Original Article

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A HORMONE-BASED THERAPEUTIC STRATEGY TO REDUCE NON-PRODUCTIVE PERIOD IN ANESTRUS BUFFALOES DURING BREEDING AND NON-BREEDING SEASON UNDER FIELD CONDITIONS

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ABSTRACT

Sixty anestrus Murrah buffaloes were evaluated for the success rate of progesterone (P_4) + estrogen (E_2) + equine chorionic gonadotropin (eCG) protocol (Breeding season, BS = 15; Non-breeding season, NBS = 15) and estradoublesynch protocol (BS = 15; NBS = 15). The buffaloes subjected to P_4+E_2+eCG had higher (P>0.05) estrus induction response compared to estradoublesynch during BS (86.0 vs 80.0%, respectively) and NBS (73.3 vs 66.6%, respectively). Estrus period score at induced estrus in buffaloes subjected to either of protocols in BS or NBS was not different (P>0.05), however, pregnancy rate was lower (P<0.05) in buffaloes exhibiting <50 estrus period score in comparison to those exhibiting >50 score. Further, pregnancy rate (induced + spontaneous estrus) was better (P>0.05) using P_4+E_2+eCG compared to estradoublesynch protocol (73.3 vs 63.3%). In addition, the interval between start of a protocol and conception in P_4+E_2+eCG was less compared to estradoublesynch (P>0.05; 13.9±1.3 *vs* 17.5±3.1 days). In summary, P_4+E_2+eCG protocol is a better hormone-based strategy compared to estradoublesynch for anestrus buffaloes during breeding and non-breeding season under field conditions.

Keywords: *Bubalus bubalis*, buffaloes, dairy household, estrus, hormones, season

INTRODUCTON

In buffaloes, breeding season starts in rainy period and winter is the favorable period, while summer remains the unfavorable period for breeding. A set of data collected from a rural veterinary hospital in Punjab on seasonal variation in artificial insemination (AI) in buffalo suggested the maximum AIs per month were in October and November, whereas the minimum AIs per month were in June and July (Ghuman and Dhami, 2017). Seasonal decline in reproductive activity was also manifested by reduced expression of estrus as the cases of abbreviated duration of estrus and unobserved/silent estrus were highest in April (70%) and lowest in December (10%; Prakash *et al*., 2005). Moreover, calving interval was longer

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for buffalo calving in February-June due to delayed resumption of post-calving ovarian activity compared to those calving in July to December (from 38 to 64 become 116 to 148 days; Singh *et al*., 2000). The minimum and maximum pregnancy rate in buffalo in a calendar year was observed in June and January and was respectively recorded as 27% and 54% (Ghuman and Dhami, 2017). To maximize productive life of a buffalo, she must be bred within 90 days after calving and thus start a new lactation every 13 months. However, their reproductive efficiency is hampered by seasonal reproductive activity. Thus, it is important to develop technologies that can alleviate seasonal suppression of reproductive activity in buffaloes.

Using transrectal ultrasound-aided monitoring of ovaries, the buffaloes during summer anovulatory period (non-breeding season) displayed dominant follicles that even after attaining ovulatory size (10.1 to 15.7 mm) undergo atresia without ovulation (Ghuman *et al*., 2010). This suggested that acyclicity in buffalo during summer season can be alleviated by fixed time artificial insemination (FTAI) using hormone-based therapeutic strategies based on progesterone (P_4), gonadotropin releasing hormone (GnRH) and prostaglandins ($PGF_{2\alpha}$), although these protocols had variable outcome with respect to conception (Warriach *et al*., 2015). Anestrus as well as subestrus buffalo responded poorly to ovsynch or $PGF_{2\alpha}$ based therapeutic strategies especially during non-breeding season (Dadarwal *et al*., 2009; Ghuman *et al*., 2009). A preliminary comparison of fertility outcome of ovsynch and a progesterone plus estradiol benzoate (P_4+EB)-based FTAI protocol during non-breeding season in buffaloes reared by small farmers revealed an overall (1^{st} AI and re-insemination) conception rate of 25% and 69.8%, respectively (Ghuman and Dhami, 2017).

