Estrus Induction and Fertility Rates in Response to Exogenous Hormonal Administration in Postpartum Anestrous and Subestrus Bovines and Buffaloes

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ABSTRACT. A total of 130 animals (82 cattle, 48 buffaloes) with histories of anestrous 60–90 days post-partum and belonging to different agroclimatic zones of Punjab were subjected to rectal palpation and blood samplings at least three times at weekly intervals. The body condition score (BCS) of each animal was also recorded. The animals were divided into two groups; viz., true anestrous (Gp-I) and sub-estrus (Gp-II) through rectal palpation of ovaries and plasma progesterone (P4) concentrations. Furthermore, the Gp I and II animals were divided into treatment (Gp Ia, 40 cattle and 16 buffaloes; Gp IIa, 12 cattle and 14 buffaloes) and control groups (Gp Ib, 20 cattle and 8 buffaloes; Gp Ib, 10 cattle and 10 buffaloes). True anestrous animals (Gp Ia) were treated with 3 injections of hydroxyprogesterone caproate (750 mg, i.m.) at 72-hr intervals followed by injection of equine chorionic gonadotropin (eCG; 750 I.U., i.m.) 72 hr after the last progesterone injection. The animals were bred at the first estrus after the induced one. The first service conception rate (FSCR), overall conception rate (OCR), services per conception and pregnancy rate of the true anestrous treated cattle (Gp Ia) were 44.4%, 48.0%, 2.08 and 60.0%, respectively. In the true anestrous control cattle (Gp Ib), only five that were observed to be in estrus failed to conceive. In the anestrous treated buffaloes (Gp Ia), the FSCR and pregnancy rate were 50.0%, 62.5%, 1.6 and 62.5%, respectively. No buffalo amongst true anestrous control (Gp Ib) showed estrus. The subestrus animals (Gp IIa) were administered Prostaglandin F2α (PGF2α, 25 mg Dinoprost, i.m.) and bred at induced estrus. Amongst the Gp IIa animals, all cattle (100%) and twelve buffaloes (85.7%) responded to treatment. Of these animals, the FSCR and pregnancy rate at induced estrus in the cattle were 50.0% each, whereas they were 66.6% and 57.1%, respectively, in the buffaloes. The subestrus control animals (Gp IIb) remained infertile. In summary, the plasma P4 profile can be used to differentiate true anestrous and subestrus animals and thus to determine a hormonal therapy. Furthermore, fertile estrus can be induced with hormonal therapy in anestrous and subestrus bovines.

KEY WORDS: anestrous, buffalo, cattle, conception rate, subestrus.

The early return to cyclicity after calving is a prerequisite for high reproductive efficiency in dairy animals, that is, cattle and buffaloes. To maintain the recommended calving intervals, the animals need to conceive as soon as possible (85–90 days for the cow and 100–150 days for the buffalo) [7]. Increased intervals from calving to conception are due to deferred commencement of ovulation and estrus expression and reduced pregnancy rates, thus increasing the culling rate of the dairy herd.

An increased calving-to-conception interval as a result of true anestrous or subestrus in bovines adversely affects the economics of the dairy sector. The incidence of true anestrous in the Indian subcontinent varies from 14–48% in cattle [9, 36] and 19–74% in buffaloes [10, 34], with the maximal cases (53%) seen in bovines reared in rural areas [13]. The incidence of silent estrus or subestrus is higher in buffaloes (6–73%) [23] than in cattle (7–44%) [2]. A variety of factors, such as breed, parity, season [28], presence of bull, negative energy balance [3] and suckling [32], have been suggested to explain the prolonged calving-to-conception intervals.

Critical differential diagnosis of true anestrous and subestrus is only possible through frequent milk or plasma progesterone assay, repeated rectal palpation and ultrasonography [19].

Treatment of true anoestrus cattle and buffaloes with equine chorionic gonadotropin (eCG) after a period of progesterone treatment to stimulate cyclicity has been attempted with variable success [5, 26, 35]. A combination of controlled internal drug release (CIDR) for 7 days followed by an injection of 1 mg estradiol benzoate [11] and, more recently, a first injection of gonadotrophin releasing hormone (GnRH) followed by prostaglandin F2α (PGF2α) on day 7 and a second injection of GnRH on day 9 (day 0, GnRH; day 7, PGF2α; day 9, GnRH) have been adopted in cattle [33]. Treatment of bovines suffering from subestrus has mainly focused on the use of natural or synthetic PGF2α [17, 23, 31]. Of late, Ovsynch protocols have been successfully utilized to treat subestrus cows [12].

