THERAPEUTIC MANAGEMENT OF ANOESTRUS AND SUBOESTRUS BUFFALOES UNDER FIELD CONDITIONS USING HORMONAL AND NON-HORMONAL REMEDIALS

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ABSTRACT

The present study was carried out during breeding season on 98 anoestrus / suboestrus buffaloes to evaluate therapeutic efficacy of various hormonal and non-hormonal drugs in inducing oestrus and conception. In all 37 and 29 true anoestrus and 17 suboestrus buffaloes were treated with Tono-Prepaline-Cyclomin-Lugols (Inj. Protone 10 ml + Inj. Vitamin-A 6 ml, 4 times each at 4-5 days interval, Cyclomin-7 bolus orally every 3rd day, and 0.5 % Lugol's iodine solution 10 ml i/ut or on os once), GnRH (Receptal 5.0 ml i/m once) and $PGF_2\alpha$ (Juramate 2.0 ml i/m once), respectively, keeping 15 animals as untreated control. Animals detected in oestrus were inseminated by the trained lay inseminators. The oestrus induction response with $PGF_2\alpha$ was the highest (100 %), followed by GnRH (89.66 %) and Tono-Prepaline-Cyclomin group (83.78 %), with the corresponding oestrus induction intervals of 3.59±0.44, 20.73±3.29 and 28.03±3.23 days, and conception rates at induced oestrus of 35.29, 26.92 and 38.71 %, respectively. The overall conception rates of 3 cycles for the three protocols were almost identical (76.47, 73.03 and 74.19 %), but with significantly ($P<0.05$) shorter fertile oestrus interval of 17.31±4.11 days in $PGF_2\alpha$ treated group as compared to GnRH (42.32±5.30 days) and Tono-Prepaline-Cyclomin (43.00±5.17 days) groups. All these results were significantly superior to those of untreated control group (oestrus expression 46.66 % and CR 57.14 % in 67.00±14.00 days). Hence all these regimes are recommended to the practicing veterinarians for their judicious use in the field to ameliorate the economically important problem of anoestrus in buffaloes. However, looking to the cost and period of response, use of $PGF_2\alpha$ was the most economic in suboestrus buffaloes and GnRH in anoestrus ones as compared to 4 doses of Tono-Prepaline with Lugol's and Cyclomin bolus.

KEY WORDS: Anoestrus, Buffaloes, Hormonal/Non-hormonal therapy, Oestrus induction, Conception.

INTRODUCTION

Livestock industry can be strengthened by proper reproductive management. Buffalo contributes major part in dairy sector; consequently good fertility management becomes inevitable. Therapeutic management of chronically infertile animals is uneconomic practice. Prolonged postpartum anoestrum is the major issue of economic loss to buffalo breeders. Data from Egypt, India and Pakistan indicate that only 34-49 % of buffaloes show oestrus during the first 90 days after calving and 31-42 % remain anoestrus for more than 150 days (El-Wishy, 2007). The incidence of true anoestrus and suboestrus has been reported to be higher in buffalo-cows (32.82 and 50.00 %) than in buffalo-heifers (22.90 and 8.16 %). Gonadotrophin releasing hormone (GnRH) treatment has a good therapeutic action to enhance early resumption of ovarian activity in anoestrus buffaloes (Shah *et al.*, 2002). The recent development and experimentation with the luteolytic agent $PGF_2\alpha$ and its analogues have given indication to the potent solution for dealing with suboestrus condition and in improving reproductive efficiency of subfertile buffaloes. Similarly, non-hormonal remedials like Lugol's iodine alone or in combination with vitamin-mineral supplementation have also been

used with varying success in inducing oestrus and fertility in anoestrus buffaloes (Mathur *et al.,* 2005; Singh *et al*., 2006). However, in most of the reports only one protocol has been tested at a time. Hence the present study was planned to evaluate the comparative therapeutic efficacy of Vitamin-minerals plus Lugols, GnRH and prostaglandin at a time in anoestrus and suboestrus buffaloes under field conditions.

MATERIALS AND METHODS

This study was conducted from August 2007 to March 2008 at 2 villages, viz., Anklav and Sandesar of Anand district in Gujarat (India). All the buffaloes managed by the farmers individually at their door-step and brought to the AI centres of these village co-operative societies for AI, pregnancy diagnosis and sexual health control camps were initially screened for their reproductive status through gynaeco-clinical examinations and detailed history was recorded. Out of total 98 buffaloes confirmed to be anoestrus/ suboestrus through per rectal examinations twice 10 days apart, 83 buffaloes (70 in Anklav and 28 in Sandesar) were selected at random for following three therapeutic protocols, keeping 15 animals as untreated control.