Induction of synchronized estrus in seasonal anestrus buffaloes may provide a potential alternative for increasing the lifetime productive period of buffalo. However, the major bottleneck in wide application of hormone-based therapeutic strategies at small farmer's doorstep is poor conception rate during non-breeding season. Hence, this study was planned to select a hormone-based therapeutic strategy to reduce non-productive period in anestrus buffaloes during breeding and non-breeding season under field conditions.

MATERIALS AND METHODS

Animals

The study was conducted on 60 lactating Murrah buffaloes with the history of anestrus during the three-month period before the start of study. The selected buffaloes were in the age group ranging from 3 to 8 years, and in 2^{nd} to 4^{th} parity with body weight ranging from 350 to 500 kg. Buffaloes were daily fed with 10 to 15 kg of chopped green fodder, 2 to 3 kg of concentrates, 50 to 60 g of mineral mixture and had free access to drinking water. The study was conducted during breeding (Winter: November to February) and non-breeding (Summer: May to August) seasons.

Methodology

Based upon history of anestrus, the buffaloes were confirmed for anestrus by rectal palpation twice at 12-day interval. Thereafter, all the buffaloes were dewormed using a per-oral bolus (fenbendazole) at 10-day interval and were fed 50 g mineral mixture per day for 30 days. These buffaloes were randomly subjected to hormone-based therapeutic strategies for the induction of estrus followed by fixed-time AI (FTAI) as detailed below:

Progesterone plus estrogen plus equine chorionic gonadotropin (P_4+E_2+eCG) protocol (Figure 1) fifteen buffalo each during breeding (Group 1_B) and non-breeding (Group 1_{NB}) season, on Day 0 of protocol, were administered (i.m.) 2 mg estradiol benzoate and 500 µg PGF2α analogue (cloprostenol sodium), and 500 IU eCG was administered (i.m.) on Day 9. Sustained progesterone release device (0.96 g hydroxy progesterone implant) was placed intra-vaginally from Day 0 till Day 9. On Day 11, 20 µg GnRH analogue (buserelin acetate) was administered (i.m.) and AI was done 14 h after GnRH analogue administration.

Estradoublesynch protocol (Figure 2). Fifteen buffalo each during breeding (Group 2_B) and non-breeding (Group 2_{NB}) season, two days before the start of protocol were administered (500 µg $PGF_{2\alpha}$ analogue, cloprostenol sodium, i.m.). On Day 0, 7 and 8 of protocol, each buffalo was administered (i.m.) 20 µg GnRH analogue, 500 µg PGF2α analogue and 1 mg estradiol benzoate, respectively, and AI was done 48 and 60 h after estradiol benzoate administration. Under both the protocols, the buffaloes failing to conceive and returning to estrus was re-inseminated at observed spontaneous estrus without any additional hormonal treatment. In non-return cases, the pregnancy was confirmed per rectally at Day 60 of last AI.

Observations

The success rate of estrus induction hormonal strategy during breeding and non-breeding season was based upon the estrus induction response, pregnancy rate at induced as well as subsequent two spontaneous estruses, buffalo turning to be anestrus at pregnancy diagnosis and the interval between start of protocol to fertile estrus.

Estrus induction response characteristics at induced and spontaneous estrus were recorded using an estrus scoring chart (adopted as per Gamit *et al*., 2015; Kumar *et al*., 2019). The farmers were well educated about the estrus signs and their importance through on-site training. Thereafter, the farmer is observed the buffaloes for various visual signs of estrus, twice per day (early morning and late evening) for 30 minutes. The scoring parameters of visual estrus signs were seeking other buffalo (3 points), restlessness (5), mounted but not standing (10), sniffing the vagina of another buffalo (10), chin resting (15), mounting other buffalo (35), standing estrus (100) and mounting head-side of other buffalo (45). The scoring scale was adopted for recording the observations of genital tract such as vulva swelling (+2: 30 points, +1: 20 points), vulva moist and congested (+2: 20, +1: 10), uterine tone (+3: 30, +2: 20, +1: 10) and cervico mucous discharge (present: 100, absent: 0). The observed score for behavioral and genital tract alterations were recorded for each buffalo. If the sum of score was >50 during two consecutive observation periods, then the buffalo was considered to be in estrus.

