USE OF PROGESTERONE IMPREGNATED INDIGENOUS SPONGES ALONG WITH PMSG FOR THE INDUCTION OF CYCLICITY IN ANESTROUS MURRAH BUFFALO

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ABSTRACT

Twenty one anestruos Murrah buffalo were equally divided to receive indigenously prepared intravaginal sponges containing 1.20, 1.40 and 1.60 g progesterone for 8 days followed by administration (i.m.) of 400 IU PMSG on the day of sponge removal. All the buffalo exhibited estrus between 36-72 h after sponge removal with average duration of estrum as 20.66±0.52 h (16-24 h). The conception rate in S1.20, S1.40, S1.60 group was 42.9, 28.6 and 42.9%, respectively on induced estrus with an overall pregnancy rate of 71.4, 28.6 and 57.1%, respectively on subsequent estrus. Plasma progesterone increased ($p<0.05$) subsequent to sponge insertion in all the groups. In brief, considering the estrus induction response, conception rate and cost effectiveness, the indigenous sponges containing 1.20 g progesterone along with PMSG may be recommended for induction of cyclicity in anestrous buffalo.

Keywords: Buffalo, Conception rate, Estrus induction, Progesterone, Sponge

INTRODUCTION

Progesterone is one of the desirable progestagens used for the induction of cyclicity in true anestrous dairy animals (Dodamani *et al.,* 2011). For this purpose, many workers have used commercially available progesterone devices containing variable concentrations of progesterone like CIDR-B containing 1.90 g progsterone (Gordon, 1999), CIDR-B 1.38 g (Haider *et al*., 2015), PRID 1.55 g (Neglia *et al*., 2003) and a progesterone containing ear implant (Thangapandiyan *et al*., 2015) with varying degree of success. In India, no indigenous preparation of progesterone releasing intravaginal device is available for the estrus manipulation in buffalo. Moreover, in anestrous buffalo, very few studies have been conducted on estrus synchronization using indigenous progesterone sponges (Visha *et al*., 2014 and Devipriya *et al*., 2015). Therefore, the present study was planned to evaluate the efficacy of indigenous progesterone sponges loaded with different concentrations of natural progesterone for the induction of estrus and subsequent conception rate in Murrah buffalo.

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MATERIALS AND METHODS

Intravaginal sponge were prepared using a synthetic flat spongy foam sheet of 6 cm thickness, which was cut into circular sponges of 10 cm diameter and tied with a cotton string of 60 cm length. Subsequently, the sponges were sterilized under UV light for 2 h and were loaded with a ethanolic solution of natural progesterone (4-Pregnene-3,20-dione, Sigma-Aldrich) containing 1.20 g, 1.40 g or 1.60 g progesterone. The sponges were left overnight for drying in a laminar flow and were packed in a neat and sterilized aluminium foil till their use. A cylindrical plastic pipe (diameter 4 cm, length 50 cm) with an end narrowed for easy insertion in the vagina was used as speculum. A plastic rod plunger (diameter 2 cm, length 60 cm) was used for pushing the sponge into vagina through the speculum (Figure 1).

For the present study, true anestrus Murrah

Figure 1: Intravaginal progesterone sponge (left) with speculum (middle) and plunger (right).

buffalo (n=21) maintained at well-organised farms in loose housing system were used between July and August. The acyclicity of buffalo was confirmed by ultrasonographic examination carried out 10 days apart. The buffalo were randomly and equally divided to receive intravaginal sponges containing 1.20 g (S1.20), 1.40 g (S1.40) or 1.60 g (S1.60) progesterone for 8 days followed by administration (i.m.) of 400 IU Pregnant Mare Serum Gonadotropin (PMSG) on the day of sponge removal.

For loading the speculum, intravaginal sponge was inserted into the narrower end of sterilized speculum by taking out sponge thread from its distal end. The perineal and vulvar region of buffalo were cleaned and liquid paraffin was applied on the outer surface of loaded speculum. Following intra-vaginal insertion of loaded speculum, progesterone impregnated sponge was expelled out of speculum into vagina near the cervical opening and sponge was kept inside vagina for 8 days.

For estrus detection, bull parading was done between 24-72 h following removal of progesterone sponges and the buffalo showing mucus hanging from vulva, bellowing or being teased by bull was considered in estrus. Simultaneously, ultrasonography of the reproductive tract was performed to confirm estrus. At 60 and 72 h after sponge removal, all the buffalo were artificially inseminated with frozen-thawed semen. Thereafter, buffalo were observed twice daily for estrus signs or non-return to cyclicity between days 18 and 30 post-AI.

