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Evaluation of Estrona Powder as Estrus Inducer in Anestrus Buffaloes under Field Conditions

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ABSTRACT

Background: Anestrus is a multifactorial problem in buffaloes. Herbal preparations yielded favorable results with estrus induction. Hence, the present study was undertaken to evaluate the therapeutic efficacy of Estrona powder (Rakesh Pharmaceutical) on induction of estrus in anestrus buffaloes under field conditions.

Methods: In total, 29 buffaloes were selected during Ambulatory clinics at village Sanoda and randomly allotted to two groups. The treatment group was fed 125g Estrona powder (n=18;101±10 Days in milk) for four days, while same amount of concentrate was fed to buffaloes of placebo group (n=11; 99±08 Days in milk). Blood from jugular vein was collected before initiation of treatment, day 0 (D0), day of estrus (DE) and on day 60 after AI/Service (D60) for estradiol (E2) and progesterone (P4) estimation.

Result: A significant number of buffaloes in Estrona treatment group induced estrus 80%(13/18) within shorter time interval (11.3±4.9 days) as compared to Placebo group 27% (3/11). No effect of treatment was observed on E2 and P4 concentrations during D0, DE and D60. However, within group, there was significant effect on D0, DE and D60 on concentrations of P4 and E2 hormones. These results indicate that Estrona powder successfully induced estrus, hence can be used for estrus induction in buffaloes.

Key words: Anestrus, Buffaloes, Estrus induction, Estradiol, Herbal preparation, Progesterone.

INTRODUCTION

Buffaloes are known for sluggish reproduction (Suthar and Dhami, 2010), however plays an important role in contributing 49% of total milk production of India. Inefficient feeding and management practices increase open days under field conditions and cause economic losses to the buffalo breeders (Parmer *et al*. 2012). Anestrus is a multifactorial problem affected by plausible factors like malnutrition, environmental stress, uterine pathology and improper managemental practices. Many nutritional formulations, vitamins and minerals, feeds with estrogenic property, herbal preparations, hormonal regimens and combinations were explored to induce/synchronize estrus in buffaloes with encouraging results (Walia *et al*. 2010, Hadiya *et al*. 2015). Herbal medicines are generally considered safe, hence scientists and research groups explore herbal medicines to enhance reproduction and reproductive functions in cattle and buffaloes. In last two decades different herbal medicines are launched in the market with success. Herbal medicines like Janova, Sepia, polyherbal drugs, herbal seeds (Dill /Suva /Fenugreek /Black paper seed/Black seed/Common grass *etc*.) were tried (Prajapati *et al*., 2018; Mane *et al*., 2018; Kumar *et al.,* 2021) and demonstrated successful induction of estrus in cattle and buffaloes. The preparation of herbal origin is known to be indigenous/tradition medicine, mostly cost effective and without any side effect.

Hence, the present study was undertaken to evaluate the therapeutic efficacy of Estrona powder (Rakesh Pharmaceutical, Kalol, Gujarat) in induction of estrus and conception rate in anestrus buffaloes, under field conditions.

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MATERIALS AND METHODS

The trial was undertaken in clinical cases presented at Ambulatory clinic of Kamdhenu University during January to October 2020. The Ambulatory clinic visits Sanoda village (Tehsil Dehgam, Dist. Gandhinagar), twice in a week and the cases reported are recorded in standard case sheets. Buffaloes presented with history of infertility were subjected to physical and per-rectal examinations. The buffaloes with apparently normal uterine conditions and smooth ovaries without persistent corpus luteum were selected and randomly allotted to two groups. The body condition score (BCS; 1 to 5 scale), parity, days in milk and other demographic information were recorded total 29 anestrus buffaloes were randomly allotted to two groups. Buffaloes of both groups were dewormed with Wormivet powder (Rakesh Pharmaceuticals, G.I.D.C, Kalol, Gujarat) 30 gm, twice daily and advised owner to feed 2.5 kg/day concentrate with green and dry fodder (50:50 ratio). Group I (Treatment; n=18) buffaloes were fed Estrona powder (Rakesh

Pharmaceutical, G.I.D.C, Kalol, Gujarat) 125 gm daily for four days. Group II (Placebo; n=11) buffaloes were fed equal amount of concentrate (125 gm) for four days. The owners were informed to record estrus signs like withholding of milk, vaginal discharge, swelling of vulvar lips etc., and report to the Ambulatory clinic.

Blood samples were collected for hormonal analysis in clot activated vacutainer from jugular vein on the day 0 (initiation of treatment; D0) and on the day of estrus (DE) and day 60 of pregnancy (D60) in buffaloes, as given in experimental design. Blood was transported at 4°C to the laboratory and extracted serum after centrifuging at 4000 rpm (Thermo Scientific Sorvall X4R Pro-MD, India) for 5 minutes and separated serum samples were labeled and stored in 2ml serum storage vials at -20°C until analyzed for estradiol (E2) and progesterone (P4). Hormonal assay was performed in a commercial laboratory using Elecsys® Estradiol III and Elecsys® P4 III by the Cobas e-411 analyzer (Roche Diagnostics, Germany). Cobas e-411 analyzer uses chemiluminisence immune assay (CLEIA) method for the analysis of the E2 (pg/ml) and progesterone P4 (ng/ml) concentrations (Colazo *et al*. 2008). The intra and inter assay coefficient of variation for E2 were 9.42% and 9.5% and for P4 were7.9% and 7.6%, respectively.