In rural India, bovines with postpartum anestrous are administered hormones for induction of estrus without attempting a differential diagnosis of true anestrous and subestrus. This field-oriented study was carried out to differentiate the two conditions on the basis of rectal palpation in conjunction with progesterone levels and thereafter to treat the animals using hormones for induction of estrus.
MATERIALS AND METHODS

Selection of animals: The present study was carried out in 18 randomly selected villages in the Malwa region of Punjab between May and August (summer months). A total of 130 anestrous animals (82 cross-bred cattle; 48 Murrah buffaloes) were subjected to rectal palpation and blood sampling on days 1 (day of presentation), 7 and 14 before commencement of the treatment. All the animals were 60–90 days postpartum and had histories of anestrous (absence of estrous symptoms; viz., bellowing and cervicovaginal mucus discharge in cattle and buffaloes and mounting in cattle only) according to the farmers. On the basis of rectal palpation and progesterone (P4) concentration, the animals were grouped into two groups, namely, the true anestrous (Group I) and subestrus groups (Group II). In Group I, ovaries were smooth and inactive with plasma P4 concentrations of less than 0.5 ng/ml at all time points. This group was further subdivided as follows. i) The treatment group (Gp Ia; 40 cattle, 16 buffaloes) contained animals that were administered exogenously 3 injections of hydroxyprogesterone caproate (750 mg, i.m.) at 72-hr intervals followed by one injection of eCG (750 I.U., i.m.) given 3 days after the last injection of hydroxyprogesterone caproate. The estrus detection method was critical observation of behavioral estrus symptoms (as mentioned above) by the farmers and later confirmation by local veterinarians of standing estrus. The animals were bred at the first estrus following the induced one. ii) The control group (Gp Ib; 20 cattle, 8 buffaloes) received no treatment, but the animals were bred at natural estrus (if any) during the period of study (70–80 days). Subestrus animals (Group II) had a palpable corpus luteum with a P4 concentration of more than 0.5 ng/ml at one or more of the three timepoints. These animals were also subgrouped. i) The treatment group (Gp IIa; 12 cattle, 14 buffaloes) contained animals that were treated with PGE2 (25 mg Dinoprost, i.m.) on the day when the CL was palpable and bred during induced estrus, and ii) the control group (Gp IIb; 10 cattle, 10 buffaloes) received no treatment, but were advised to be bred if they came in to estrus during the period of study.

The body condition scores (BCS) of all the animals were also measured as suggested by Edmondson et al. [4]. The animals were subjected to pregnancy diagnosis 90 days post breeding by rectal examination.

Progestosterone estimation: The plasma progesterone concentrations were measured by radioimmunoassay using the precoated tube method with the help of RIA kits supplied by FAO/IAEA Agricultural and Biotechnology, Seibersdorf, Austria. The mean intra- and inter-assay coefficients of variation were 6.71 and 10.08%, respectively. The sensitivity of the assay was 0.032 ng/ml.

Statistical analysis: The data for estrus response and conception rates were analyzed by chi-square test with a 95% level of significance [6].

RESULTS

Out of 130 animals that presented with anestrous, 60/82 (73.2%) cattle and 24/48 (50.0%) buffaloes were diagnosed as true anestrous, whereas, subestrus was observed in 22/82 (26.8%) cattle and 24/48 (50.0%) buffaloes (Table 1). Statistical analysis revealed a higher percentage of true anestrous in cattle than in buffaloes. The BCSs of the true anestrous cattle and buffaloes varied between 2.0–2.5 (poor) and 2.0–3.5 (poor to good), respectively. However, subestrus cattle and buffaloes had very good BCSs (3.0–3.5). Out of the 40 cows in Gp Ia, 36 (90%) came into estrus (1st) 48–72 hr after treatment and all of them showed estrus (2nd) 18–21 days after the first estrus; 16 of these animals conceived during the first insemination and yielded a first service conception rate (FSCR) of 44.4% (Table 2). Amongst the remaining 20 cattle, only 12 came into estrus a third time 19–24 days after the second estrus (8 cattle underwent anestrous), and 6 of the animals conceived. Out of the remaining 6 cattle, 2 came into estrus a 4th time (4 cattle underwent anestrous) 19–21 days after the third estrus and conceived. The overall conception rate (OCR), services per conception and pregnancy rate were 48.0%, 2.08 and 60.0%, respectively. However, in the Gp Ib cattle (n=20), only 5 animals (25%) came in estrus, but none of them conceived. Amongst the Gp Ia buffaloes (n=16), 14 animals (87.5%) exhibited estrus (1st) after 48–96 hr in response to treatment, and subsequently, 12 buffaloes (2 underwent anestrous) showed estrus (2nd) after 19–21 days; 6 of these animals conceived at first service, rendering a 50.0% FSCR. Out of the remaining 6 buffaloes, 4 conceived during the 3rd estrus (20–22 days after second estrus) and 2 underwent anestrous again. OCR, services per conception and pregnancy rate were estimated to be 62.5%, 1.6 and 62.5%, respectively, in the buffaloes (Table 2). No buffalo in Gp Ib (n=8) showed estrus. The estrus induction response, conception rates (FSCR & OCR) and pregnancy rates of the Gp Ia animals were significantly (P<0.05) higher than those of Gp Ib (control).

