USE OF ESTRUS INDUCTION STRATEGY IN TRUE ANESTRUS BUFFALO FOR REDUCING NON-PRODUCTIVE PERIOD

K.S. MUNGAD[1], J.A. PATEL[2], A.J. DHAMI[3]*, K.K. HADIYA[4], D.N. BORKHATARIYA[1] AND N.P. SARVAIYA[2]

Department of Animal Reproduction, Gynaecology and Obstetrics
Anand Agriculture University, Anand - 388 001

Received: 22.09.2016 Accepted: 18.11.2016

ABSTRACT

True anestrus buffalo were subjected to three hormone protocols (Ovsynch, Heatsynch and Triu-B protocol) during summer and winter season for the induction of synchronized estrus followed by fixed-time AI (n=12 in each group). Estrus induction response was similar (75.0-91.6%, $p>0.05$) between protocols during summer and winter. Overall conception rate in response to AI at induced estrus and at subsequent two spontaneous estruses was between 25.0-33.3% in summer and 50.0 - 58.3% during winter. In comparison, 8.3% and 25.0% untreated control buffalo exhibited spontaneous estrus and conceived during summer and winter season, respectively. Plasma progesterone profile confirmed anestrus status of buffalo recruited in the study as well as the success of hormone protocols and subsequent pregnancy status ($p<0.05$). In conclusion, estrus induction protocols combined with fixed-time AI can be used in true anestrus buffalo to improve their reproductive efficiency, however, an effective protocol is required for summer season.

Keywords: Buffalo, Estrus synchronization, Fertility, Progesterone, Season

INTRODUCTION

A major limiting factor in the wide spread application of artificial insemination (AI) in buffalo is the difficulty in detection of estrus especially during hot and humid months (Barile, 2005). Recently, the use of various combinations of hormones has been advocated for the induction of synchronized estrus in buffalo, thus, facilitating the use of fixed time AI in this species without any need for estrus detection (Baruselli and Carvalho, 2005) However, a meagre amount of work has been carried out for the evaluation of comparative efficacy of various hormone protocols to induce synchronized estrus in buffalo during summer and winter season (Jabeen *et al.,* 2012). Therefore, the present study evaluated the estrus induction response, conception rate and plasma progesterone profile in anestrus buffalo subjected to three different hormone protocols during summer and winter season.

[1]Research Scholar, [2]Professor, [3]Professor and Head, [4]Assistant Professor; *ajdhami@aau.in

MATERIALS AND METHODS

This study was carried out in the villages of milk-shed areas of Amul and Panchamrut Dairies of Gujarat during peak summer and peak winter season during March 2015 to February 2016. After day 90 postpartum, buffalo (n=96) exhibiting true anestrus were identified by rectal palpation at a 10-day interval. Thereafter, buffalo were dewormed using Ivermectin 100 mg s/c, and were administered elemental phosphorus 80 mg i/m and a 10 ml vitamin AD_3E preparation i/m, and were fed multi-mineral bolus @ 1 bolus p/o for 7 days. These buffalo were randomly divided (n=12 in each group) during summer and winter season and were subjected to hormone protocols aimed at induction of synchronized estrus along with fixed time AI (FTAI), viz. Ovsynch (d0 and d9, buserelin 10 µg; d7 $PGF_{2\alpha}$ 500 µg; d10 FTAI), Heatsynch (d0, buserelin 10 µg; d7 $PGF_{2\alpha}$ 500 µg; d9 EB 1 mg; d11 FTAI) and Triu-B (d0, 0.96 g hydroxy progesterone implant i/vaginal; d6 $PGF_{2\alpha}$ 500 µg, d7 implant removal and EB 1 mg; d8 FTAI). Twelve anestrus buffalo in each season were kept as untreated control and were inseminated at spontaneous estrus. In all the groups, buffalo detected in estrus subsequent to AI at first induced or spontaneous estrus were re-inseminated up to two subsequent cycles. In non-return cases, the pregnancy was confirmed per rectally at day 60 of last AI. For plasma progesterone analysis, the jugular vein blood samples were collected thrice during administration of protocols and on day 21 post-AI. Plasma was stored at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma progesterone was estimated using standard Radio-Immuno-Assay (RIA) technique. Labelled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech, France. The sensitivity of assay was 0.1 ng/ml. The intra- and inter-assay coefficients of variation were 5.4 and 9.1%, respectively. The data on estrus induction response and conception rates (Chi-square test) and plasma progesterone between different treatments and seasons (ANOVA and 't' test) were analyzed using online SAS software version 20.00.

