COMPARATIVE EFFICACY OF OVSYNCH AND OVSYNCH+CIDR IN POST-PARTUM ANOESTRUS BUFFALOES

PRAVEEN KUMAR M[1], VEERA BRAMHAIAH K AND M[2]. MUTHARAO[2]
Veterinary Gynaecology & Obstetrics, College of Veterinary Science, Tirupati
Sri Venkateswara Veterinary University

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ABSTRACT

Synchronization of post-partum anoestrus buffaloes with ovsynch and ovsynch+CIDR showed that theestrus response, time taken for onset of estrus and duration of estruswere 71.43 and 71.43 %; 62.4±1.21 and 57.25±1.25 h and 18.80±0.66 and 20.25±1.75 h, respectively and its differencebetween protocols did not show any significant difference.The mean serum progesterone concentrations on different days of synchronization viz., on treatment initiation day, at the time of $PGF_{2\alpha}$ administration and on the day of AI were 0.74±0.50 and 0.01±0.01; 3.54±0.47and 4.33±0.74and 1.17±0.47 and 0.95±0.74 ng/ml, respectively and its concentration difference between protocols was not significant (P>0.05) and within each protocol was significant (P<0.05).Resumption of ovarian activity and development of functional dominant follicle at synchronized estruswas good with Ovsynch+CIDR with overall conception rate of 71.43%. While the same was 57.14%with Ovsynch protocol.

Key words : Buffaloes, Postpartum anestrus, Ovsynch, Ovsyncha+CIDR, Conception

INTRODUCTION

India is the home tract of the world's best dairy buffaloes (Chawla, 1998) and contributes significantly to rural economy. Major limitation to the success of rebreeding after each calving is postpartum anestrus (Singh and Sahni, 1995) with functional disorder of ovaries and constitute to about 19 to 74% (Vale, 1994). Various research workers have used different hormonal preparations to stimulate hypothalamic-endocrine axis and initiate ovulation and resumption of normal cyclicity of anestrus in buffaloes (Aminudeen, 1991). Among these ovsynch protocol was commonly used to synchronize ovulation (Kumar *et al.,* 2010) but GnRH may fail to turnover dominant follicles late in oestrus cycle leading to premature estrus in 11-14% treated animals (Gabor *et al.,* 2002). To overcome this, present study was undertaken to compare the efficacy of Ovsynchwith Ovsynch+CIDRin post-partum anoestrus buffaloes.

MATERIALS AND METHODS

Fourteen post-partum anestrus buffaloes having 60 days and above post-partum period without initiation of ovarian cyclicity and maintained under uniform managemental and husbandry conditions were utilized. All the animals were thoroughly examined for reproductive health and ovarian status both by per rectal examination and transrectal ultrasonography (sonoray DS-30 plus Portable LCD B/W Ultrasound Scanner with 6.5 MHz linear-array transducer) before starting the experiment. Later, these buffaloes were examined for the absence of corpus luteum in two successive intervals 10 days apart to consider them under postpartum anestrus condition. These were divided randomly into two groups and assigned as Group I (Ovsynch; n=7) and Group II (Ovsynch+CIDR; n=7).

Group I buffaloes were administered with 10 µg of GnRH (Receptal, Intervet, Holland)intramuscularly on day 0, 500 µg of Cloprostenol Sodium (Vetmate, Vet care, India)intramuscularly on day 7 and again 10 µg of GnRH on day 9. Fixed time AI (FTAI) was performed at 16 to 24 h after 2ndGnRH injection.Group II buffaloes were administered with 10 µg of GnRHand also inserted Control Internal Drug Release (CIDR) device (EAZI-Breed, Pfizer Animal Health, NewYork) impregnated with1.38 g of progesterone in the silastic coil on day 0, 500mcg of Cloprostenol Sodium and removed the CIDR on day 7 and again 10 µg of GnRH on day 9. Fixed time AI (FTAI) was performed at 16 to 24 h after 2ndGnRH injection.All the Buffaloes inseminated were observed for estrus symptoms closely and those which returned to estrus were again inseminated.All the buffaloes were subjected to transrectal ultrasonography to assess the ovarian inactivity during synchronization.

