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Evaluation of the Heatsynch protocol in Murrah buffaloes (*Bubalus bubalis*) during hot summer season

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ABSTRACT

The objective of this study was to evaluate the effect of the Heatynch protocol on plasma estrogen concentration in Murrah buffaloes and to evaluate if the Heatsynch protocol induces estrus in Murrah buffaloes. All treated animals responded to the treatment by displaying obvious signs of estrus. The mean plasma estrogen concentration among the individual buffalo was 191.9 ± 68.3 pg / ml after Estradiol benzoate (EB) injection. The plasma progesterone profile of these animals indicated that out of 16 animals 8 animals were acyclic before Heatsynch treatment (8/16) as plasma progesterone concentrations in these animals were basal (<0.24±0.01 ng/ml) for most of the sampling period during the course of treatment. Following Heatsynch treatment only two buffalo out of sixteen were acyclic (2/16) on the basis of their plasma progesterone profile. These results indicated that Heatsynch protocol is useful in inducing cyclicity in acyclic buffaloes. The seven buffalo out of sixteen (7/16) were conceived after treatment with Heatsynch protocol in summer. So, the treatment might have potential field application value to overcome poor estrus symptoms in summer.

Keywords: Cyclicity, Estradiol benzoate, GnRH, Heatsynch, Buffalo

Buffaloes play a prominent role in rural livestock production in Asia and India in particular. Silent estrus is perhaps the most important factor leading to poor reproductive efficiency in buffaloes (Kanai and Shimizu, 1983; Prakash *et al.,* 2002) especially during hot summer months (Madan and Prakash, 2007). Murrah buffaloes however are sluggish breeders, beset with reproductive problems (Madan and Prakash, 2007). A high percentage of buffalo cows (30-40%) experience a prolonged period of anoestrus with the Indian farmers incurring an estimated loss of 19-20 million tones of milk each year due to this problem. As the signs of estrus in buffaloes are less obvious than in cattle (Hafez, 1954), the estrus detection accuracy is one of the major problems limiting the use of A.I. in

this species (Jainudeen, 1986). Very recently an estrus synchronization protocol called Heatsynch in cattle has been developed (Pancarci *et al.,* 2002) which makes use of a combination of GnRH-PGF^{2a}-Estradiol cypionate injection. Barros *et al.* (2000) and Fernandes *et al.* (2001) had also successfully tested a similar protocol using estradiol benzoate in place of estradiol cypionate.

One benefit of using Estradiol benzoate in a Heatsynch protocol instead of the second GnRH in a traditional Ovsynch protocol is the reduction of costs. Another benefit of Heatynch protocol is that all injections and AI are performed in 24 h interval and thus are given at the same time of the day. However, in an Ovsynch you are also able to inject and breed in 24 to 48 h intervals. Heatsynch protocol has not been attempted in buffaloes. Furthermore, no report is available on evaluating the cyclicity in buffaloes. The objectives of the present investigation was (1) to evaluate the effect of the Heatsynch protocol on plasma estrogen concentration in Murrah buffaloes and (2) to evaluate if the Heatsynch protocol induces estrus in Murrah buffaloes.

MATERIALS AND METHODS

The study was carried out at the experimental herd of the National Dairy Research Institute, Karnal, Haryana, India from May to July 2006. The maximum environmental temperature varied from 42.5° to 45.3° C during this period. Climatic data viz., dry bulb temperature (C_{db}), & wet bulb temperature (C_{wb}) in degree Celsius was recorded at 6.30 AM and 2.30 pm every day. The National Dairy Research Institute Farm, which is located at 250 m above mean sea level, latitude 29.43 N and longitude 77.02 E. Sixteen repeat breeding Murrah buffalo were used for this experiment. All buffaloes were maintained on available concentrate mixture consisting of maize grain, groundnut cake, mustard cake, wheat bran, mineral mixture, salt and roughages (sorghum, berseem , maize or oat fodder as per the availability at farm). Drinking water was available ad libitum in the paddock. The institute ethical committee approved the experiment.

