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EFFECT OF DIFFERENT ROUTES OF PGF2α ADMINISTRATION AND PROGESTERONE ON THE TREATMENT OF INFERTILITY IN BUFFALOES

M. M. MANSOUR and G. A. ABDO

Department of Theriogenology, Faculty of Veterinary Medicine.
Cairo University, Giza P. O. 12211 Egypt.

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SUMMARY

The study was conducted on 28 suboestrous and 14 anoestrous buffaloes. The suboestrous buffaloes (Experiment I) were divided into three groups; group I (n=10): 25 mg PGF2α intramuscular (i.m), group 2(n=13); 5 mg PGF2α intrauterine (i.u.) and group 3 (n=5) as control. The aneostrous buffaloes (Experiment II) were placed in two groups: group 1 (n=9): progestogen (ear implant) with pregnant mare serum gonadotrophin (PMSG) regimen and group 2 (n=5): as control. Fixed - time inseminations by frozen semen at 72-96 hr after PGF2α treatment and 48-72 hr after implants removal were done.

Strong estrous intensity was observed in 69.2% of i.u. PGF2α group followed by 40% in i.m. PGF2α group. After progestogen + PMSG regimen 44.4% anoestrous buffaloes showed a moderate estrous intensity. Overall conception rates of suboestrous buffaloes treated with different routes of PGF2α were 69% and 60% in group 2 and group 1, respectively compared to 20% in the control group. After treatment of anoestrous buffaloes with progestogen + PMSG, the conception rate was 67%.

In conclusion, fixed-time insemination following use of prostaglandin (PGF2α) and progestogen with PMSG regimen can be effectively used for treatment of suboestrous and anoestrous buffaloes. Moreover, the intrauterine route of PGF2α administration appeared to be equally efficient to the intramuscular route to induce fertile oestrous and increase fertility rate in suboestrous buffaloes.

Key words: Buffaloes, Oestrous, PGF2α, Suboestrous, Synchro-Mate (SMB), Anoestrous.

INTRODUCTION

One of the important restricting factor for the widespread use of AI in buffalo is related to the determination of the ideal time for semen deposition in the female genital tract. Overt signs of estrus in buffaloes are less obvious than in cattle and anoestrus is a major problem in buffalo breeding (Jainudeen, 1986). The incidence of anoestrus is higher in herds using AI rather than natural service and this might indicate that the problem may often lie with estrous detection rather than with the animal itself. In relation to ovarian function, anoestrus can be divided into various categories including functional ovaries with undetected estrus (suboestrus) and non-functional ovaries (true-anoestrus), ovaries with persistent corpus luteum and cystic ovaries (Sahasrabundhe and Pandit 1997). According to Shah et al.,(1990), the most important cause of anoestrus due to management is inadequate estrous detection. One approach to hand such undesirable problem in achieving successful insemination of infertile buffaloes is to induce estrus and inseminate at a fixed or predetermined time. Several studies have revealed the effectiveness of prostaglandin F2α for cattle with unobserved oestrus. However, comparison of fertility rates between different routes of prostagland F2α injection in buffaloes have still under investigation.

The purpose of this study was to investigate the effect of prostaglandin F2α and Norgestomet plus pregnant mare serum gonadotrophin (PMSG) on the reproductive performance of infertile buffaloes.

MATERIAL AND METHODS

Animals:

The present investigation was carried out on fourty-two buffaloes. A part of the present study was carried out on Neonatal Calves Disease Research Center (NCDRC), Faculty of Veterinary Medicine, Cairo University while the other part was carried out on a private farm. The animals were 4-5 years old, and weighed 330-450 kg. All animals were managed similarly and were apparently healthy.

All buffaloes in the present study were subjected to rectal examination twice/weekly for three successive weeks to follow up the reproductive status of each animal.

All animals had history of low reproductive performance due to poor heat detection associated with long intercalving periods. According to the ovarian findings, the animals were classified into two main approaches. Experiment (I): buffaloes with structures on their ovaries (suboestrus)and experiment (II):

buffaloes without any structure on their ovaries (true-anoestrous).

Experiment (I):

Twenty eight buffaloes without any signs of estrus (subestrus) were subjected to two different routes of natural prostaglandin F2α treatments. Buffaloes were assigned to one of three groups and were maintained as a single experiment throughout the working period according to Sahasrabundhe and Pandit (1997). Group 1 (n = 10) animals were received two intramuscular (im) injections of prostaglandin F2α (PGF2α) 11 days apart(25 mg) per injection dinoprost tromethamine (Lutalyse, Upjhon Co.). Buffaloes ingroup 2 (n=13) were received double intrauterine infusions using recto-vaginal technique of 5 mg dinoprost tromethamine (Lutalyse) ipsilateral corpus luteum with 11 days apart. Animals in group 3 (n=5) served as untreated control.