Synchronized estrussymptoms were evaluated for the intensity and duration of estrus (Rao and Rao, 1981)at the time of AI and estrus response was calculated by the percentage of buffaloes exhibited the estrus out of the total buffaloes synchronized. The Interval between treatment and onset of estrus was calculated in hours by taking the time gap between the time of administration of Prostaglandins and exhibition of estrus symptoms.The duration of estrus was estimated in hours from the time of first appearance of behavioural symptoms to disappearance of symptoms of estrus. The intensity of estrus was measured by using a score card out lined by Callesen*et al.* (1993) with slight modifications and classified as weak, intermediate and intense estrus based on the type of the symptoms exhibited by the animal.All

[1.]MVSc, Scholar, [2.] Professor

the inseminated buffaloes were monitored regularly and those animals which did not return to estrus were subjected to pregnancy diagnosis by ultrasonography after 30 days of insemination. The conception rate was calculated by the percentage of buffaloes conceived out of the total buffaloes inseminated as mentioned below:Pregnancy percentage = (No. of buffaloes diagnosed as positive / Total no. of buffaloes inseminated) ×100.

Blood samples were collected (5 ml) from post-partum anestrus Buffaloes in heparinized vacutainers at the time of day of initiating treatment (day 0), day 7, day 9, day of AI and day 21. Serum was separated immediately after collection by centrifugation at 3000 rpm for 15 min and stored at -20^0 C until analysis. Serum progesterone concentrations were determined by ELISA (Enzyme Linked Immune Sorbent Assay) based commercial diagnostic kit (Calbiotech Progesterone ELISA, USA). The parameters of different protocols were analysed by t-test to understand the difference between groups on the day of treatment, two-way analysis of variance to assess overall difference between the groups and one-way analysis of variance to understand the difference within each group with reference to follicular diameter and hormone profiles. SPSS 15.0 for windows was employed in the analysis. The significance of all the parameters was measured at P<0.05 level significance.

RESULTS AND DISCUSSION

The estrus response after synchronization of postpartum anestrusuffaloes with Ovsynch and Ovsynch+CIDR protocols was similar. The difference in the interval between treatment and onset of estrus between groups was 4 h (P<0.05) with the earlier onset in buffaloes synchronized with Ovsynch+CIDR.The difference in the duration of estrusin buffaloes synchronized was 2.5h between these two protocols but it was shorter in Ovsynch.The intensity of estrus was intense in 60.00 and 80.00% and intermediate in 20.00 and 20.00%with Ovsynch and Ovsynch+CIDR, respectively and none of the buffaloes exhibited weak estrus with Ovsynch+ CIDR but 20.00%buffaloes exhibited weak estruswith Ovsynch(Table No. 1 and Fig. 1).

Similarity in estrus response with Ovsynch and Ovsynch+CIDR might be due to small group of animals in the present study and also the ability of progesterone produced either by the luteinized follicle or corpus luteum developed after first dose of GnRH to induce cyclicity (Lamb *et al.,* 2007) and action of progesterone on the ovary to increase LH secretion for better follicular development (Kundulkar*et al.,* 2016) in both the groups. *I*n addition, $PGF_{2\alpha}$ on day 7 might have induced luteolysis leading to faster serum progesterone drop (Bakr *et al.,* 2015), increase in the concentration of estradiol (Taponen*et al.,* 1999) and optimal LH surge (Bakr *et al.,* 2015) for estrus response.Still the estrus response with Ovsynch in this study is lower than the findings of Vikash *et al.* (2016) and Mujawar *et al.* (2019) (100%) and with Ovsynch+CIDRKumar *et al.* (2015) (100%) and Vikash *et al.* (2016) (95.74%).It might be due to small, less estrogenic dominant follicle (Brantmeier *et al.,* 1987), age and stage of oestrous cycle (Vasconcelos *et al.,* 1999) and also possibly be due to variation in the breed of animal, environmental conditions, managemental factors and plane of nutrition.

The mean time taken for onset of estrusafter synchronization was insignificantly earlier with Ovsynch+CIDR compared to Ovsynch. But in contrast with the present findings,Azawi *et al.* (2012b) observed earlier onset with Ovsynch protocol.The mean duration of estrusbuffaloes synchronized with Ovsynch in the present study is nearly similar to the mean durations recorded by Dhami *et al.* (2015) (64.13±1.33 h) and Mujawar *et al.* (2019) (66.62±5.23 h) in post-partum anestrus buffaloes. While, Kumar *et al*. (2015) (48.50±5.17 h) recorded lower duration than the present values of Ovsynch protocol. However, Dhami *et al.* (2014) (75.17±4.75 h) and Buhecha *et al.* (2016) (70.60±1.30 h) reported higher values with Ovsynch protocol.Same with Ovsynch+CIDR in the present study is nearly similar to the mean durations recorded by Kumar *et al*. (2015) (54.5±2.91 h) and in contrast, Naseer *et al*. (2012) (64±12.3 h) reported higher values.