All infertile animals identified were first dewormed using Albandazole 3000 mg (Helmiguard 3000, Vetcare India Ltd.) and were vaccinated against HS and FMD. They were also treated for ectoparasites, if any, by using Flumethin (Flupor, Vetnex-RFCL India Ltd). Owners of the ear-marked animals were supplied with mineral mixtures (Amul brand) for supplementing to their animals. Problem breeders were then divided into 4 groups and were subjected to different therapeutic regimes as detailed below.

Group-I: Nutritional Supplementation Therapy

Thirty seven apparently healthy true anoestrus buffaloes were subjected to Tono-Prepaline-Cyclomin plus Lugol's iodine (TPCL) treatment, which consisted of Lugol's iodine 0.5% solution 10 ml on os cervix or i/ut once, plus Inj. Protone 10 ml i/m (0.2 g sodium salt of phosphoric acid/ml, Vetnex-RFCL India Ltd) and Inj. Vitamin-A 6 ml i/m (each 2 ml contain 6 lacs IU of Vitamin-A as palmitate, Virbac Co.), 4 injections each at 4-5 days intervals and Cyclomin-7 (Alved Pharma) bolus one every third day orally for one month.

Group-II: GnRH Therapy

Twenty nine true anoestrus buffaloes were treated with single i/m dose of 20 µg GnRH, i.e Inj. Receptal 5 ml (Buserelin acetate 4.2 µg/ml, Intervet India Pvt Ltd).

Group-III: Prostaglandins $F_2\alpha$ Therapy

Seventeen buffaloes, with history of suboestrum and presence of CL on one of the ovaries, were subjected to single i/m dose of 500 µg $PGF_2\alpha$, i.e. Inj. Juramate 2 ml (250 µg Cloprostenol/ml, Jurox Pvt. Ltd., Australia) followed by fixed time AI twice at 72 and 96 hrs.

Group-IV: Untreated Control

Fifteen apparently healthy true anoestrus (11) and suboestrus (4) buffaloes were kept as control, without any of the above specific treatment, but were analyzed for their reproductive status at the end of experiment.

The animals detected in oestrus were inseminated at induced or spontaneous oestrus by the trained lay inseminators of the concerned village societies. All the animals were followed for at least 3 months post-treatment or for two natural cycles after induced oestrus. Oestrus induction response and conception rates overall and at induced oestrus were calculated group-wise and compared by using chi-square test. The data on oestrus induction intervals were analyzed using completely randomized design (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

The results obtained are presented in Table 1 and illustrated by figures 1 and 2.

Effect of Lugol's Iodine and Mineral-Vitamin Supplements

Out of 37 true anoestrus buffaloes treated with TPCL, 83.78 % responded with a mean oestrus induction interval of 28.03±3.23 days. The oestrus induction response and oestrus induction intervals of buffaloes of two villages did not differ significantly. The conception rate at induced oestrus and overall of 3 cycles was 38.71 % and 74.19 %, respectively, with a mean treatment to fertile oestrus interval of 43.00±5.17 days. The overall conception rate and fertile oestrus interval for buffaloes of Anklav were significantly higher than those of Sandesar (80.95 vs 60.00 % and 45.18±6.43 vs 36.83±6.75 days; Table 1, Fig. 1-2).

These findings compared favourably with the reports of Akhtar *et al.* (2004) and Srivastava (2008). Mathur *et al*. (2005) recorded oestrus induction response of 80 and 67 % within 10 and 21 days following Tono-Prepaline therapy for 2 weeks with 100 % fertility in Frieswal and Sahiwal cows, respectively. However, the oestrus induction response and conception rate were reported to be very poor (0-30 %) with simple oral supplementation of macro-micro minerals (Biswas *et al*., 2005) or UMMB (Kang *et al.,* 2005) for 30 days in summer anoestrus buffaloes. Utero-ovarian massage and Lugol's iodine painting of os-cervix is also reported to induce oestrus in 45 to 50 % buffaloes within 8-15 days with 67 to 93 % conception (Rathour *et al.,* 2005). Singh *et al.* (2006) reported only 47 % oestrus response after 30 days of Supplevite-M oral therapy with 72 % conception in anoestrus buffaloes. Patel *et al*. (2007) found 33 % oestrus induction in anoestrus buffaloes treated with mineral (Flomin-C) supplement orally for six days as compared to 17 % in control group with 50 and zero % conception.