Experiment (II):

In this experiment fourteen buffaloes without any structure on their ovaries were included. They were diagnosed as true-anoestrus according to Shah et al. (1990). The animals were divided into two groups. Group (I), nine buffaloes were subjected to Norgestomet treatment. Each buffalo received an intramuscular injection of norgestomet (3 mg) and estrdaiol valerate (5 mg) plus a subcutaneous ear implant SynchroMate-B (SMB) containing 6 mg progestagen norgestomet (Creaster, Intervet). The implant was subcutaneously implanted on the convex surface of the ear and left in situ for 9 days. The SMB implant was removed, 9 days after insertion, and at this time each buffalo was intramuscularly injected with 1000 IU of pregnant mare serum gonadotrophin (PMSG, Folligon, Intervet). Group (2) five buffaloes served as untreated control animals.

Estrus evaluation:

Response of different animal groups to various treatments were evaluated. Estrus detection including injection of the external sexual organs and careful observation of animal behaviour were done for at least 20 min. early in the morning, at mid-day and in the evening after start of the experiment. Also, the animals were exposed to a buffalo bull daily for detection of the post-treatment estrus signs. According to Callesen et al. (1993), the buffaloes were examined closely for signs of estrus both internally per rectum for uterine tonicity (highly tonic ++, tonic + and no tonicity 0) and externally for vulvar edema (highly edematous (++), edematous (+), and not edematous (0), for mucus {large volume, stringy)++), moderate volume, stringy to viscous (+), spare or no volume (0)}: and for estrus behaviour (clear signs of restless, alert and tail elevation (++), moderate signs (+), and no signs (0). Based on these findings, estrus intensity was classified into

Table (1): Evaluation of estrus intensity.

Estrus intensity*	Internal signs	External signs		
	Uterine tonicity	Vulvar edema	Mucus	Behaviour
Strong	++	++	++	++
Moderate	+	+	+	+
Not clear	0	0	0	0

* The ++/+/0 designations indicate the maximal level of the estrus signs achieved during the observation period of the animal.

three categories: strong, moderate and not clear.

Fixed-time insemination:

All buffaloes were inseminated (one inseminator) with 0.5 ml frozen semen from a bull of proven fertility. Fixed-time insemination was done for all treated buffaloes at 72 and 96 hours after injection of second dose of PGF2α in experiment (I) and after 48 and 72 hours after implant removal in experiment (II) irrespective of estrus behaviour (Shah et al. 1990). All buffaloes were tested for pregnancy 60 days after AI.

Fertility measures including the time interval between end of the treatmen to onset of estrus, estrus intensity, number of insemination/conception and conception rate were reported.

Statistical analysis:

All data were statistically analyzed by the least-squares analysis of variance using the General Linear Models Procedures (GLM) of the Statistical Analysis System (SAS, 1990).

RESULTS

Table (2) presents the clinical signs of oestrus

Table (2): Clinical signs of estrus in suboestrus and anoestrus buffalo after prostaglandin (PGF2α) and Synchro-Mate (SMB) treatment respectively.

	Exp (I) Suboestrus			Exp (II) Suboestrus	
	Group 1	Group 2	Control	Group 1	Control
No. of treated buffaloes	10	13	5	9	5
No. of buffaloes showed clear estrus	8 (80%) (b)	12 (92.3%) (a)	3 (60%) (ab)	7 (77.7%) (c)	0(0%)
Hours from treatment to onset of estrus (M±SE)	73.4 ± 4.2 (a)	56.2 ± 9.2 (b)	-----	43.7 ± 14.85 (b)	-----
Estrus within 72 after PGF2α	6(60%) (a)	11(84.6%) (a)	-----		-----
Estrus within 48 h after SMB	----	----	-----	6(66.6%)	-----
Estrus intensity					
(A) Strong	4(40%) (a)	9(69.2%) (b)	2(40%) (ab)	3(33.3%) (a)	-----
(B) Moderate	4(40%) (a)	3(23.1%) (a)	1(20%) (a)	4(44.4%) (a)	-----
(C) Not clear	2(20%) (a)	1(7.7%) (a)	0(0%) (a)	2(22.2%) (a)	-----

Figures with different subscripts within rows were significantly different. (a) Vs (b) at $p<0.05$ (c) Vs (d) at $p<0.01$

Table (3): Clinical respones of suboestrous buffaloes after prostaglandin (PGF2α) treatment.