The buffalo (n=16) were assigned to an Heatsynch protocol as described by Mohan *et al.* (2009). It consist of an injection of a GnRH analogue (Buserelin, 10 µg, Receptal VET, Intervet India Pvt. Ltd., Pune, Maharashtra, India) at any stage of the estrous cycle (day of GnRH treatment set as day 0) followed by an injection of Dinoprost (25 mg, Lutalyse, Pharmacia and Upjohn, Puurs, Belgium) on day 7 and an injection of Estradiol benzoate (1 mg, Sigma, USA) given on day 8. All animals were assigned to a timed AI at 48 to 60 h following the Estradiol benzoate administration (Mohan *et al*., 2009).

Blood samples were collected from the jugular vein on alternate days from 20 days prior to treatment. Subsequent samples were drawn once daily from the day of first GnRH administration up to day 8, further the blood samples (5 ml) were collected

at 2 h intervals starting from the time of EB injection for 60 hrs and subsequently every alternate day for another 30 days. Blood samples were collected in EDTA coated tubes, cooled on ice and then centrifuged at 4° C. The plasma was frozen and stored in 2 aliquots at -20° C until analysis for total estrogen and progesterone.

Enzymeimmunoassay (EIA) for estrogen

Highly sensitive heterologous EIA procedure for estrogen estimation in buffalo plasma using the second antibody coating technique followed (Mondal *et al*., 2006). The procedure employed 50 µl of extracted and reconstituted plasma samples, antiserum against estradiol 17β-17-HS-BSA. This procedure used estradiol 17β-horse radish peroxidase as the enzyme conjugate. The sensitivity of the assay in extracted plasma was 0.2 pg/50 µl/well which corresponds to 1.45 pg/ml of plasma. The intra- and interassay coefficients of variation of the assays were 6.3% and 9.5% for total estrogen.

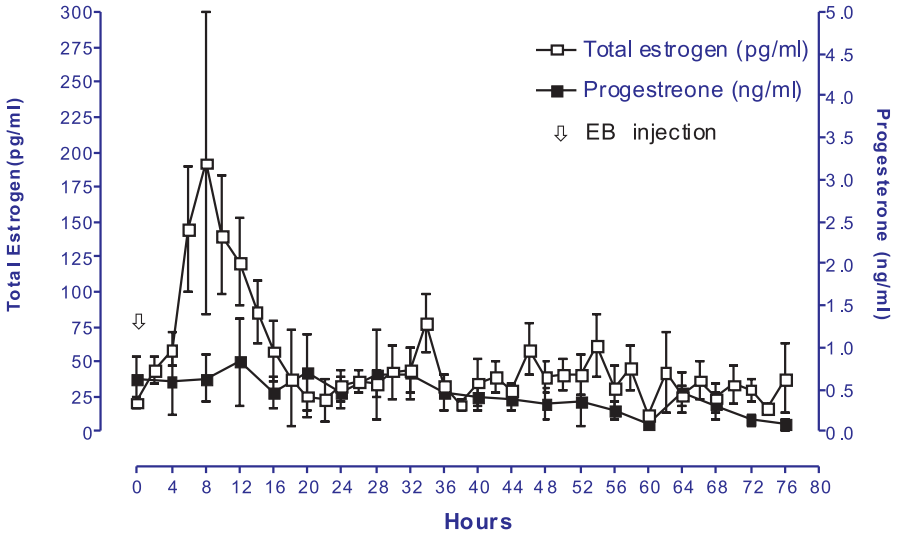

Figure 1. Changes in plasma total estrogen and progestreone (Mean±SEM) profile after administration of Estradiol benzoate in buffaloes (n=16) treated with Heatsynch protocol.

Radioimmunoassay for progesterone

Progesterone was estimated by direct radioimmunoassay following a previously validated procedure (Kamboj and Prakash, 1993). Radioimmunoassay of progesterone estimation was carried out in plasma using 20 µl of plasma. The

sensitivity of the assay for progesterone was 4 pg/tube, which corresponds to 0.2 ng/ml of plasma. The intra and inter-assay coefficients of variation for progesterone were 8.4% and 12.0%, respectively.

