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Short Communication

Induction of Ovulatory Oestrus in True Anoestrous Buffaloes during Low Breeding Season

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ABSTRACT

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A fertility treatment trial of anoestrous buffaloes was undertaken on a well managed dairy farm in the low breeding season. Forty lactating buffaloes, 12 each from first and second parity and 16 from third or higher parity, not seen in oestrus for at least 3 to 5 months were diagnosed anoestrous by rectal examination. A subcutaneous ear implant containing 3 mg of norgestomet was inserted, accompanied by an intramuscular injection of 3 mg norgestomet and 5 mg of oestradiol valerate (Syncro-mate B, Intervet). After 9 days the implant was removed and 600 IU of PMSG (Intervet) were given intramuscularly. Irrespective of oestrous behaviour, the animals were inseminated with frozen semen at two fixed times: 48 and 72 h after removal of the implant. Thirty animals having calved in the same period as the experimental animals and not seen in oestrus since then served as controls. Milk samples for progesterone analysis were taken on the day of insertion of the implant, the day of removal and on days 10 and 22 after the second A.I. Progesterone determination by means of an enzyme immunoassay (EIA) revealed that 33 buffaloes out of 40 (82.5%) were in true anoestrus. Ovulation occurred in 21 buffaloes out of 30 (70%). Three buffaloes could not be followed up further. The overall conception rate was 53.3%. The parity-wise conception rate was 40%, 44.4% and 72.2% in first, second and third or higher parity, respectively. None of the control animals displayed oestrus during the course of the study.

INTRODUCTION

According to Ishaq (1957), quoted by Rife (1959), in Pakistan the female buffalo is sexually inactive from March to the end of June, with only 3% coming into heat. In a village herd in India the period of highest sexual activity was from September to February, when 76% of the animals came in heat, while the period of lowest sexual activity was from March to August with only 24% of

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the animals showing oestrus (Rao and Rao, 1970). In Bangladesh, only 2% of the buffaloes were found in heat during summer (Hossain and Ahmed, 1971).

In Pakistan no results are available on the relationship between nutrition and ovarian activity during different seasons. In India, in an experiment on buffaloes receiving sub-maintenance rations, the percentage of buffaloes with a constant basal level of progesterone in the peripheral blood, indicating ovarian inactivity, was higher in summer (Kaur and Arora, 1984). In the summer season the responsiveness of the hypothalamic hypophyseal system to exogenous GnRH is lowered (Aboul-Ela et al., 1983). Summer anoestrus is a common problem in the dairy buffalo in Egypt, Pakistan, India and Bangladesh.

Anoestrus in cows has been successfully treated either with an ear implant containing norgestomet or with an intravaginal device containing progesterone (Gonzalez-Padilla et al., 1975; Mulvehill and Sreenan, 1978; Smith et al., 1979; Willemse et al., 1982). norgestomet was effective in the induction of oestrus in suckling beef cows (Miksch et al., 1978). In dairy buffaloes a conception rate of 43.7% was obtained during the normal breeding season with fixed time A.I. at 48 and 72 h after withdrawal of a norgestomet ear implant, followed by injection of PMSG (Rao and Rao, 1979). The norgestomet–PMSG regimen has been attempted in buffalo heifers and buffalo cows not seen in oestrus for at least 3 months (Rao and Sreemannarayana, 1983). In this experiment the conception rate after insemination in two consecutive oestrous periods was 46.2%. The influence of the environment, especially nutrition, was highlighted by Singh et al. (1983) as conception rates to fixed time A.I. after a norgestomet–PMSG treatment were 40% in village herds compared to 61% for a well managed herd.

The present trial was designed to induce a synchronized ovulatory oestrus in anoestrous Nili-Ravi buffaloes in a well managed herd during the summer season in Pakistan.

MATERIALS AND METHODS

The experiment was performed on a herd consisting of 550 Nili-Ravi buffaloes. The animals were hand-milked twice a day and received a formulated ration and green fodder. They were housed indoors, but were allowed to remain outside in open sheds daily, for some hours. One stockman was responsible for the care, feeding and management (including oestrus detection twice daily with the help of a teaser bull) of 10–15 buffaloes.

For the experiment, lactating buffaloes not seen in oestrus for at least 3 to 5 months were selected. Internal genitalia of these buffaloes were palpated rectally and 40 animals with inactive ovaries and a toneless uterus were selected for the experiment. Thirty animals having calved in the same period as the experimental animals and not seen in oestrus since, served as controls. The trial was carried out in May (summer season).

The buffaloes were at an average of 192.5 ± 57.7 days after calving and none of them had shown any oestrous symptoms since. In the experimental group 12 animals were of the first parity, 12 of the second, and the remaining 16 had higher parities.

