EVALUATION OF OVSYNCH PROTOCOL ON REPRODUCTIVE PERFORMANCE OF ANESTROUS BUFFALOES DURING BREEDING SEASON IN CHITWAN, NEPAL

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ABSTRACT

This study evaluated the effectiveness of Ovsynch/FTAI protocol (day 0 fertirelin inj. 100µg I/M, day 7 cloprostenol inj. 500µg I/M, day 9 fertirelin inj. 100µg I/M, and day 10 fixed time artificial insemination 18 hours after second injection of fertirelin) in anestrous buffaloes of Chitwan, Nepal (n=14). Protocol was evaluated in terms of estrus characteristics, ovulation and pregnancy rate. Responses were analyzed by grouping the buffaloes based on type of anestrus, length of anestrus, body condition score (BCS), milking status, parity, age, management condition, follicle count on day of second GnRH treatment and site of semen deposition. Intensity of estrus characteristics on day of AI was mild. Major estrus signs observed were uterine tonicity, alertness, mucus discharge on rectal manipulation of internal genitalia, swelling of vulva and teat engorgement. Mucus discharge on rectal manipulation of internal genitalia and teat engorgement were clearly noticed in 50 % buffaloes. There was higher tendency (P=0.08) of estrus expression by buffalos with anestrous period of 10 months or lower compared to those with anestrous period longer than 10 months. Overall ovulation rate was 85.7%. Pregnancy rate to FTAI was 28.6% (22.2% and 40% in true anestrous and silent estrus buffaloes). 64.3% (66.7% and 60% of true anestrous and silent estrus) buffaloes were pregnant when checked at 102 days of FTAI including subsequent breeding. Although Ovsynch/FTAI seems to be alternative breeding technique in silent estrus buffaloes, further study with increased sample size is recommended. This protocol can potentially be used for resumption of ovarian cyclicity in true anestrus buffaloes.

Keywords: cloprostenol, FTAI, ovisynch, anestrus

INTRODUCTION

Buffaloes contribute over 68% to the total milk production of Nepal, and over 58% to country's total meat production (Government of Nepal, 2014). Nepal ranked fourth in buffalo population and fifth in buffalo milk and meat production worldwide, worth 942 million USD as of 2013 Production, 2015. Infertility in buffalo was mostly due to anestrous condition (Devkota, Bohora and Yamagishi, 2012). True anestrous and silent estrous conditions were prevalent among Nepalese anestrous buffaloes (Sah & Nakao, 2010). After the work of Pursley *et al.,* in 1995, GnRH based protocol of ovulation synchronization become established as Ovsynch and widely used in dairy industry for timed AI of lactating dairy cow (Wiltbank & Pursley, 2014) which is schematically represented in (Fig 1).

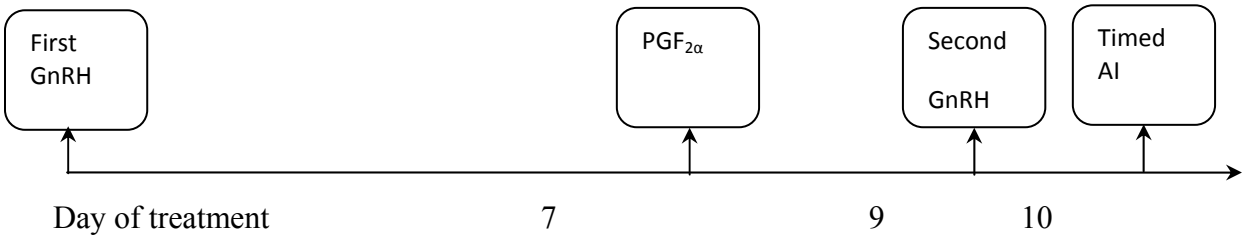

Fig 1. Schematic representation of Ovsynch protocol

Fixed time AI programs like Ovsynch without the need for estrus detection may be introduced in buffalo. There are few studies of Ovsynch protocol in different countries for fixed time insemination of buffaloes. There are scarce reports evaluating Ovsynch protocol in anestrous buffaloes during breeding season.In a study with 111 buffaloes, pregnancy rate of 36% was obtained when AI was performed 18 and 42 hours of second GnRH injection in Ovsynch protocol (Neglia *et al*., 2003). Neglia *et al*., (2003) rarely found estrus signs in Ovsynch treated buffaloes.Ovsynch protocol tested in 62 cyclic and 21 non cyclic buffaloes resulted in conception rate of 43.7% and 17% respectively (De Rensis *et al*., 2005).