RESULTS AND DISCUSSION

The proportion of anestrus buffalo exhibiting induction of synchronized estrus as well as prostaglandin $F_{2α}$ ($PGF_{2α}$) administration to estrus induction interval using three hormone protocols were similar between groups and between seasons (p>0.05, Table 1). The observed estrus induction response during winter was in harmony with earlier results in buffalo (Buhecha *et al.,* 2016 and Thorat *et al*., 2014), whereas others documented poor estrus induction response using Heatsynch in cyclic and acyclic buffalo (Mirmahmoudi *et al.,* 2014). The estrus induction rate achieved during summer season using Triu-B protocol was comparable to Kajaysri *et al.* (2015), but was higher than 35% reported by others with Ovsynch (Azawi *et al.,* 2012). Estrus induction intervals observed in present study during summer and winter season were similar to earlier reports in anestrus buffalo during breeding season (Nakrani *et al.,* 2014 and Buhecha *et al.,* 2016) and non-breeding season (Azawi *et al.,* 2012), whereas, a relatively shorter estrus induction interval was observed in other report (Kajaysri *et al.,* 2015).

The conception rate at induced estrus using three hormone protocols was 16.7% estruses varied between 25.0 - 33.3% during summer and 50.0 - 58.3% during winter (Table 1). The conception rate obtained at induced estrus under all three protocols was higher than earlier observations during winter (Buhecha *et al.,* 2016 and Ali *et al*., 2012), and summer (Ali *et al*., 2012). In the present study, the overall conception rate subsequent to the use of hormone protocols during summer and winter was 27.8% (10/36) and 55.5% (20/36), respectively. Furthermore, in untreated anestrus control buffalo each during summer season, and varied between 25.0 - 41.7% during winter season (Table 1). The overall conception rate of induced and two subsequent spontaneous during summer and winter, the buffalo exhibiting estrus and subsequently conceiving were 8.3% (1/12) and 25.0% (3/12), respectively (Table 1). The poor results during summer season may be attributed to a deep acyclic condition characterized by an absent or strongly reduced follicle turnover (Presicce *et al.,* 2005).

The interval (days) between start of protocol to fertile estrus was higher in Triu-B protocol (26.0**±10.1) as compared to** Heatsynch (17.7±7.7) and Ovsynch (16.7±6.7) during summer season. However, during winter season it was higher in Heatsynch protocol (23.3±6.5), followed by Triu-B (19.0±6.2) and Ovsynch protocol (15.0±6.2). In the control group the service period was higher during summer as compared to winter (185 *vs*. 155.1±18.1 days). Thus, the application of different synchronization protocols curtailed 45-60 unproductive days of anestrus buffalo in comparison to untreated controls, which is economical to the farmers. The numbers of animals again turned out to be anestrus following treatment during summer was more compared to winter (14 *vs.* 8), indicating favourable effect of breeding season in establishing cyclicity/ pregnancy in hormonally treated animals.

The basal plasma progesterone concentrations at the start of hormone protocols (day 0) in buffalo of all the groups confirmed their anestrus status (Table 2). As expected, plasma progesterone on day 7 was elevated compared to day 0 concentration ($p<0.05$, Table 2), indicating the success of day 0 hormone treatment. In fact, day 0 treatment might have lead to luteinization of growing follicles and/or ovulation of dominant follicle and formation of corpus luteum under the influence of GnRH (Pursley *et al*., 1995 and Buhecha *et al*., 2016). On the day of AI, plasma progesterone again decreased to basal concentrations ($p<0.05$, Table 2), thus, suggesting the success of day 7 treatments. On day 21 post-AI, plasma progesterone was higher compared to concentrations at the time of AI ($p<0.05$, Table 2). Moreover, on day 21 post-AI, plasma progesterone was higher in conceiving buffalo as compared to their non-conceiving counterparts ($p<0.05$, Table 2). Comparatively lower plasma progesterone recorded on day 7 and day 21 of present study during summer compared to winter season ($p>0.05$, Table 2) was suggestive of adverse impact of heat stress on steroidogenesis and associated conception rate (Presicce *et al.,* 2005, and Ahmadi and Ghaisari, 2007). Nevertheless, plasma progesterone profile with respect to estrus synchronization/induction protocols corroborated with earlier observations in cyclic and anestrus buffalo under similar protocols particularly during favourable breeding season (Nakrani *et al*., 2014 and Buhecha *et al*., 2016).