Insignificantly higher mean duration of estrus (23.4±0.80 h) with Ovsynch+CIDR observed in the present investigationmight be due to the variation of breeds, number of postpartum days and different methods used to record these observations.

The intense estrus observed with Ovsynch+CIDR is comparable with the report of Murugavel *et al. (*2010) (63.64%). In contrast, Kalaswa *et al.* (2017) and Mujawar *et al.* (2019) recorded 40.00 and 12.50 % of intense estrus, respectively with Ovsynch protocol. The variations in the intensity of estrusin various studies might be due to variation in the methods of scoring, age, breed of the animal and climate. Higher intensity of estrus in CIDR-based regimen might be due to the priming of hypothalamo hypophyseal pituitary gonadal (HPG) axis with adequate amounts of progesterone is beneficial for the recovery of HPG axis function at induced-estrus (De Rensis *et al*. 2005).

The mean serum progesterone concentrations were the highest on the day of $PGF_{2\alpha}$ administration and lower on the day of AI in Ovsynch+CIDR synchronized buffaloes. The difference in the concentration during all the days of synchronization between Ovsynch and

Ovsynch+CIDR groups was not significant (P>0.05). But, the same at the time of initiation of treatment, on the day of PG administration and on the day of insemination was insignificantly (P>0.05) higher in ovsynch+CIDR Group buffaloes. While, the difference within buffaloes synchronized with each protocol was significant (P<0.05). The presence of higher progesterone on day7 in the CIDR-based regimen compared to ovsynch group of buffaloes could be due to supplementary contributions from the CIDR despite the release of progesterone from the CL (Ghuman *et al.,* 2012) (Table No. 2 and 3).

All the buffaloes were timely inseminated and the first service conception rate was higher in buffaloes synchronized with Ovsynch+CIDR and second service conception rate wassimilarin buffaloes synchronized with both protocols. The overall conception rate was higher in Ovsynch+CIDRgroup when compared to Ovsynch group. Postpartum anestrus usually might be due to absence of optimal LH surge and inhibition of progesterone and prolactin on LH secretion (Bakr, 2015). In the present study, the Ovsynch+CIDR might have induced estrus,wherein GnRH might have mediated the return of optimal LH level to normal and CIDR would have induced –ve feedback at both pituitary and ovarian levels (Bakr, 2015) and removal of CIDR would have prohibited progesterone –ve feedback for more luteal activity leading to the initiation of ovarian activity after PGf*2α* administration. After 2nd GnRH this in turn lead to a sort of ovarian estrous cyclicity and heat induction mechanism (Bakr, 2015) with provision of favourable conditions for follicular growth and enhanced production of estrogens (Garcia-Winder *et al.,* 1986) for LH surge (Table No. 1).

Conception rates at induced estrus with Ovsynch protocol in the present study is nearly similar to the findings of Dhami *et al.* (2015) (50%). In contrast, Vikash *et al.* (2016) (61.7%) recorded higher conception rates at induced estrus with Ovsynch protocol. While, lower conception rates were also recorded by Kumar *et al.* (2015) (16.66%) and Buhecha *et al.* (2016) (33.33%) with ovsynch protocol compared to present study.The overall conception rate of 57.14% achieved with Ovsynch protocol in postpartum anestrus buffaloes in the present study is in agreement with the findings of Buhecha*et al.* (2016) (58.33%) and Prasad *et al.* (2019) (60%). However, Gupta *et al.* 2015) (40%) and Kumar *et al.* (2015) (33.33%) observed lower conception rate than the present study. This conception rates obtained with Ovsynch might be due to initiation of new wave of follicle (Twagiramungu *et al*., 1994) followed by emergence and selection of a dominant follicle that becomes preovulatory (Savio *et al*., 1993).

The higher conception rate observed in the present study with Ovsynch+CIDR might be due to reduced problem of premature follicular maturation and ovulatory problems by increasing the progesterone concentration through CIDR until $PGF_{2\alpha}$. In addition it would have adjusted the period of luteolysis and ovulation through CIDR over ovsynch protocol (Stevenson *et al.,* 2006). Further, supplementation of progesterone might have caused release of more LH after GnRH resulting in increased ovulatory response and in turn conception rates by concentrations of estradiol -17 beta (Thompson *et al.,* 1999) and by reducing incidence of persistence of follicle (Martinez *et al.,* 2001).

