disease condition with normal reproductive organs. Ovarian structures screening was carried at 12 days apart 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. Then, selected buffaloes were randomly categorized in to two treatment groups with fifteen animals in each group. In Group 1, the buffaloes were administered with PMSG (Folligon, 400 IU i.m., MSD Animal Health) on 3 day, GnRH (Buserelin acetate, 10 µg, i.m., Receptal VET, MSD Animal Health) on day 0, PGF2α (cloprostenol, 500 µg, i.m., Vetmate, Vetcare) on day 7, and on day 9 hCG (Follison, 2000 IU i.m., Indian Immunological Ltd). Thereafter, timed artificial insemination (TAI) was done at the time of hCG injection and once more at 24 h later. In Group 2, the buffaloes were administered with PMSG (Folligon, 400 IU i.m.) on 3 day, GnRH (Buserelin acetate, 10µg, i.m., Receptal VET) on day 0, PGF2α (cloprostenol, 500 µg, i.m.) on day 7, and on day 9 hCG (Follison, 2000 IU i.m.). Additionally, progesterone device (TRIU-B, Virbac pharmaceuticals Ltd.) was kept into vagina on day 0 and was removed on day 7 of the protocol. Thereafter, TAI was done same as in Group 1. Furthermore, blood samples were collected on 3, 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^{o}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 Group 1, out of 15 animals that received Modified Cosynch Plus protocol treatment, 13 (86.6%) animal responded and exhibited sign of heat after the prostaglandin administration and out of which six animal (46%) exhibited very good symptoms, four animal (30.7%) showed good category estrus and only three animal (23.07%) responded with poor estrus symptoms. Similarly in Group 2, all 15 animals (100%) that received Modified Cosynch Plus protocol along with progesterone device for 7 days responded to treatment and expressed sign of heat and out of which seven buffaloes (46.6%) responded with very good symptoms, six animal (40%) showed good estrus symptoms and only two animals (13.4%) expressed poor estrus (Table 1).

In present study, overall estrus induction rate was marginally higher (100% vs 86.6%), in Group 2 compared to Group 1. Similarly, Co-synch Plus protocol in anestrous buffaloes during low breeding season resulted 100% estrus induction rate (Sharma *et al*., 2017) and during breeding season resulted 93.7% estrus induction rate (Kumar *et al*., 2016). It means as far as onset of heat symptoms are concerned incorporation of progesterone in Co-synch Plus protocol does not have additional much benefit to treat anestrus condition in buffaloes during breeding and non-breeding season. However, in another study under similar field conditions, use of intra-vaginal device in Modified Co-synch protocol significantly improved the heat induction (80% vs 100%) in anestrous buffaloes during summer season (Sharma *et al*., 2019).

Rathore *et al*. (2017) also reported 88% estrus induction response in anestrus Murrah buffaloes with Ovsynch Plus protocol in non-breeding season, however, in heifers the induction rate was only 66.67% suggesting an efficacy of treatment on parity of animals. Similarly, in anestrous buffalo heifers, use of intra-vaginal device resulted 100% estrus induction within 48 to 72 h (Singh *et al*., 2009) suggesting role of progesterone in improving onset of heat symptoms. Use of progesterone device (CIDR) in Ovsynch protocol also resulted increased follicular size and better estrous behavior in anestrous lactating buffaloes (Ghuman *et al*., 2012).

When the effect of treatment on intensity of heat symptoms was looked into and data were analyzed between estrus intensity categories of both the groups, again we could not find any significant differences ($P<0.05$) between the groups. This suggests that administration of PMSG three days before first GnRH resulted in development of follicle which was luteinized and transformed in to CL by first GnRH which acted as source of progesterone. The amount of progesterone secreted by this CL was sufficient enough to induce heat symptoms and additional supplementation of progesterone to improve heat intensity is not required in Co-synch plus protocol during non-breeding season. However, in our another study under similar field conditions, use of intra-

In Group 1, at the start of treatment (day 3) and on the day of first GnRH injection, plasma concentration as expected were recorded at basal level i.e. 0.26±0.04 and 0.27±0.04 ng/ml, respectively. These levels rose to maximum on day 7 (2.30±0.24 ng/ml) which were significantly higher ($P<0.01$) than day 3 and day of first GnRH injection (Figure 1). These values again came down significantly to basal level on day 9 (0.27±0.06 ng/ml) and on day 10 (0.22±0.03 ng/ml) when the animals were inseminated. The possible explanation for higher progesterone on day 7 is because of PMSG treatment 3 days before first GnRH that initiated follicular development which were luteinized by the first GnRH and transformed in to CL which acted as source of progesterone raising the plasma progesterone to highest on day 7 and PGF 2 alpha treatment resulted luteolysis of this CL to bring the progesterone to basal level on day of insemination (Sharma *et al*., 2017).