In brief, induction of synchronized estrus followed by fixed time AI can be used in true anestrus buffalo to reduce the non-productive period in their life time. However, these hormone protocols are more efficacious in winter and use of an effective protocol during summer season requires further investigations.

ACKNOWLEDGEMENTS

We thank Dr. A.M. Thaker, Dean of the Faculty and Dr. A.J. Dhami, PI of the project “AICRP on Nutritional and Physiological Interventions for Enhancing Reproductive Performance in Animals” for the facilities and financial support.

Table 1: Estrus induction (EI) and subsequent conception rate in response to hormonal protocols administered to anestrus buffalo (n=12 in each group) during summer and winter season

Protocol	Estrus induction response, %	$PGF_{2\alpha}$ Inj to estrus induction interval, h	Conception rate				Buffalo turned to be anestrus by 60 d post-treatment, n	Start of protocol to fertile estrus interval, day
			Induced estrus, %	2nd estrus, %	3rd estrus, %	Overall, %		
Summer								
Ovsynch	75.0 (9/12)	71.7±3.3	16.7 (2/12)	12.5 (1/8)	0.0 (0/4)	25.0 (3/12)	5 (41.7%)	16.7±6.7
Heatsynch	83.3 (10/12)	56.9±2.1	16.7 (2/12)	14.3 (1/7)	0.0 (0/3)	25.0 (3/12)	6 (50.0%)	17.7±7.7
Triu-B	83.3 (10/12)	63.2±2.5	16.7 (2/12)	11.1 (1/9)	16.7 (1/6)	33.3 (4/12)	3 (25.0%)	26.0±10.1
Control	8.3 (1/12)	-	8.3 (1/12)	0.0 (0/11)	0.0 (0/11)	8.3 (1/12)	11 (91.7%)	185*
Winter								
Ovsynch	83.3 (10/12)	69.8±3.4	41.7 (5/12)	16.7 (1/6)	33.3 (1/3)	58.3 (7/12)	3 (25.0%)	15.0±6.2
Heatsynch	91.7 (11/12)	54.1±2.5	25.0 (3/12)	28.8 (2/7)	25.0 (1/4)	50.0 (6/12)	3 (25.0%)	23.3±6.5
Triu-B	83.3 (10/12)	62.1±2.8	41.7 (5/12)	16.7 (1/)	25.0 (1/4)	58.3 (7/12)	2 (16.7%)	19.0±6.2
Control	25.0 (3/12)	-	16.7 (2/12)	10.0 (1/10)	0.0 (0/9)	25.0 (3/12)	9 (75.0%)	155.1±18.1*

*$p<0.05$ service period; $PGF_{2\alpha}$, Prostaglandin $F_{2\alpha}$

Table 2: Plasma progesterone profile in anestrus buffalo subjected to estrus induction protocols. C, conceived; NC, non-conceived; O, overall

Protocol	Status	Plasma progesterone, ng/ml (day 0, start of protocol)			
		d0	d7, $PGF_{2\alpha}$	d9/10, AI	d21 post-AI
Summer					
Ovsynch	C, n=2	0.50±0.20	4.35±0.45	0.40±0.02	4.00±0.20^{q}
	NC, n=10	0.41±0.06	2.09±0.44	0.53±0.10	1.50±0.38^{p}
	O, n=12	0.43±0.06^{a}	2.47±0.45^{b}	0.51±0.09^{a,*}	1.92±0.42^{b}
Heatsynch	C, n=2	0.63±0.29	3.65±0.15^{q}	0.35±0.06	4.50±0.10^{q}
	NC, n=10	0.49±0.04	1.93± 0.35^{p}	0.57±0.09	1.15±0.25^{p}
	O, n=12	0.51±0.05^{a}	2.21±0.33^{b}	0.53±0.08^{a}	1.71±0.43^{b}
Triu-B	C, n=2	0.29±0.05	3.95±0.55	0.28±0.02	4.19±0.19^{q}
	NC, n=10	0.47±0.10	2.45±0.33	0.74±0.25	1.33±0.31^{p}
	O, n=12	0.44±0.09^{a}	2.70±0.33^{c}	0.66±0.21^{a}	1.39±0.40^{b}
Winter					
Ovsynch	C, n=5	0.47±0.06	3.80±0.36^{q}	0.63± 0.13	4.59±0.57^{q}
	NC, n=7	0.39±0.09	2.15±0.29^{p}	0.96±0.18	1.48±0.67^{p}
	O, n=12	0.42±0.06^{a}	2.84±0.33^{b}	0.82±0.12^{a,*}	2.78±0.64^{b}
Heatsynch	C, n=3	0.20±0.02	3.87±0.69^{q}	0.25±0.05	4.36± 0.24^{q}
	NC, n=9	0.42±0.09	1.79±0.17^{p}	0.51±0.1	1.57±0.36^{p}
	O, n=12	0.36±0.07^{a}	2.31±0.33^{b}	0.45±0.08^{a}	2.27±0.45^{b}
Triu-B	C, n=5	0.53±0.10	3.82±0.29^{q}	0.39±0.07	4.42±0.28^{q}
	NC, n=7	0.43±0.11	2.20±0.38^{p}	0.46±0.06	1.29±0.31^{p}
	O, n=12	0.47±0.07^{a}	2.88±0.34^{b}	0.43±0.05^{a}	2.59±0.51^{b}