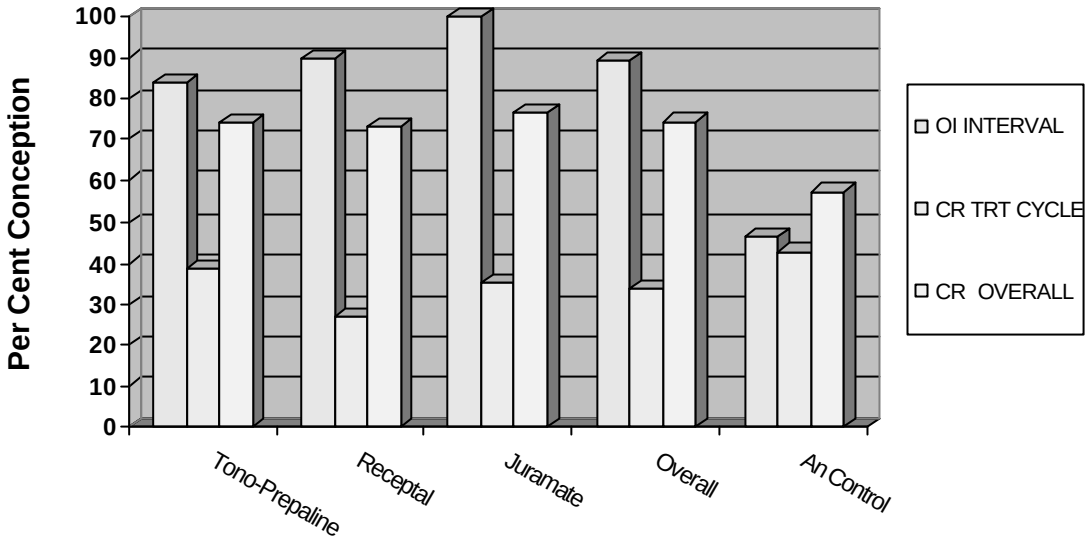
The mechanism by which Lugol's solution acts on the reproductive system is not well understood (Mwaanga *et al.*, 2004). Intrauterine infusion of Lugol's solution causes hyperaemia of uterine mucosa, a sign of enhanced circulation, which consequently leads to high degree of drug absorption. The absorbed iodine probably stimulates production of thyroid hormones, which increase body's metabolic rate. This increased metabolic activity could be one of the triggering factors of ovarian function since one of the main causes of the ovarian dysfunction is energy utilization imbalance. The enhanced uterine blood circulation might also influence ovarian activity. In the present study, deworming of all animals was done prior to initiation of treatment and the therapy was found significantly beneficial as compared to untreated control group (mean oestrus induction 87.50 vs 47.00 % in 28 vs 65 days and conception rate 74.19 vs 57.14 % in 43 vs 67 days).

Effect of Gonadotropin Releasing Hormone - GnRH

Out of 29 true anoestrus buffaloes treated with single i/m dose of 20 µg GnRH, 89.66 % responded with oestrus induction interval of 20.73±3.29 days and resulted in the conception rate of 26.92 % at induced oestrus. The overall conception rate was 73.07 % with treatment to fertile oestrus interval of 42.32±5.30 days. The oestrus induction response was lower (87.50 vs 100 %), and oestrus induction interval (23.14±3.81 vs 10.60±3.65 days) and fertile oestrus interval (46.94±5.42 vs 17.67±9.22 days) were significantly ($P<0.05$) higher in buffaloes of Anklav than those of Sandesar (Table 1, Fig. 1-2). Better results in buffaloes of Sandesar may be due to less number of animals included and peak breeding season when trial was conducted.