Reduced conception rate with Ovsynchin the present study might be due to premature follicular maturation before 2nd GnRH, ovulation before TAI (Moreira *et al.,* 2000) and inability of 1st GnRH to turn over the dominant follicle leading to premature estrus (Geary *et al.,* 2000). In addition, failure of dominant follicle present at the time of second GnRH treatment to express LH receptors leading to failure of ovulation suggesting that GnRH did not induce ovulation due to asynchronous wave emergence and/or a small dominant follicle without LH receptors (Colazo *et al.,* 2004). Finally, it is concluded that to synchronize postpartum anestrus buffaloes Ovsynch+CIDR protocol is able to produce functional dominant ovulatory follicle leading to the enhanced conception rate over Ovsynch protocol.

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Table No 1: Mean (±SE) estrus response, time of onset, duration & intensity of estrus and conception rate in Post-partum Anestrus Buffaloes synchronized with Ovsynch and Ovsynch+ CIDR

Parameter	Ovsynch N=5	Ovsynch + CIDR N=4	t – value / Chi-square value
Estrus response (%)	71.43 (5/7)	71.43 (5/7)	0.14^{NS}
No response (%)	28.57 (2/7)	28.57 (2/7)	
Interval between treatment and onset of estrus (h)	62.40±1.21	57.25±1.25	2.65^{NS}
Duration of estrus	18.80±0.66	20.25±1.75	-1.04^{NS}
Intensity of estrus			
Intense (%)	60.00 (3/5)	80.00 (4/5)	
Intermediate (%)	20.00 (1/5)	20.00 (1/5)	
Weak (%)	20.00 (1/5)	0 (0)	
Conception rate			
First Insemination (%)	42.86 (3/7)	57.14 (4/7)	
Second Insemination (%)	14.29 (1/7)	14.29 (1/7)	
Overall conception (%)	57.14 (4/7)	71.43 (5/7)	

Fig. 1: Comparative estrus response and conception rate in post-partum anestrus buffaloes synchronized with Ovsynch and Ovsynch+CIDR protocols

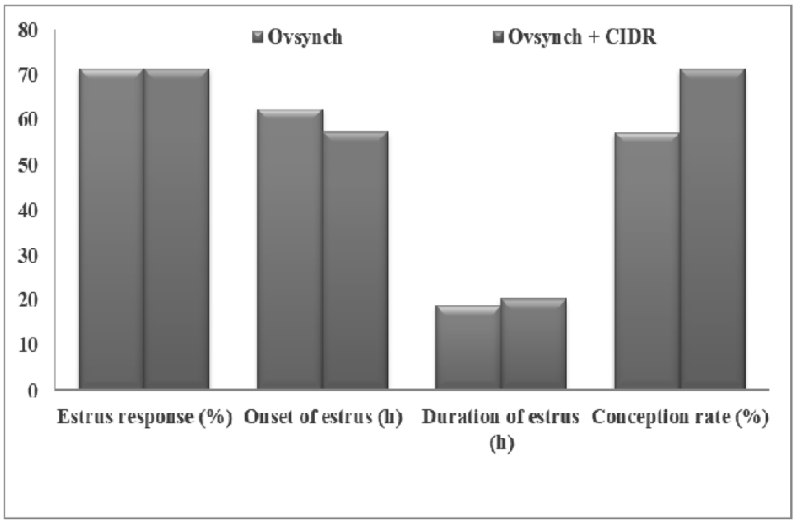

Table No 2: Progesterone (ng/ml) concentrations in Post-partum anestrus Buffaloes synchronized with Ovsynch and Ovsynch+ CIDR

S.No	Parameters	Day of treatment	Group I	Group II	T test value
1	Progesterone (ng/ml)	0 Day (n = 7)	0.74 ± 0.50^{aA}	0.01 ± 0.01^{aA}	1.48^{NS}
		7 Days (n = 7)	3.54 ± 0.47^{bA}	4.33 ± 0.74^{bA}	-0.85^{NS}
		10 Days (n = 7)	1.17 ± 0.47^{aA}	0.95 ± 0.74^{aA}	-0.22^{NS}
		Overall(n=3x7:21)	1.81 ± 0.38^{A}	1.76 ± 0.53^{A}	

Means bearing different superscripts (a, b) within a column differ significantly P≤ 0.05

Means bearing different superscripts (A, B) within a row differ significantly P ≤ 0.05

Table No 3 : Analysis of variance of Progesterone (ng/ml) in Post-partum anestrus Buffaloes synchronized with Ovsynch and Ovsynch+ CIDR

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Value
Between Groups	1	0.03	0.03	0.01^{NS}
Error	36	239790.60	6660.85	
Within Group 1	2	31.82	15.91	27.81*
Error	20	60.47		
Within Group 2	2	72.16	36.08	14.02*
Error	20	118.50		

*: Significant (P≤0.05) NS: Non-significant (P≥0.05)