Pregnancy rate is a new and recent method of measuring fertility in buffaloes as compared to traditional estimation of days open and conception rate. Pregnancy rate measures the percentage of buffaloes eligible to become pregnant and actually do become pregnant in a given time period. The interval between start of protocol to fertile estrus was calculated for each hormonal strategy used in the present study. The interval in terms of days was calculated from the start of protocol (Group 1: Day 0; Group 2: Day 2) till the day of AI (at induced or spontaneous estrus) which lead to pregnancy in buffalo.

Statistical analysis

The numerical data is represented as AV±SE, and the differences were considered significant at $P<0.05$. Statistical analysis was performed using GIMMIX procedure of SAS version 9.3 (SAS/STAT) statistical software. The data of various responses like estrus induction response, estrus period score, fate of buffalo failing conceive at induced estrus and the interval between start of protocol and pregnancy was analyzed between therapeutic strategies as well between seasons, using ANOVA. The pregnancy rate and the relation between estrus period score and pregnancy rate was subjected to chi squared test.

RESULTS AND DISCUSSIONS

The success rate of two hormone-based therapeutic strategies during the breeding (winter) and non-breeding (summer) season in lactating buffaloes having the history of anestrus for three months was evaluated based upon the estrus induction response and the conception rate at induced as well as subsequent two spontaneous estruses. In addition, interval between the start of each hormonal strategy to fertile estrus was recorded.

Estrus induction response

In present study, the buffaloes subjected to P_4+E_2+eCG had higher ($P>0.05$) estrus induction response compared to estradoublesynch during breeding (86 vs 80%, respectively) as well as non-breeding season (73.3 vs 66.6%, respectively). The administration of a small dose of eCG in P_4+E_2+eCG protocol about 3 to 4 days before the expected ovulation might have lead to competitive binding along with FSH to the FSH receptors of granulosal cells (Butnev *et al*., 1996). This response might have resulted in a decreased rate of granulosal apoptosis (Tilly *et al*., 1995), increased IGF1 (Sun *et al*., 2013) and estrogen production, thus, resulting in an increased diameter of the pre-ovulatory follicle and ultimately better estrus response (De Rensis and Lopez-Gatius, 2014). In previous studies, a better estrus induction response was reported following the use of P_4+E_2+eCG protocol in anestrus buffalo during breeding season (98%; Khan *et al*., 2018) compared to non-breeding season (83.3%; Mungad *et al*., 2016). Furthermore, estrus induction response following the use of estradoublesynch and doublesynch protocols in anestrus Gir cattle during non-breeding season was reported as 95 and 85%, respectively (Chaudhary *et al*., 2018).

Nevertheless, the detailed analysis of estrus period score at induced estrus in buffaloes subjected to P_4+E_2+eCG or estradoublesynch protocol in breeding or non-breeding seasons revealed that their estrus period score at induced estrus was not different ($P>0.05$), despite the fact that the proportion of buffaloes showing estrus induction response was higher ($P>0.05$) during breeding season as well as following the use of P_4+E_2+eCG protocol (Table 1). In addition, the estrus period score of buffaloes failing to conceive at induced estrus and subsequently returning to spontaneous estrus was not different ($P>0.05$) between seasons or protocol used (Table 1). On the other hand, the estrus period score of buffalo at induced estrus was higher ($P>0.05$) compared to the score exhibited by buffalo at spontaneous estrus (Table 1). In fact, in the buffaloes at spontaneous estrus, the expression of estrus was low due to the consistent failure of buffaloes to exhibit 'standing estrus', thus leading to a mean estrus score of <100 during most of the spontaneous estruses. However, in the present study, buffaloes at spontaneous estrus exhibited estrus behavioral signs like sniffing, chin resting, mounting but not standing, mounting on other buffalo except standing estrus. In addition, the genital tract signs like vulva swelling, moist vulva and uterine tone was better at spontaneous estrus as compared to induced estrus. Others have suggested that in dairy cattle with suprabasal plasma progesterone, animals would not stand to be mounted, but the secondary signs of estrus were observed for an extended period due to prolonged growth of ovulatory follicle and increased release of estradiol (Duchens *et al*., 1995).