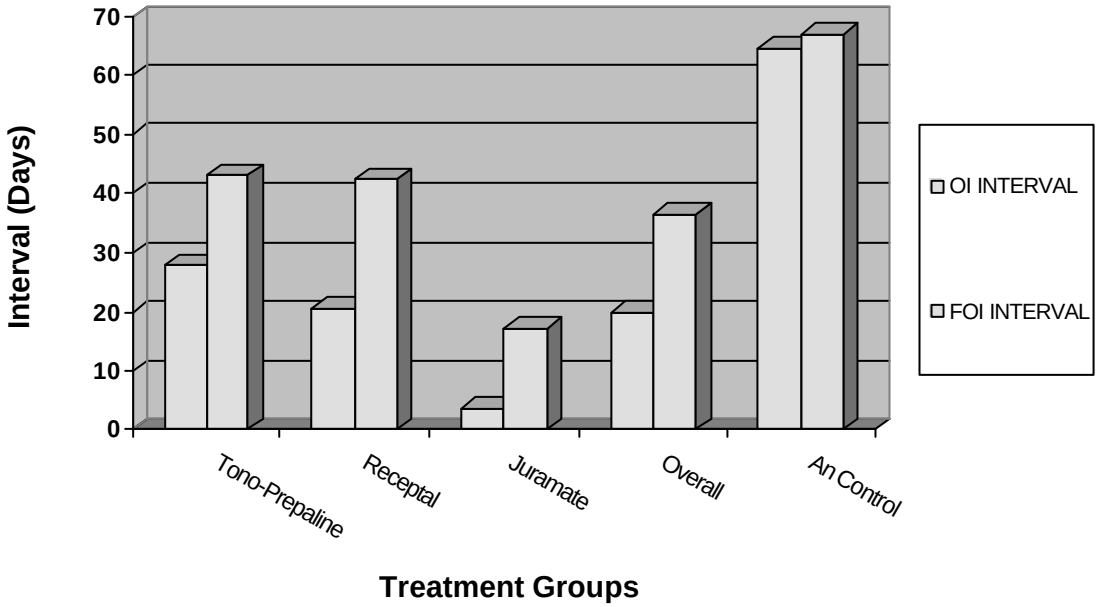
The present findings with GnRH corroborated well with the reports of Nautiyal *et al*. (1997) and Khasatiya *et al*. (2004), and partly with those of El-Shamma *et al.* (1996), Mohmmed *et al*. (1999) and Dugwekar *et al.* (2006), wherein either oestrus induction response or conception rate was comparable with the present findings, but not both. Dhoble and Gupta (1987), however, found oestrus response only in 12 of 53 anoestrus buffaloes within 15 days of GnRH (21 µg) treatment.

Fig. 1. Oestrus induction (OI) response and conception rate (CR) overall and at induced oestrus in anoestrus buffaloes treated with different hormonal and non-hormonal drugs



Zaghloul *et al*. (1993) recorded only 60 % oestrus response within 25 days of GnRH (500 µg Fertagyl) treatment, but with 100 % fertility in postpartum acyclic buffaloes, while Reddy *et al*. (1994) found 50 % oestrus with only 40 % conception using 5 ml Receptal. Though the present results could not be compared as such with the available literature, the findings of oestrus induction response and conception rate clearly indicated that ovarian cyclicity with ovulatory oestrus can be effectively induced with GnRH in anoestrus buffaloes under field conditions.

Fig. 2. Oestrus induction (OI) interval and fertile oestrus interval (FOI) in anoestrus buffaloes treated with different hormonal and non-hormonal drugs



Effect of Prostaglandins $F_2\alpha$

Of the 17 suboestrus buffaloes treated with $PGF_2\alpha$, 100 % buffaloes responded with a mean oestrus induction interval of 3.59±0.44 days only, and resulted in first service conception rate of 35.29 % with fixed time insemination twice at 72 and 96 hrs post-treatment. The overall conception rate in 3 cycles was 76.47 % with treatment to fertile oestrus interval of 17.31±4.11 days (Table 1, Fig. 1-2). Though the oestrus induction response of buffaloes of both the village was 100.00 %, with almost identical mean oestrus induction intervals of 3.67±0.56 and 3.40±0.75 days, the overall conception rate amongst buffaloes of Anklav (91.66 %) was significantly higher ($P<0.05$) than that of Sandesar (40.00 %), with the mean treatment to fertile oestrus intervals of 18.55±4.64 and 10.50±7.52 days, respectively.

$PGF_2\alpha$ or its analogues induce luteolysis and were found to induce ovulatory oestrus and improve reproductive efficiency in subfertile buffaloes (Singh *et al.*, 1979). Merits of this property have been taken to treat buffaloes that are suboestrus following 15 weeks of parturition. The luteolytic action is most potent between days 5 and 17 of the bovine cycle and most of the animals show ovulatory oestrus within 3-4 days of treatment. The present findings with $PGF_2\alpha$ corroborated well to the reports of Khasatiya *et al*. (2004) and to some extent to those of Kharche and Srivastava (2001) and Sharma (2002), who all noted oestrus induction response between 72 to 90 % and conception rates of 65 to 77 % within 2-5 days of $PGF_2\alpha$ treatment in suboestrus animals. Dhoble and Gupta (1987), however, noticed only 56.52 % detectable oestrus, 17.39 % silent oestrus and 26.09 % no response at all in suboestrus buffaloes following use of 5 ml Dinoprost. Pant and Singh (1991) similarly found higher first service conception of 54.80 % with natural service, comparable with that of normal cyclic buffaloes (59.1%). Sathiamurthy *et al.* (2007) could achieve 66.6 % oestrus induction response with first service conception of 50 % in suboestrus buffaloes using Lutalyse.