All the data documented on standard record sheets of Ambulatory clinics was entered in Microsoft excel spreadsheet and statistical analysis was performed using SPSS for windows (Version 26 SPSS Inc. IBM Pvt Ltd. Bengaluru, India). The descriptive function of analysis menu in SPSS was used to generate descriptive analysis of the two groups. The effects of the two treatments (Placebo vs Estrona powder treatment) on estrus induction and hormone levels (P4 and E2) during D0, DE and D60 was evaluated using multivariate generalized linear model. Random effect of buffaloes within group were included in the model. Effect of parity (3.0 ± 1.0 vs 3.0 ± 1.1), BCS (3.0 ± 1.0 vs 3.0 ± 1.14) and days in milk (99.0 ± 8.0 vs 101.0 ± 10.0) evaluated in the model were found non-significant (P > 0.05), hence excluded from the analysis. The effect of treatment (placebo and treatment) on the first service conception rate was evaluated as percentage.

RESULTS AND DISCUSSION

The study trial reported significant effect of the Estrona powder treatment on induction of estrus from D0 to DE in buffaloes, as compared to placebo treated buffaloes (P<0.05; Table 1). Out of 11 buffaloes, only three buffaloes (27%) expressed estrus within 20 days with an average interval of 42.1 ± 17.9 days between D0 to DE after placebo treatment. In the Estrona powder treatment group, 16 buffaloes (88%) expressed estrus within 20 days with an average interval of 11.3 ± 4.9 days between D0 to DE (P<0.05; Table 1). Overall Estrona powder treatment in all buffaloes (n =18) demonstrated estrus within 7 to 23 days. Non-significant effect of treatments (Placebo vs Estrona powder) was observed on the behavioral signs of estrus (P < 0.01) in buffaloes. On the day of estrus either AI / natural service was carried out by the owner. The first service conception rate was measured confirming pregnancy on day 60 by rectal palpation. In placebo group 33% (01/03) buffaloes were confirmed pregnant, while in Estrona treatment group 56% (09/13) buffaloes were pregnant. The results showed significant effect of Estrona powder treatment as compared to placebo group for the estrus induction in buffaloes. Earlier similar results were observed with Estrona bolus in bovines (cow/buffaloes) evaluated in Maharashtra state (Mane *et al*., 2018). Moreover, estrus response was observed within 15 days in 83% (15/18) animals, while 17% (3/18) animals showed estrus within 19 to 23 days after treatment. Kumar *et al*. (2021) observed that polyherbal preparation with mineral mix could induce estrus with higher conception rate than the hormonal treatment alone in anestrous cows and reported 60% estrus induction and 80% conception rates. Our results indicate similar estrus induction rate, whereas lower conception rate. The lower conception rate in our study might be due to first service conception rate against three service (AI) conception rate parameter used by Kumar *et al*. (2021). In earlier studies different herbal seeds, preparations and combinations were tried and reported 50 to 87% estrus indication response in bovines (Despande *et al*., 2000; Pugashetti *et al*. 2009; Srivastava *et al*., 2003; Sahatpure *et al*., 2016). The results of our study on estrus induction are similar in range. Our study is in line with the previous studies where conception rate in buffaloes was reported from 40 to 80%. (Ravikumar *et al*., 2007; Pugashetti *et al*., 2009; Kumar *et al*., 2021).

Hormone profile (E2 and P4) during the D0, DE and D60 is depicted in Table 2. As compared to the placebo group there was no effect of Estrona powder treatment on E2 and P4 concentrations at day 0, day of estrus and day 60 of pregnancy check (P > 0.05). However, within group, there was significant effect of D0, DE and D60 on levels of P4 and E2 hormones (P < 0.01). During DE, low P4 and maximum E2 concentrations were observed in buffaloes of both groups (P< 0.001; Table 2) and during D60, higher P4 (P< 0.01; Table 2) and lower E2 were observed as compared to D0 and DE. These results are in accordance to the earlier

Table 1: Effects of placebo and Estrona powder treatment on the estrus induction in buffaloes.

Group	Percentage (number) of buffaloes in estrus	Duration of estrus induction (Days: Mean ± SD)
I (Placebo; n=11)	27% (03/11) [a]	42.1 ± 17.9[a]
II (Estrona powder; n=18)	88% (16/18)[b]	11.3 ± 4.9[b]

[a,b] Within column different superscripts show significant difference (P<0.05). I Placebo treatment; II Estrona powder (Rakesh Pharmaceuticals, G.I.D.C, Kalol, Gujarat).

Table 2: Estradiol and progesterone levels during initiation of treatment, day of estrus and day of pregnancy check in buffaloes of Placebo and Estrona treatment groups.

Hormone profile	Groups			
	Placebo	Treatment	Placebo	Treatment
	Estradiol (pg/ml)		Progesterone (ng/ml)	
Day 0	22.67 ± 1.93[a]	24. 40 ± 2.5[a]	0.7 ± 0.2[a]	0.45 ± 0.04 [a]
Day of Estrus	42.77 ± 4.92 [b]	35.60 ± 6.5[b]	0.17 ± 0.03[b]	0.19 ± 0.01 [b]
Day 60: Pregnancy	32.67 ± 1.31[c]	33.4 ± 2.9 [b]	3.00 ± 0.39[c]	3.72 ± 0.4 [c]

[a,b, c] Within column different superscripts showed significant difference (P<0.01). I Placebo treatment; II Estrona powder (Rakesh Pharmaceuticals, G.I.D.C, Kalol, Gujarat).

reports on Jafrabadi (Dugweker *et al*. 2008), Murrah (Nagvekar *et al*. 2015) and Surti (Saxena *et al*. 2017) buffaloes.

CONCLUSION

The results from our study indicate that Estrona powder treatment successfully induced estrus, hence can be used for estrus induction in buffaloes.

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Conflict of interest

None

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