Pregnancy rate

The use of P_4+E_2+eCG protocol in buffaloes lead to better (P>0.05) pregnancy rate at induced estrus (66.6%) compared to estradoublesynch protocol (53.3%, Table 2). Moreover, the overall pregnancy rate was better (P>0.05) using the P_4+E_2+eCG protocol compared to estradoublesynch protocol (73.3 vs 63.3%, Table 2). In previous studies, the pregnancy rate using P_4+E_2+eCG protocol was recorded as 45.4% in anestrus Surti buffaloes (Patel *et al*., 2018) and 40.6% in anestrus Murrah buffaloes (Murugavel *et al*., 2009). Further, following the use of estradoublesynch protocol, the pregnancy rate was recorded 27 to 40% in anestrus buffalo (Patel *et al*., 2018; Prajapati *et al*., 2018).

In the present study, the higher pregnancy rate with P_4+E_2+eCG protocol could be due to the administration of eCG at removal of the P_4 device which increases the diameter of the dominant follicle and ovulation rate (Nunez-Olivera *et al*., 2014). Furthermore, P_4+E_2+eCG protocol also increases the corpus luteum (CL) growth, early luteal phase P_4 concentrations and pregnancy rate (Carvalho *et al*., 2013).

The present study targeted to decide a better estrus induction protocol out of the two protocols used for buffaloes during the breeding as well as non-breeding season. Our results suggested that the overall pregnancy rate including the pregnancy rate at induced and spontaneous estrus was higher (P>0.05) during the breeding as well as non-breeding season following the use of P_4+E_2+eCG protocol compared their counterparts subjected to estradoublesynch protocol during the respective seasons (Table 2). In earlier studies, pregnancy rate in anestrus Brazilian Murrah buffaloes subjected to P_4+E_2+eCG protocol was 66.7 and 62.7% in breeding and non-breeding season, respectively (Carvalho *et al*., 2016). Also, the crossbred Murrah buffaloes had pregnancy rate of 64.2 and 60.2% in breeding and non-breeding season, respectively (Monteiro *et al*., 2018). Using estradoublesynch protocol, the pregnancy rate was recorded as 60 and 64% in anestrus crossbred cattle and buffalo, respectively (Sahoo *et al*., 2017). The pregnancy rate in cattle which were subjected to estradoublesynch or doublesynch protocols during non-breeding season was 55.0 and 45.0%, respectively (Chaudhary *et al*., 2018).

The fate of buffaloes failing to conceive at induced estrus

Out of thirty buffalo subjected each to P_4+E_2+eCG protocol or estradoublesynch protocol, a comparatively less (33.3%, P>0.05) proportion of buffaloes failed to conceive at estrus induced with P_4+E_2+eCG protocol compared to their estradoublesynch protocol counterparts (46.7%; Table 3). Similarly, in previous studies, about 54.6% buffalo failed to conceive following P_4+E_2+eCG protocol and this proportion was

72.7% following estradoublesynch protocol (Patel *et al*., 2018). In the present study, following the use of two estrus induction protocols, it was revealed that there is a marginal difference (P>0.05, 50 vs 57%) in buffaloes returning to spontaneous estrus following the failure of conception at induced estrus (Table 3). This suggested that either of protocol can be used in buffaloes with respect to the proportion of buffalo returning to spontaneous estrus after the failure of conception at induced estrus. It was suggested that the failure of buffalo to return to spontaneous estrus following use of estrus induction/synchronization protocol is a major drawback in popularity of these hormonal protocols in field conditions (Ghuman and Dhami, 2017). Moreover, in present study, the proportion of buffalo conceiving at spontaneous estrus was almost similar (P>0.05) in both the protocol groups (Table 3).