The findings clearly support that $PGF_2\alpha$ analogues have definite standing in successful management of suboestrus condition in buffaloes. However, the variations observed in oestrus induction response and fertility in different studies could be due to many factors such as stage of cycle, product potency, oestrus detection efficiency, nutritional status, general and genital health, breeding time and quality of semen used, season/ climate, and luteal activity or sustainability leading to embryonic mortality etc.

Overall Treatment Response and Comparison of Control Vs Treatment Groups

Out of overall 83 anoestrus/suboestrus buffaloes treated with different nutritional supplementation therapy and hormones, 89.15 % responded with a mean oestrus induction interval of 20.08±2.06 days. The conception rate at induced oestrus was 33.78 % and overall 74.32 %, with a mean treatment to fertile oestrus interval of 36.69±3.30 days. The overall conception rate (81.48 vs 55.00 %) was better in Anklav, but with longer fertile oestrus interval (39.16±3.82 vs 26.82±5.53 days) as compared to that of Sandesar (Table 1; Fig. 1-2).

As regards relative efficacy of different treatment protocols, oestrus induction response with $PGF_2\alpha$ was the highest (100 %), followed by GnRH (89.66 %) and Lugol's plus Tono-Prepaline-Cyclomin group (83.78%), with the corresponding oestrus induction intervals of 3.59±0.44, 20.73±3.29 and 28.03±3.23 days, respectively. The overall conception rates for the three protocols were almost identical (76.47, 73.03 and 74.19%), but with significantly ($P<0.05$) shorter oestrus induction interval of 17.31±4.11 days in $PGF_2\alpha$ treated group as compared to other two groups (42-43 days; Table 1, Fig. 1-2). All these results were significantly better or superior than those of control untreated group (oestrus expression 46.66 % in 64.57±9.36 days; and conception rate 57.14 % in 67±14 days).

Studies on the comparative efficacies of different treatment protocols of anoestrus and suboestrus buffaloes on farm or even field conditions are scanty in the literature. The present relative findings

with $PGF_2\alpha$ (Cloprostenol) and GnRH (Busereline) therapy coincided well with the results of Khasatiya *et al.* (2004). However, Dhoble and Gupta (1987) found very poor oestrus induction response in both anoestrus and suboestrus buffaloes treated with GnRH (22.6 %) and $PGF_2\alpha$ (73.91 %), respectively. Patel *et al*. (2007), on the contrary, recorded 33.3 and 50.0 % oestrus induction response with 50.0 and 66.6 % conception rate in 6 buffaloes each treated with Iliren and Receptal, respectively, as against 16.6 and zero % conception in untreated control anoestrus animals.

Conclusions

The present findings suggest that nutritional deficiency/ imbalance plays a major role in causing infertility and hence, area specific vitamin-mineral supplementation should be the part of treatment schedule of anoestrus buffaloes as was used in the present study. The results of this protocol were even at par with any other hormone therapy. The findings of oestrus induction response and conception rate with GnRH indicated that ovarian cyclicity with ovulatory oestrus can be effectively induced with GnRH in anoestrus buffaloes under field conditions, thereby reducing their service period and calving interval towards achieving the goal of economic return. Further, the results of Juramate suggest that $PGF_2\alpha$ analogues have definite standing in successful management of suboestrus condition in buffaloes with fix time insemination on 3rd and 4th day post-treatment, since these drugs induce mostly ovulatory oestrus with or without clinical manifestations. Moreover, looking to the cost and period of response, use of $PGF_2\alpha$ was the most economic in suboestrus buffaloes and GnRH in anoestrus ones as compared to 4 doses of Tono-Prepaline with Lugol's and Cyclomin bolus. Although village-wise some variation in response rate was noted for the three protocols. Hence, all the three protocols can be equally recommended, provided body condition score of anoestrus animals is sound for GnRH therapy and palpable CL exists on the ovary for $PGF_2\alpha$ therapy.

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