A subcutaneous ear implant containing 6 mg of norgestomet was inserted, accompanied by an intramuscular injection of 3 mg norgestomet and 5 mg of oestradiol valerate (Syncro-mate B, Intervet). After 9 days the implant was removed and 600 IU of PMSG were given intramuscularly. Fixed time insemination with frozen semen was done at 48 and 72 h after withdrawal of the implant, irrespective of oestrous behaviour.

Milk samples were taken on the day of insertion of the implant, the day of removal and on 10 and 22 days after the second A.I. In those samples progesterone values were determined by an enzyme immunoassay as described earlier by Van de Wiel and Koops (1986).

The EIA procedure for direct (i.e. without extraction) measurement of progesterone in buffalo milk was validated by comparing progesterone concentrations in 32 buffalo milk samples as measured by EIA and by a previously validated direct radio-immunoassay (RIA) (Van de Wiel et al., 1978). The equation of the regression line was $EIA = 0.89\ RIA + 1.33$ (ng/ml) with a correlation coefficient $r = 0.969$.

RESULTS

The clinical diagnosis of true anoestrus was confirmed by milk progesterone levels in 33 out of 40 animals (82.5%). Among these 33 buffaloes, one lost the ear implant and two others were excluded from the experiment as they were suffering from endometritis.

Ovulation was induced in 21 buffaloes out of 30 (70%) as estimated from progesterone values in milk samples taken on day 10 after A.I. The parity-wise success rate of induction of ovulation was 60%, 77.7% and 72.7% in first, second and third or higher parity, respectively. Out of 30 anoestrous animals 16 conceived subsequently giving an overall conception rate of 53.3% (Table 1). A buffalo was considered as pregnant when progesterone values both on the 10th day and 22nd day after A.I. were more than 5.0 ng/ml. These pregnancies were confirmed by rectal examination on day 60 after A.I.

The conception rate was highest in the third or higher parity buffaloes as all the buffaloes ($n = 8$) of this group in which ovulation was induced, conceived (100%). The parity-wise overall conception rates in all the treated animals were 40%, 44.4% and 72.7% in first, second and third or higher parity, respectively.

TABLE 1

Oestrus induction with a progestagen-containing ear implant in 30 Nili-Ravi buffaloes with true anoestrus

Treatment	No. of buffaloes	Implant insertion	Buffaloes with > 5 ng/ml milk progesterone 10 days post-A.I.		Buffaloes with > 5 ng/ml milk progesterone 22 days post-A.I.	
			(*n*)	(%)	(*n*)	(%)
			←ovulation→		←pregnancy→	
Control	30	—	—		—	
Treated (Total)	30	30	21	70.0	16	53.3
1st Parity	10	10	6	60.0	4	40.0
2nd Parity	9	9	7	77.77	4	44.44
3rd Parity	11	11	8	72.72	8	72.72

DISCUSSION

Seven animals were incorrectly diagnosed as being non-cycling. Obviously the presence of a functional corpus luteum was not detected in these animals during rectal palpation. The same discrepancy was found in an experiment with true anoestrous cows (Willemse et al., 1982).

Progesterone values were basal (0.18 ± 0.23 ng/ml) in all 30 buffaloes at the time of insertion and at the time of withdrawal of the implant, which confirms the true anoestrous state in these animals. Twenty-one buffaloes showed elevated values (13.22 ± 6.74 ng/ml) at 10 days after withdrawal of the implant, indicating that ovulation had occurred.

These results are in complete agreement with those obtained by Singh et al. (1983). The particular treatment regimen was used to study the efficacy of the drug in the best milk-producing breed of buffaloes, i.e. Nili-Ravi.

We obtained an overall conception rate of 53.3% with fixed time A.I. after induced ovulation while Singh et al. (1983) reported a conception rate of 50.13% over three oestrous periods after treatment, and Rao and Sreemannarayana (1983) achieved an overall pregnancy rate of 46.2% after insemination in an induced plus the following spontaneous oestrous period (32.5% in heifers and 68% in adults). In our experiment the increase in conception rate with advancement of parity supports the earlier findings of Cady et al. (1983) in Nili-Ravi buffaloes.

The present results of the treatment of anoestrous buffaloes with norgestomet in the low breeding season are encouraging. We conclude that a norgestomet treatment regimen was able to restore ovarian activity, resulting in ovulation in the majority of anoestrous buffaloes (70%). In earlier studies it has also been reported that a great proportion of animals will continue to cycle following treatment with norgestomet (in buffaloes: Rao and Sreemannarayana, 1983; Singh et al., 1983; Rao, 1985; in cows: Willemse et al., 1982). The advantage of this treatment can be appreciated especially as none of the 30 control animals displayed oestrus over the same period of time.

The results achieved in our study in a herd with high standards of management and nutrition would probably not be obtained in animals kept under prevailing conditions in village herds.

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