Nevertheless, in present study, the pregnancy rate at spontaneous estrus was less in comparison to the pregnancy rate at induced estrus (Table 2 and 3). Also, in the previous studies, the pregnancy rate at induced and subsequent spontaneous estrus following P_4+E_2+eCG protocol was 80.3 and 61.6%, respectively (Ghuman and Dhami, 2017). Similar results in non-breeding season using the same protocol were reported in Brazilian Murrah buffalo (Carvalho *et al*., 2016). This suggests that there is need to make better strategies to increase the pregnancy rate in buffalo at spontaneous estrus following the use of a hormonal protocol. Some researchers have used resynchronization strategies in buffalo failing to conceive at first induced estrus (Neglia *et al*., 2018), while others have used ovsynch and CIDR-GnRH protocol to resynchronize buffalo failing to conceive at first induced estrus (Arshad *et al*., 2017).

The detailed analysis of data in the present study with regard to the fate of buffalo failing to conceive at induced estrus in breeding and non-breeding season revealed that P_4+E_2+eCG protocol had edge over estradoublesynch protocol in terms of better pregnancy rate at spontaneous estrus in breeding as well as non-breeding seasons (Table 3). The use of eCG hormone in P_4+E_2+eCG protocol might have alleviated the adverse impact of season by improving the ovulatory follicular growth rate and hence better follicle diameter and better luteal profile subsequently (Sa Filho *et al*., 2010).

The interval between start of an estrus induction protocol and conception

Another part of the present study was to evaluate the efficacy of P_4+E_2+eCG and estradoublesynch protocol in terms of the interval (days) between the start of protocol and conception during breeding and non-breeding season. The lesser the number of days between start of protocol and conception, the better is the protocol in terms of economics of the dairy herd. The results of present study suggested that the interval between start of a protocol and conception in P_4+E_2+eCG protocol was 13.9±1.3 days as compared to 17.5±3.1 days for estradoublesynch (P>0.05). This interval pattern was same in both the protocols in breeding and non-breeding season (P>0.05). Thus, it can be inferred that P_4+E_2+eCG protocol had better efficacy in terms of getting the animal pregnant at less interval as compared to estradoublesynch. In previous studies, the interval between start of protocol to pregnancy was 15.0±3.0, 15.6±3.6 and 24.0±5.7 days for doublesynch, estradoublesynch and ovsynch, respectively (Prajapati *et al*., 2018).

Table 1. Estrus period score (av±se) of induced and spontaneous estrus following the use of estrus induction protocols during breeding (winter) and non-breeding (summer) season in anestrus buffaloes. P_4: Progesterone; E_2: Estrogen; eCG: Equine chorionic gonadotropin; B = Breeding; NB = Non-breeding.