Means bearing uncommon superscripts within a row (a, b, c) or a column (p, q) for a protocol differ significantly, p<0.05; *between seasons within a protocol, p<0.05

REFERENCES

Ahmadi, M.R. and Ghaisari, H.R. (2007). Heat stress and different timed-AI methods influence on pregnancy rates of dairy cows. *Veterinarski Arhiv.* **7**(4): 327-335.

Ali, R., Shukla, S.P. and Nema, S.P. (2012). Hormonal induction of ovarian cyclicity and conception rate in postpartum anoestrus buffaloes. *Indian J. Field Vets.,* **7**(4): 44-46.

Azawi, O.I., Ali, M.D., Oday, S.A., Al-Hadad, A.S., Mouayad, S.J. and Hussien, A.S.A. (2012). Treatment of anoestrus in Iraqi buffaloes using Ovsynch alone or in combination with CIDR. *J. Adv. Vet. Res.,* **2**(1): 68-72.

Barile, V.L. (2005). Review article: Improving reproductive efficiency in female buffaloes. *Livestock Prod. Sci.*, **92**: 183-194.

Baruselli, P.S. and Carvalho, N.A.T. (2005). Biotechnology of reproduction in buffalo (*Bubalus bubalis*). *Rev. Bras. Reprod. Anim.*, **29**: 4-17.

Buhecha, K.V., Dhami, A.J., Patel, M.D., Hadiya, K.K., Shah, R.G. and Kharadi, V.B. (2016). Study on different estrus induction protocols with respect to fertility and plasma progesterone profile in anoestrus buffaloes. *Indian J. Dairy Sci.,* **69**(2): 197-201.

Jabeen, S., Anwar, M., Andrabi, S.M.H., Mehmood, A., Murtaza, S. and Shahab, M. (2012). Determination of Ovsynch efficiency for oestrus synchronization by plasma LH and P_4 levels in Nili-Ravi buffalo during peak and low breeding seasons*. Pakistan Vet. J.*, **33**(2): 221-224.

Kajaysri, J., Chumchoung, C. and Photikanit, G. (2015). Oestrus and ovulation responses in anoestrus postpartum swamp buffaloes following synchronization with a controlled internal drug release device and prostaglandin $F_{2\alpha}$ based protocols. *Buffalo Bull.,* **34**(3): 357-368.

Mirmahmoudi, R., Souri, M. and Prakash, B.S. (2014). Comparison of endocrine changes, timing of ovulations, ovarian follicular growth, and efficacy associated with Estradoublesynch and Heatsynch protocols in Murrah buffalo cows (*Bubalus bubalis*). *Theriogenology*, **82**: 1012-1020.

Nakrani, B.B., Panchal, M.T., Dhami, A.J., Hadiya, K.K., Patel, J.A., Gosai, R.K. and Shah R.G. (2014). Influence of controlled breeding techniques on oestrus induction response, conception rate and plasma progesterone profile in anoestrus buffaloes*. Global J. Med. Res.,* **14**(3): 1-6.

Presicce, G.A., Senatore, E.M., De Santis, G. and Bella, A. (2005). Follicle turnover and pregnancy rates following oestrus synchronization protocols in Italian buffaloes (*Bubalus bubalis*). *Reprod. Dom. Anim.,* **40**(5): 443-447.

Pursley, J.R., Mee, M.O. and Wiltbank, W.C. (1995). Synchronization of ovulation in dairy cows using PGF_2**α and** GnRH. *Theriogenology,* **44**: 915-923.

Thorat, K., Patil, A.D., Kumbhar, U.B. and Ghoke, S.S. (2014). Improving fertility in postpartum Marathwadi anoestrus buffaloes using Ovsynch and Selectsynch protocols. *Indian. J. Anim. Reprod.,* **33**(2): 58-60.