RESULTS AND DISCUSSION

The Plasma total estrogen and progesterone concentration are presented in Figure 1 and Table 1.The buffaloes (n = 16) were treated with the Heatsynch protocol to evaluated the efficacy of the Heatsynch protocol to induce estrus. All treated animals responded to the treatment by displaying obvious signs of estrus. Mean plasma estrogen concentration was 191.9 ± 68.3 pg / ml, and ranges between 35.6 – 701.3 pg/ml of plasma after injecting Estradiol benzoate (Figure 1, Table 1). The plasma progesterone profile of these animals indicated that out of 16 animals 8 animals were acyclic before Heatsynch treatment (8/16) as plasma progesterone concentrations in these animals were basal (<0.24 ± 0.01 ng / ml) for most of the sampling period and during the course of treatment. Following Heatsynch treatment only two buffalo out of sixteen (2/16) were acyclic on the basis of their plasma progesterone profile.

To our knowledge this is the first study which evaluated the efficacy of the Heatsynch protocol to induce estrus in Murrah buffaloes. All treated animals responded to the treatment by displaying obvious signs of estrus. The exhibition of estrus symptoms in all animals was probably an effect of potent estradiol benzoate treatment which also resulted in all animals exhibiting high total estrogen peak concentrations in blood plasma. Earlier studies in cows (Pancarci *et al*., 2002; Evans *et al*., 2003; Cerri *et al*., 2004) demonstrated better expression of estrus using the Heatsynch protocol.

Table.1. Plasma estrogen and progesterone concentration in buffaloes (n=16) treated with the Heatsynch protocol

Endocrine parameters	Mean ± SEM	Range
Estrogen (pg/ml)	191.9 ± 68.3	35.6 – 701.3
Duration of estrogen surge (h)	18.2 ± 1.7	11 – 25
Progesterone (ng/ml)	_	0.2 – 2.41
Onset of estrogen surge after EB injection (h)	8.8 ± 0.8	6 – 15

The plasma total estrogen mean concentrations among the individual buffalo was 191.9 ± 68.3 pg / ml of plasma after estradiol benzoate injection.

Estradiol benzoate is an esterified form of estradiol 17 β that is available for use in animals in some countries. When low doses of EB are injected in cows under a low progesterone environment, it induces an LH surge from the brain (Chenault *et*

al., 1975; Hansel and Convey, 1983; Kinder *et al*., 1991) A similar response has been demonstrated in cows.

To assess whether the buffaloes were cyclic during the period of experimentation plasma progesterone was monitored on all experimental animals. The plasma progesterone profile of these animals indicated that out of 16 animals 8 animals were acyclic before Heatsynch treatment. Following Heatsynch treatment only two buffalo out of sixteen were acyclic. These results indicated that Heatsynch protocol might have the potential in inducing cyclicity in a acyclic buffaloes.

The seven out of sixteen buffaloes (7/16) were conceived after treatment with Heatsynch protocol in summer on the basis of transrectal palpation 60 days post insemination. The efficacy of Heatsynch protocol in terms of conception rate suggest that the Heatsynch protocol could be very useful in buffaloes considering the fact that the incidence of silent heat in this species is very high and particularly in summer it goes up to 70% (Prakash *et al.*, 2005; Qureshi *et al*., 2008). This study indicates that Heatsynch protocol is capable of inducing estrus cycle in anestrous. The Heatsynch protocol can have distinct advantage in enhancing the fertility of buffaloes especially since the technique can circumvent the need for heat detection. So, the treatment has potential field application value to overcome poor estrus symptoms in buffaloes during low breeding season.

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REFERENCES

Barros CM, Moreira MBP, Figueredo RA, Teixeira AB and Trinca L A. 2000. Synchronization of ovulation in beef cows (*Bos indicus*) using GnRH, $PGF_{2\alpha}$ and estradiol benzoate. *Theriogenology*, **53**:1121-1134.

Cerri RLA, Santos JEP, Juchem SO, Galvao KN and Chebel RC. 2004. Timed artificial insemination with estradiol cypionate or insemination at estrus in high producing dairy cows. *J. Dairy Sci.*, **87**(11): 37004-3715.