	Group 1	Group 2	Group 3
No. of buffaloes	10	13	5
buffaloes inseminated at first estrus	10 (100%) (c)	13 (100%) (c)	3 (60%) (d)
Buffaloes conceived after fixed-time AI	4 (40%) (a)	8(62%) (a)	0 (0%) (a)
Buffaloes conceived after subsequent AI	2(20%) (a)	1(7.7%) (a)	1 (33.3%) (a)
Overall conception rate	6 (60%)(ab)	9(69%)(a)	1 (20%) (b)
Number of insemination/conception	1.6 ± 0.8	1..4 ± 0.7	3 ± 1.7

Figures with different subscripts within rows were significantly different (a) Vs (b) at $p<0.05$, (c) Vs (d) at $p<0.01$

after prostaglandin (PGF2α) and Synchro-Mate (SMB) treatment of suboestrus and anaoestrus buffaloes respectively.

Eighty and 92.3 % of the suboestrus buffaloes showed estrus behaviour after intramuscular and intrauterine route of PGF2α respectively, while 77.7% of trueanoestrus animals were seen in estrus after SMB implant removal (table 2). At fixedtime of insemination a non significantly greater number of buffalo of intrauterine (i.u) PGF2α group and 66.6% in SMB group.

Strong estrus intensity was observed in 69.2% of i.u PGF2α group followed by 40% in i.m PGF group. After Synchro-Mate - B treatment of anoestrous buffaloes, 44.4% showed moderate estrus intensity while 33.3% had strong estrus intensity, estrous symptoms and behavioural changes during induced estrus are similar to those observed in spontaneous estrus in the untreated animals.

Amongst the treated suboestrous buffaloes (table 3) maximum conception rate was obtained in intrauterine PGF2α treated group (69%) followed by intramuscular PGF2α group (60%) in contrast with untreated control group (20%). In suboestrus buffaloes, 5mg of natural PGF2α (Lutalyse) by intrauterine route was as effective as 25 mg of the same drug by intramuscular dose. Overall conception rate of 23 suboestrous buffaloes treated with different routes of PGF2α was 65.2% (15/23).

Of the 9 treated true-anoestrus buffaloes (table 4), 5 (56%) conceived with fixed - time AI after induced ovulation, and one (11 %) buffalo

Table 4: Clinical responses of true-anoestrous buffaloes treated with Synchro Mate-B (SMB)

	Treated group (1)	Control group (2)
No. of buffaloes	9	5
Buffaloes inseminated at first estrus	9(100%)	---
Buffaloes conceived after fixed-time AI	5(56%)	---
Buffaloes conceived after subsequent AI	1(11%)	---
Overall conception rate	6(67%)	---
Number of insemination/ conception	1.6	---

conceived at subsequent AI. Of the total number of true-anoestrous buffaloes in the experiment (II) (67 %) conceived.

Overall conception rate:

Of the total number of treated buffalo (32) in the experiment (I) and (II), 65.6% conceived of these, 53% (17/32), conceived after fixed time insemination following hormonal treatment, while 12.5% (4/32) conceived at subsequent estrus. During the 2 month period of the experiment, 10% (1/10) of the buffaloes in the control groups conceived. The difference between control and treated groups was highly significant ($p<0.01$).

DISCUSSION

According to our findings, suboestrous buffaloes with active corpus luteum showed estrus at an average of 63.1 ± 13.3 h after PGF2α treatment. The present results confirm those of the previous reports, which promote the onset of estrus in suboestrus buffaloes (Chauhan et al., 1982; Singh et al., 1979 and Shah et al., 1990) and cows (Humblot and Thibier, 1980 and Seguin et al., 1978). Jindal et al. (1990) and Bicudo and Oba (1992) recorded estrus as occurring 72-96 h after administration of 500 µg of cloprostenol or 25 mg of PGF2α to lactating buffaloes. Pant and Singh (1991) treated 40 suboestrus buffaloes with 25 mg prostaglandin, 31 (77.5%) exhibited oestrus 69 h afterwards. The present results showed that the PGF2α administration through the different routes in suboestrous buffaloes initiated normal expression of oestrus although Jindal et al (1990) found that the external signs of induced estrus after prostaglandin treatment were less apparent than at the spontaneous oestrus. In the present study, 77% of the treated anoestrous buffaloes showed oestrus response and 66% were in oestrus during 48 hours after removal of ear implant. These results go in parallel with the findings of Rao (1985).