Protocol	Group	Induced estrus			Spontaneous estrus	
		<50	>50	Overall	First	Second
Estrus behaviour score						
P_4+E_2+eCG	Gp I (n=30)	27.8±2.3 (n=6)	106.0±5.7 (n=24)	90.9±7.6 (n=30)	79.2±5.8 (n=5/10)	63.0±0.0 (n=2/8)
	Sub-gp I_B (n=15)	29.0±4.0 (2)	107.2±8.3 (13)	96.8±10.8 (15)	86.5±11.5 (2/4)	63.0±0.0 (1/3)
	Sub-gp I_{NB} (n=15)	27.2±3.2 (4)	106.0±8.2 (11)	85.0±10.5 (15)	74.3±6.3 (3/6)	63.0±0.0 (1/5)
Estradoublesynch	Gp II (n=30)	32.4±3.1 (8)	110.7±5.9 (22)	89.8±7.6 (30)	84.0±6.5 (8/14)	84.0±6.8 (5/13)
	Sub-gp II_B (n=15)	31.6±4.4 (3)	111.0±6.2 (12)	95.1±11.1 (15)	80.3±7.4 (4/6)	90.0±10.4 (3/5)
	Sub-gp II_{NB} (n=15)	32.8±4.6 (5)	110.3±11.1 (10)	84.4±11.1 (15)	87.2±11.6 (4/8)	75.0±0.0 (2/8)
Genital tract score						
P_4+E_2+eCG	Gp I (n=30)	22.8±5.6 (n=7)	105.6±10.0 (n=23)	86.3±10.1 (n=30)	74.0±4.0 (n=5/10)	60.0±0.0 (n=2/8)
	Sub-gp I_B (n=15)	23.0±5.0 (2)	96.1±10.6 (13)	86.6±14.3 (15)	75.0±5.0 (2/4)	60.0±0.0 (1/3)
	Sub-gp I_{NB} (n=15)	26.0±7.5 (5)	111.0±15.7 (10)	96.0±14.3 (15)	73.3±6.7 (3/6)	60.0±0.0 (1/5)
Estradoublesynch	Gp II (n=30)	23.6±3.9 (11)	107.9±10.5 (19)	77.0±10.1 (30)	82.5±8.0 (8/14)	84.0±11.7 (5/13)
	Sub-gp II_B (n=15)	30.0±5.5 (5)	101.0±14.2 (10)	77.3±14.5 (15)	75.0±6.4 (4/6)	93.3±18.4 (3/5)
	Sub-gp II_{NB} (n=15)	18.3±4.8 (6)	115.5±16.0 (9)	76.6±14.5 (15)	90.0±14.7 (4/8)	70.0±0.0 (2/8)

$P>0.05$: between and within groups, Figures in parenthesis indicate number of buffaloes.

Table 2. Pregnancy rate (%) following induced and spontaneous estrus in buffaloes subjected to estrus induction protocols during breeding (winter) and non-breeding (summer) season. P_4: Progesterone; E_2: Estrogen; eCG: Equine chorionic gonadotropin; B = Breeding; NB = Non-breeding.

Protocol	Group	Induced estrus	Spontaneous estrus		Overall Pregnancy rate
			First	Second	
P_4+E_2+eCG	Gp I (n=30)	66.6 (n=20)	40.0 (n=2/5)	0.0 (n=0/2)	73.3 (n=22)
	Sub-gp I_B (n=15)	73.0 (11)	50.0 (1/2)	0.0 (0/1)	80.0 (12)
	Sub-gp I_{NB} (n=15)	60.0 (9)	33.3 (1/3)	0.0 (0/1)	66.7 (10)
Estradoublesynch	Gp II (n=30)	53.3 (16)	12.5 (1/8)	40.0 (2/5)	63.3 (19)
	Sub-gp II_B (n=15)	60.0 (9)	25.0 (1/4)	33.3 (1/3)	73.3 (11)
	Sub-gp II_{NB} (n=15)	46.0 (7)	0.0 (0/4)	50.0 (1/2)	53.3 (8)

$P>0.05$: between and within groups, Figures in parenthesis indicate number of buffaloes.

Table 3. The fate of buffaloes failing to conceive at induced estrus during breeding (winter) and non-breeding (summer) season. P_4: Progesterone; E_2: Estrogen; eCG: Equine chorionic gonadotropin; B = Breeding; NB = Non-breeding.

Protocol	Group	Buffalo failing to conceive at induced estrus	Buffalo returning to spontaneous estrus	Buffalo conceiving at spontaneous estrus
P_4+E_2+eCG	Gp I (n=30)	33.3% (n=10)	50.0% (n=5/10)	28.5% (n=2/7)
	Sub-gp I_B (n=15)	26.6% (4)	50.0% (2/4)	33.3% (1/3)
	Sub-gp I_{NB} (n=15)	40.0% (6)	50.0% (3/6)	25.0% (1/4)
Estradoublesynch	Gp II (n=30)	46.7% (14)	57.1% (8/14)	23.0% (3/13)
	Sub-gp II_B (n=15)	40.0% (6)	66.7% (4/6)	28.5% (2/7)
	Sub-gp II_{NB} (n=15)	53.3% (8)	50.0% (4/8)	16.6% (1/6)

$P>0.05$: between and within groups, Figures in parenthesis indicate number of buffaloes.