Similarly in Group 2, again at the start of treatment (day 3) and on the day of first GnRH injection, plasma progesterone concentrations were at basal level i.e. 0.25±0.04 and 0.21±0.02 ng/ml, respectively which attained significantly ($P<0.01$) highest level on day 7 (4.16±0.32 ng/ml) and again came down to basal level on day of insemination i.e. day 9 and 10. (0.27±0.04 and 0.23±0.04 ng ml) (Figure 1). There was no significant difference between the progesterone concentration on day 3, at the time of first GnRH and on the day of insemination. When progesterone concentration were compared between the two Groups, there was no difference in values on day 3, day of first GnRH injection and on day of insemination, however, values on day 7 were significantly higher ($P<0.05$) in Group 2 compared to Group 1 (4.16±0.32 vs 2.30±0.24 ng/ml; Figure 1). This higher progesterone in Group 2 is due to supplementary contribution by progesterone device inserted for 7 days. Similar observations have also been reported by Ghuman *et al*. (2012, 2015) in lactating anestrous/subestrous buffaloes where CIDR was used as progesterone releasing device.

In Group 1, out of 13 animals which were inseminated after synchronized estrus, six animals (46.2%) were found pregnant and two animals (28.6%) conceived following AI during subsequent estrous. Thus, 61.5% overall conception rate was recorded in this group (Table 2). Kumar *et al*. (2016) also reported a conception rate of 56.3 % at first AI and over all conception rate including subsequent AI was 75% which was slightly better than our investigation. In previous studied in anestrus peri-pubertal buffalo heifers during breeding season, various researchers observed 50 to 66% pregnancy rate at first AI following synchronized estrus with Ovsynch-plus protocol (Sharma *et al*., 2004; Singh *et al*., 2004) which are also slightly better than to our findings. In our study, we have used Modified Cosynch Plus protocol where second GnRH has been replaced with hCG injection and study has been conducted during non-breeding season in pluriparous anestrous buffaloes. Human chorionic gonadotropin (hCG) and GnRH has been used for synchronization of ovulation in GnRH based protocol owing to their relatively similar effects on the ovary (Fricke *et al*., 1993), but hCG is considered more potent ovulating agent as hCG acts independently of the pituitary gland and triggers ovulation by binding directly to LH receptors on the follicle (Ireland and Roche, 1983) while ovulatory response to a GnRH injection in part depends via the pituitary release of LH, which is affected by circulating progesterone concentration (Giordano *et al*., 2012). However, in synchronization Ovsynch protocol, replacement of 2nd GnRH with LH or hCG neither affected the ovulation rate nor conception rate in cyclic buffaloes during breeding season and anestrous buffaloes during non-breeding season (Berber *et al*., 2002; Carvalho *et al*., 2007; Ghuman *et al*., 2012). However, 15.38% conception rate was observed following Ovsynch or Ovsynch-plus in anestrus buffalo heifers during summer season (Carvalho *et al*., 2007; Malik, 2012; Saini *et al*., 2018). The differences in conception rate in these studies and our investigation might be attributed to the season and parity of the animals (Baruselli *et al*., 2001; Warriach *et al*., 2008; Berber *et al*., 2002). However, in another study in pluriparous anestrus buffaloes under same field conditions during breeding season (Sharma *et al*., 2019) where PMSG was not given 3 days before first GnRH but 2nd GnRH has been replaced with hCG resulted only 33.35 conception rate. It indicates that PMSG before first GnRH is important factor for improvement in pregnancy rate.

In Group 2, out of 15 animals, eight buffaloes (53.3%) were found to be pregnant following induced estrous and three animals (42.8%) conceived following AI at subsequent estrous. Thus, 73.3% overall conception rate was recorded in this group (Table 2). The conception rate at induced estrus was found to be similar in Group 1 (46.2%) and Group 2 (53.3%) and not statistically different. However, over all conception rate was found to be marginally higher (P>0.05) in Group 2 (73.3%) compared to Group 1 (61.5%). In contrast to this, use of progesterone (PRID) in Ovsynch protocol improved the conception rate from 19 to 30% during non-breeding season in anestrous buffaloes (Presicce, 2007) but this was still low compared to breeding season (45%; Ghuman *et al*., 2015). In another study, addition of progesterone (CIDR) in Ovsynch protocol improved conception from 44% to 70.6% in anestrous lactating buffloes (Ghuman *et al*., 2012). Similarly, priming with progesterone before 2nd GnRH injection resulted ovulation 80% animals within 72 h and a conception rate of 70% in anestrous buffaloes (Ghuman *et al*., 2015). Thus, we were expecting better conception rate in Group 2 compared to Group 1 but it did not happen. It means the amount of progesterone secreted by the luteinized follicles in response to first GnRH in Group 1 was sufficient enough to have desired effect on conception rate, however, progesterone profiles on day seven were significantly higher in Group 2 compared to Group 1.