Chenault JR, Thatcher WW, Kalra PS, Abrams RM and Wilcox CJ. 1975. Transitory changes in plasma progestins, estradiol and luteinizing hormone approaching ovulation in the bovine. *J. Dairy Sci*., **58**(5): 709-717.

Evans A.C.O., L'keeffe O, Mihm M, Roche JF, Macmillan KL and Boland MP. 2003. Effect of oestradiol benzoate given after prostaglandin at two stages of follicle wave development on oestrus synchronization, the LH surge and ovulation in heifers. *Anim.*

Reprod. Sci., **76**(1/2): 13-23.

Fernandes P, Teixeira AB, Crocci AJ and Barros CM. 2001. Timed artificial insemination in beef cattle using GnRH agonist, PGF2 alfa and estradiol benzoate (EB). *Theriogenology*, **55**:1521-1532.

Hafez ESE. 1954. Oestrus and some related phenomena in the buffalo. *J. Agric. Sci. Cambridge*, **44**: 165 – 172.

Hansel W and Convey M. 1983. Physiology of the estrous cycle. *J. Anim. Sci.*, 57:404-423.

Jainudeen, MR. 1986. Reproduction in water buffalo. In : Current Therapy in Theriogenology (D.A. Marrow, ed.) W.B. Saunders, Philadelphia, pp. 443 – 449.

Kamboj M and BS Prakash. 1993. Relationship of progesterone in plasma and whole milk of buffaloes during cyclicity and early pregnancy. *Trop. Anim. Hlth. Prod.*, **25**: 185-192.

Kanai Y and Shimizu H. 1983. Characteristics of the estrous cycle of the Swamp buffalo under temperate conditions. *Theriogenology.*, **19**(4):593-602.

Kinder JE, Garcia-Winder M, Imakawa K, Day ML, Zaleskg DD, D'Occhio ML, Stumpf TT, Kittok RJ and Schanbacher BD. 1991. Circulating concentrations of 17-estradiol influence pattern of LH in circulation of cows. *Domest. Anim. Endocrionl.*, **8**: 463-469.

Mohan K, Sarkar M and Prakash BS. 2009. Efficiency of Heatsynch for synchronization of estrus, timing of ovulation, endocrine profile and timed artificial insemination in Murrah buffaloes (*Bubalus bubalis*). *Asian-Aust. J. Anim. Sci.*, **22**(6) : 774-780.

Madan M.L. and Prakash BS. 2007. Reproductive endocrinology and biotechnology applications among buffaloes. In: *Reproduction in Domestic Ruminants*, **6**: 261-281 (Ed. JI Juengel, JF Murray and MF Smith. Nottingham University Press, Nottingham, UK).

Mondal M, Rajkhowa C and Prakash BS. 2006. Relationship of plasma estradiol-17β , total estrogen and progesterone to estrus behaviour in mithun (*Bos frontalis*) cows. *Hormones and Behavior*, **49**:626-633.

Pancarci SM, Jordon ER, Risco CA, Schonten MJ, Lopes FL, Moreira and Thatcher WW. 2002. Use of Estradiol Cypionate in a presynchronized Timed Artificial Insemination Program for Lactating Dairy Cattle. *J. Dairy Sci.*, **85**: 122-131.

Prakash BS, Paul V and Anandlaxmi N. 2002. Development and validation of a simple, sensitive, second antibody format enzyme immunoassay for LH determination in plasma. *J. Immunol. Methods.*, **270**: 281-290.

Prakash BS, Sarkar M, Paul V, Mishra DP, Mishra A and Meyer HHD. 2005. Postpartum endocrinology and prospects for fertility improvement in the reverine buffalo (*Bubalus bubalis*) and Yak (Bos grunniens). *Livest. Prod. Sci.*, **98**: 13-23.

Qureshi MS and Ahmed N. 2008. Interaction among calf suckling use of oxytosin, milk production and reproduction in dairy buffaloes. *Anim. Reprod. Sci.*, **106**: 380-392.