For progesterone estimation, 5 ml blood sample in heparinized tubes was collected on day 0 (immediately before the insertion of intravaginal sponge), day 4, day 8 (day of sponge removal) and finally about 48 h after sponge removal. Blood was centrifuged at 3000 rpm for 10 minutes to separate plasma and stored at -20˚C until estimation of progesterone by ELISA kit (Calbiotech Company, USA).

Plasma progesterone was compared between groups within time interval and within group between time interval by ANOVA using SPSS (16.0) system for windows. All numerical data was presented as mean±SE. Differences at a P-value less than 5% ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

In the present study, 100% retention of intravaginal sponges was recorded and no abnormal discharge was seen at the time of sponge removal in majority buffalo (n=18/21). The opaque and translucent discharge observed in remaining buffalo became clear and transparent within 48 h of sponge removal.

All the buffalo exhibited estrus after sponge removal, thus suggesting that progesterone concentration in sponge had no impact on estrus induction in buffalo. Various authors have reported induction of estrus in buffalo using progesterone release device with or without other hormonal preparations (Ghuman *et al.,* 2012; Vikash *et al*., 2014 and Buhecha *et al*., 2016). Nevertheless, in the present study, the intensity of estrus was moderate and almost similar in all the buffalo. Regarding overt estrus sign recording, only bellowing was present in six buffalo at the time of estrus.

Table 1: Plasma progesterone (ng/ml, Mean±SE) **following insertion of intravaginal sponges for the induction of estrus in buffalo.** 'S' indicates sponge with progesterone concentrations

Group	Day 0	Day 4	Day 9	Day 11
S1.20	$0.76\pm0.04^{ab,A}$	$1.40\pm0.24^{c,A}$	$1.12\pm0.16^{bc,A}$	$0.55\pm0.06^{a,A}$
S1.40	$0.65\pm0.07^{a,A}$	$1.76\pm0.26^{b,A}$	$1.24\pm0.22^{b,A}$	$0.56\pm0.08^{a,A}$
S1.60	$0.64\pm0.08^{a,A}$	$1.70\pm0.25^{c,A}$	$1.22\pm0.17^{b,A}$	$0.60\pm0.10^{a,A}$

$^{a,b,c}p<.05$, with in row; ^{A}similar superscript do not differ ($p<0.05$) within a colum

It was cited that PMSG supplementation at the time of progesterone implant removal enhances ovarian stimulation thus resulting in earlier onset and tighter synchrony of estrus (Murugavel *et al*., 2009). Similar findings were recorded in the present study in buffalo as all the buffalo exhibited synchronized estrus within 36 to 72 h after sponge removal. Administration of PMSG probably helps in the complete recovery of the hypothalamus-pituitary-gonadal axis function already stimulated by the progesterone treatment. The average duration of estrum in the present study was 20.66±0.52 h (16-24 h) which was in agreement with an earlier study suggesting similar duration of estrus using CIDR (Romano and Fahning, 2013). Again, the observed tight synchrony of estrus may be ascribed to the effect of PMSG.

In the present study, the conception rate at induced estrus in S1.20 and S1.60 group was 42.9% each and in S1.40 group was 28.6%, with their respective pregnancy rates as S1.20 - 71.4%, S1.40 - 28.6% and S1.60 - 57.1%. No similar work exists to compare the present findings. However, an overall conception rate of 57% was reported in true anestrous buffalo using 1.5 g progesterone sponges and $PGF_{2\alpha}$ administration a day before the sponge removal (Devipriya *et al*., 2015). Nevertheless, in the present study, a similar estrus response and higher pregnancy rate observed after using 1.20 g progesterone sponge is suggestive of their efficacy for estrus synchronization in true anestrous buffalo.

The basal plasma progesterone on day 0 (<1ng/ml, Table 1) in all the buffalo confirmed the acyclic nature of these animals. However, plasma progesterone increased ($p<0.05$, Table 1) following insertion of vaginal sponges as also reported earlier (Singh *et al*., 2010). Nevertheless, similar ($p>0.05$) plasma progesterone was observed on days 0, 4, 9 and 11 between the groups (Table 1), although plasma progesterone concentration in sponges was different between groups.

The calculation of cost effectiveness suggested that the cost of self-made intra-vaginal sponges varied between around Rs. 151-196, however, the cost of commercially available intra-vaginal progesterone device like CIDR is Rs. 700. Therefore, the sponges used in present study were cost effective for the induction of estrus as the cost was approximately 70-80% less than these commercially available progesterone devices.

In conclusion, on the basis of cost effectiveness, easy preparation, estrus induction response and conception rate, indigenous sponges containing 1.20 g progesterone along with PMSG may be recommended for the induction of cyclicity in true anestrous Murrah buffalo.

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