Suboestrus is one of the most common infertility factors encountered in buffaloes and the incidence may vary from 40% (Roa and Roa, 1979) to 73% (Shah et al., 1990) according to different breeding seasons of the year. In the present treated suboestrous buffaloes, 5 mg PGF2α intrauterine was effective as 25 mg of the same drug intramuscularly. It is believed that by using this route there is an immediate transfer of PGF2α from the uterus to the ovarian artery (via the counter current exchange mechanism operating between the uterine vein and ovarian artery) resulting in CL luteolysis (Sahasrabudhe & Pandit 1997). The intramuscular route required more amount of the drug as compared to the intrauterine route. The intrauterine route requires proper sterilization of the drug deposition equipment and trained personnel for the drug administration. Hence, the intrauterine route in the present study appeared to be equally efficient, more convenient and one-fifth economical to the intramuscular route. Rao and Venkatramaiah (1989) reported that the intravulvosubmucosal route of PGF2α injection can allow the effective dose of cloprostenol to be reduced to 20% (10 μg) administrated by the intravulval route in river buffaloes was also reported by Rao and Rao (1979) who found the dose to be as effective as the larger one (500 μg) given by intramuscular injection. The decline in progesterone concentration and the onset of oestrus after prostaglandin treatment was found by Dhaliwal and Sharma (1990) to be slower in buffaloes treated by the intravulvosubmucosal route (8 mg dose) than in those injected intramuscularly (25 mg). Oestrus control has been attempted with PGF2α administered by the intravaginal route as well as by intramuscular injection (Subramaniam et al 1989); according to such work, 12.5 mg of prostaglandin injected intramuscularly on the same side as the active corpus luteum was as effective as 5 mg administered intravaginaly. The role of PGF2α in the induction of oestrus was previously reported by El-Menoufy et al. (1985), and Ghallab et al. (1985); while the application of PGF2α in the treatment of suboestrous buffaloes is described by Elsayed et al. (1985); and Ghallab et al. (1985); while the application of PGF2α int he treatment9f suboestrous buffaloes is described by Elsayed et al. (1985); El-Wishy et al. (1985); Shah et al. (1990); Pant and Singh (1991); Sahasrabudhe and Pandit (1997). The overall fertility rate (65.2 %) obtained in the present study after different routes of PGF2α treatment is higher than 52.5 % and 52 % that obtained by Rao and Rao (1978) and El-Menoufy et al. (1985) in normal buffaloes, respectively and higher than 44 % and 50 % reported by Roa and Roa (1979) and El-Wishy et al. (1985) in suboestrous buffaloes respectively. Meanwhile, the present results agreed with 62.7 % and 63.6 % that reported by Shah et al. (1990) and Sahasrabudhe and Pandit (1997) in suboestrous buffaloes respectively. The different resutls of fertility rate were due to the different source and route of administration of prostaglandin F2α.

In the present study 77.7 % of the treated anoestrous buffaloes were able to restore ovarian activity and 56 % conceived after fixed time AI. The results agreed with Roa and Sreemannarayana (1983); Roa (1985) and Shah et al. (1990) found that the majority of the animals (70 %) continue to cycle after treatment with norgestomet. Singh et al. (1988) reported that follicular development, ovulation and uterine responses were recorded after norgestomet-PMSG treatment of anoestrous buffaloes. The animals exhibited a good ovarian and uterine response with the majority of ovulation occurring between 48-and 72 h post-treatment. The efficacy of hormonal (PMSG, oestradiol valerate, Synchromate-B) and non-hormonal (Lugol's iodine solution) methods in the induction of oestrus in 100 anoestrous Murrah buffaloes is detailed in a report by Yadav et al. (1994). Synchromate-B was the most effective treatment, with 75 % of the animals showing oestrus.

In suboestrous buffaloes, animals showed standing estrus within 72 hours after PFG2α treatment according to Jindal et al. (1990), Shah et al. (1990) and Bicudo and Oba (1992), so fixed-time insemination in the present study was done at 72 hours post-treatment. In true anoestrous buffaloes, the decision in this study to commence fixed-time insemination at 48 hours after implant removal was based on observations of a previous study in buffaloes (Roa, 1985; Jainudeen, 1986, Shah et al., 1990) and in cow (Kerr et al., 1991; McGowan et al., 1992) in which most of the animal treated with the SMB system were seen in standing estrus approximately 30-40 hours after implant removal. The results of both present experiments (Exp I & Exp II) showed that high proportion of buffaloes were exhibiting signs of estrus at the time of insemination and the pregnancy rate (53 %) achieved after fixed time insemination were higher than the rate (40 %) achieved by McGowan et al. (1992) but lower than that 59.3 % reported by Mikeska et al. (1988).

In conclusion, a satisfactory overall pregnancy rate was achieved in infertile buffalo inseminated at a fixed time following the use of prostaglandin (PGF2α) and synchro-mate B (SMB) to overcome suboestrous and anoestrous problems respectively. The intrauterine route of administration of PGF2α can be effectively used for the treatment of suboestrous in buffaloes.

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