Table 4. The relation between estrus period score (induced and spontaneous estrus) and pregnancy rate in buffaloes subjected to estrus induction protocol in breeding (winter) and non-breeding (summer) season.

Estrus period score	Pregnancy rate (%) based upon	
	Estrus behaviour score	Genital tract score
<50	21.4[a] (3/14)	35.3[d] (6/17)
50-100	72.2[b] (13/18)	78.3[e] (18/23)
>100	89.3[c] (25/28)	85.0[f] (17/20)

P<0.05: values with different superscripts within a column differ from each other, figures in parenthesis indicate number of buffaloes.

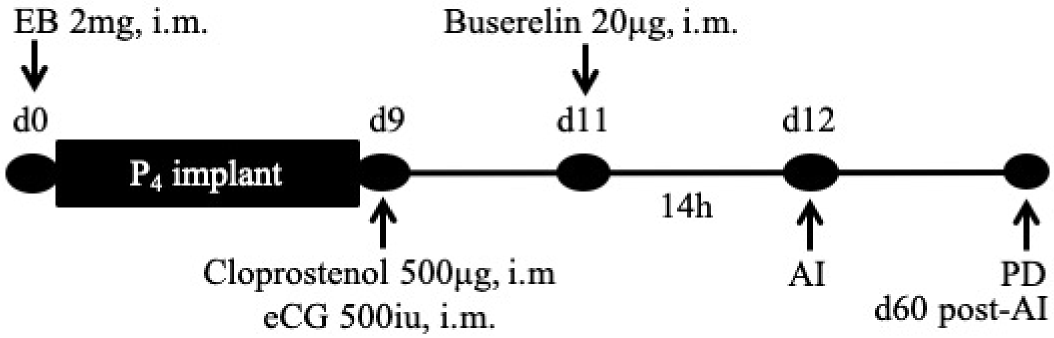

Figure 1. P_4+E_2+eCG protocol. Treatment on day 0, 9 and 11 was done at 7 pm and AI was done at 9 am.

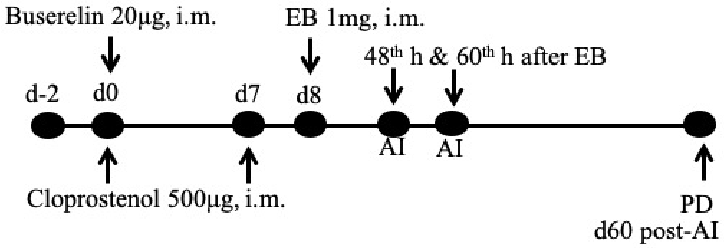

Figure 2. Estradoublesynch protocol.

The relation between estrus period score and pregnancy rate

The buffaloes exhibiting better signs of overt estrus had better chance of getting conceived was suggested earlier (Kumar *et al*., 2016). This is due to the fact that the estrus behavioral and genital tract alteration on the day of estrus is related to circulatory estrogen concentration (Saxena *et al*., 2017). A better developed ovulatory follicle will have higher steroidogenic capacity and subsequently highly steroidogenic corpus luteum, which are prerequisite for successful conception (Nayak *et al*., 2009). In the present study, the pregnancy rate was lower in buffalo exhibiting <50 estrus period score (estrus behavior and genital tract score) in comparison to their counterparts exhibiting estrus period score in the range of 50 to 100 or >100 (P<0.05, Table 6 and Figure 5). This suggested that estrus period score even at induced estrus is related to subsequent chances of successful conception. In previous studies, pregnancy rate based on the intensity of estrus response *viz*., intense, or moderate estrus response, was 80 vs 33% with cosynch and 71.4 vs 66.6% with cosynch plus protocol, respectively (Nayak *et al*., 2009; Kumar *et al*., 2016).

In summary, under field conditions, P_4+E_2+eCG protocol is a better hormone-based therapeutic strategy compared to estradoublesynch for anestrus buffaloes during breeding as well as non-breeding season. Pregnancy rate subsequent to insemination at induced or spontaneous estrus was associated with estrus period score in buffaloes.

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