Over all conception rate including AI at induced estrus and at subsequent estrus was found to be marginally higher in Group 2 (73.3%) compared to Group 1 (61.5%), however, values were not statistically different. Only two animals in Group 1 and only three animals in Group 2 conceived during subsequent estrus it also indicates that effect of treatment in both Groups did not differ. However, in another study in pluriparous anestrus buffaloes under same field conditions during non-breeding season (Sharma *et al*., 2017) where PMSG was not given 3 days before first GnRH but 2nd GnRHwas replaced with hCG with or without progesterone for 7 days before 2nd GnRH, three animals per group conceived following AI at subsequent estrus. It indicates that type of treatment protocol initiates the cyclicity in anestrus buffaloes but does not influence the conception rate following AI at subsequent estrus. However, other workers have suggested that size of CL and progesterone profiles following AI determines the conception rate during subsequent estrous (Pandey *et al*., 2011; Saini *et al*., 2018). Modified Cosynch Plus protocol treatment during non-breeding season resulted in very good induction and acceptable conception rate

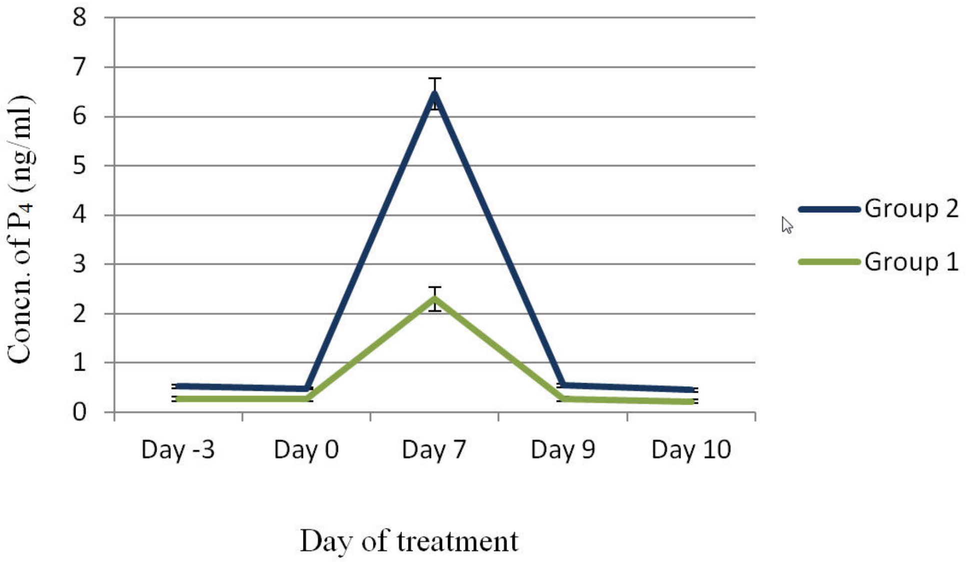

Figure 1. Graph showing variation in progesterone concentration on day 7 of both the groups.

Table 1. Estrus induction rate depending upon intensity of heat symptoms following synchronization of estrous cycle through Modified Cosynch Plus protocol with (Group 2) and without progesterone supplementation (Group 1) in buffaloes during summer season.

Group	Overall estrus induction rate (%)	Animals showed excellent estrus (%)	Animals showed medium estrus (%)	Animals showed poor estrus (%)
Group 1 (n=15)	13/15 (86.6)	6/13 (46)	4/13 (30.7)	3/13 (23.07)
Group 2 (n=15)	15/15 (100)	7/15 (46.6)	6 /15 (40)	2/15 (13.4)

Table 2. Conception rates (%) following synchronization of estrous cycle through Modified Cosynch Plus protocol with (Group 2) and withoutprogesterone supplementation (Group 1)in buffaloes (CR: Conception rate; FTAI: Fix Timed Artificial Insemination).

Group	CR after FTAI (%)	CR after subsequent AI (%)	Overall CR (%)
Group 1 (n=15)	7/13 (53.8)	2/6 (33.3)	69.2
Group 2 (n=15)	5/15 (33.8)	8/10 (80)	86.6

in anestrous buffaloes. Additional supplementation of progesterone in Modified Cosynch Plus protocol treatment during non-breeding season does not influence induction and conception rate in anestrous buffaloes.

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