Amongst the Gp Ia animals, 12/12 (100%) cattle and 12/14 (85.7%) buffaloes responded to the treatment (Table 3) after 36–96 hr. Of them, 6 cattle (50.0%) and 8 buffaloes (66.6%) conceived at induced estrus. The pregnancy rates of the treated subestrus cattle and buffaloes were 50.0% and 57.1%, respectively. The statuses of the remaining cattle and buffaloes (6 each) could not be followed as they were culled. The control animals in Gp Ib (10 cattle and 10 buffaloes) remained infertile. The incidences of induced estrus, conception rates (FSCR) and pregnancy rates were signifi-

<p>| Table 1. Distribution of true anestrous and subestrus cattle and buffaloes |
|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Conditions</th>
<th>Cattle n (%)</th>
<th>Buffaloes n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>True anestrous</td>
<td>60 (73.2)*</td>
<td>24 (50.0)</td>
</tr>
<tr>
<td>Subestrus</td>
<td>22 (26.8)</td>
<td>24 (50.0)</td>
</tr>
</tbody>
</table>

* Significantly (P<0.05) greater than buffaloes.
INDUCTION OF ESTRUS AND FERTILITY IN BOVINES AND BUFFALOES

...cantly (P<0.05) greater in the prostaglandin-treated animals (Gp IIa) than in the controls (Gp IIb).

**DISCUSSION**

Post-partum ovarian quiescence either due to true anestrous or subestrus constitutes the major reproductive failure in cattle [8, 12] and buffaloes [23]. The onset of post-partum ovarian activity is highly dependent on re-establishment of LH stores [37] for which several hormonal and non-hormonal approaches [14, 22, 35] have been attempted so far, but the success of the treatment is highly dependent on differential diagnosis of both the conditions [30]. Conventionally, the method of choice for diagnosis of true anestrous and subestrus is rectal palpation of ovaries, but this may cause a high proportion of misdiagnosis with even the most skilled palpators [18] leading to incorrect treatment. The plasma progesterone concentration and palpation of ovaries for corpora lutea per rectum were 77–87% compatible; however, 18% and 37% of the cows were assigned incorrectly to the treatment and non-treatment groups, respectively [15]. Determination of progesterone in milk or blood is another valuable means of preventing incorrect diagnosis and treatment [15, 19]. Taking into consideration the fact that all the animals in the present study were brought for treatment of anestrous, only 26.8% of cattle and 50.0% of buffaloes were actually found to be in subestrus when routine rectal palpation findings were augmented with the plasma progesterone concentrations. This highlighted the need for measurement of progesterone as an aid to rectal screening of genitalia for differential diagnosis of true anestrous and subestrus.

The higher percentage of cattle observed in anestrous compared with buffaloes in the present study can be attributed to the comparatively poor BCSs of the cattle. The BCSs of the anestrus buffaloes were also low in the present study. It is recommended that the dairy cow have a BCS of 3.25–4.0 at the time of breeding [4]. Poor BCS in the post-partum period is a reflection of the negative energy balance that cattle/buffaloes experience soon after parturition, as dry matter intake is routed to meet the demand of milk production and thus reproduction becomes secondary. It is now thought that anestrous is largely due to failure of the dominant follicle to ovulate rather than being due to their absence [21]. Negative energy balance in post partum animals has been related to decreased LH pulse frequency that leads to decreased estradiol production by the dominant follicle [32] and thus failure of ovulation, but the exact underlying physiology is largely unknown.

The estrus response of true anestrous animals to the hormonal therapy (progesterone + eCG combination) in the present study was comparable to several previous reports in cattle [16, 22] and buffaloes [23, 26, 27]. The underlying mechanism is that subluteal levels of progesterone by way of injections or implants increases LH pulse frequency leading to maturation of the dominant follicle [37]. The combination of progesterone with eCG causes increased estradiol production leading to behavioural estrus followed by ovulation and thus favours conception [24]. The reason for commencement of estrus in some (n=5) cattle in control Gp Ib...
that failed to conceive could not be ascertained; however, it might have been due to repeated rectal ovarian massage [14].

Following hormonal treatment of the true anestrous cattle, a relatively lower FSCR (44% vs 49–60%) and OCR (48% vs 63%) was observed in the present study compared with previous studies [1, 29], this might be attributable to poor BCS [8, 33]; on the other hand, the conception rates of the buffaloes were comparable to the previous studies [23, 27].

In the present study, the subestrus animals were successfully treated with PGF2α (100% of cattle; 85.7% of buffaloes). Treatment of subestrus animals with PGF2α, on the day a palpable and functional CL is present favours the onset of estrus [8, 23]. The previously reported conception rates at induced estrus in subestrus cattle [20] and buffaloes [25] were comparable to those in the present study.

It can be concluded from the present study that rectal palpation together with plasma progesterone estimation are needed to distinguish true anestrous from subestrus, and this is possible if the ‘Cowside’ progesterone kits used in developed countries are made available at the field level.

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REFERENCES