Paul and Prakash (2005) showed that 9 out of 10 non-lactating cyclic buffaloes ovulated. Ovsynch protocol is more effective in multiparous buffaloes than in primiparous ones (De Araujo Berber, Madureira and Baruselli, 2002). De Araujo Berber *et al*., (2002) recorded the ovulation rate after second GnRH to be 93.3%.In a study designed to know the effect of parity to the efficacy of Ovsynch protocol in buffalo in Egypt, all 8 heifers ovulated to second GnRH whereas only 88.8% parous buffaloes ovulated out of 9 (Derar *et al.,*2012). Conception rate of heifers was 62.5% out of 686 and that of parous buffaloes was 22.7% out of 273 buffalo cows (Derar *et al*., 2012). Results obtained in study of Derar *et al*., (2012) showed higher count of follicles in heifers compared to buffalo cows.

This protocol narrows down the window of ovulation and able to achieve the pregnancy rate of 66.7% in subestrous animals (Brar & Nanda, 2010) when pregnancy was checked 90 days including two subsequent breedings. All of anestrus buffalo heifers achieved ovulation but with poor conception rate of 18 percent (Ghuman*et al.,* 2009). Pregnancy rates of 20% at first AI and 60% upto third AI were obtained in applying Ovsynch protocol to 15 anestrous Murrah graded primiparous buffaloes (Malik *et al*., 2011). Malik *et al*., (2011) used 500 IU of eCG 3 days before first GnRH injection of the Ovsynch protocol and they named it Ovsynch Plus. All buffaloes were true anestrus animals in the study of recorded estrous signs like uterine tone and mucus discharge only in 40% of the animalMalik *et al*.,(2011).

In a study with 8 and 11 Nili Ravi buffaloes during peak and low breeding season ovulation rates of 87.5% and 36.36% respectively were reported as determined by LH peak and plasma progesterone rise using ELISA (Jabeen *et al*., 2013). Jabeen *et al*., (2013) included all parous buffaloes in their study. In a study with 10 cyclic lactating Nili Ravi

buffaloes, estrus expression rate of modified Ovsynch protocol was found to be 30% and conception rate was found to be 40% (Ullah, 2013). Ullah modified the protocol by presynchronization with $PGF_{2\alpha}$ 13 days before starting Ovsynch and AI was done at the day of second GnRH.

In Nepal, seven Murrah buffaloes of IAAS livestock farm were treated with the protocol in December 1999 (Sah, 2000). Sah used 50 μg fertirelin and 25 mg $PGF_{2\alpha}$ for treatment. Sah found only one pregnancy out of seven buffaloes. Devkota *et al.,*2009 tested Ovsynch protocol in 5 lactating multiparous buffaloes of Institute of Agriculture and Animal Science livestock farm, Rampur, Chitwan during the breeding season. Devkota and Bohora confirmed ovulation by USG 24 hour after insemination. Devkota and Bohora reported ovulation in 40% of 5 animals and pregnancy in both ovulated buffaloes. There was absence of behavioral and physical estrus signs in Ovsynch treated buffaloes (Devkota *et al.,*2009).

This study was designed to evaluate Ovsynch protocol in anestrous buffaloes of Chitwan for FTAI in silent estrus buffaloes and for resumption of ovarian cyclicity in true anestrous buffaloes. This study evaluates the protocol based on estrus characteristics, ovulation rate, and pregnancy rate.We did not know the quality of semen used for insemination. This study does not cover hormonal assay. Pregnancy loss or gain after 102 days of FTAI could not be incorporated in the study due to time limit.

MATERIALS AND METHODS

Study period and site

Ovsynch protocol was executed from 17 July 2015 to 26 July 2015 and study was continued till November 6, 2015 at AFU livestock farm of Rampur, Sharadanagar VDC and private farms of adjoining Shibanagar VDC of Chitwan district.

Maximum and minimum temperature during the study period recorded from 17 July, 2015 to 6 September, 2015, humidity percentage during July, and daily average hours of bright sunshine during July averaged 33.7 ± 1.0^0C, 26.5 ± 1.0^0C, 83.3±6.7, and 5.0±3.2 respectively in our study site Rampur (Department of hydrology and meteorology, 2015). The study sites in surrounding areas of Rampur are supposed to have similar agro climate. Treatment period is of decreasing day length, day length being slightly longer than the night. This agro-climate is representative of southern part of Nepal.

Animals

All six buffaloes in AFU livestock farm had passed at least eight months from the date of parturition without breeding. Buffaloes were kept in single row tie-stall, weaned calves being isolated. Buffaloes in Shivanagar area were either heifers or passed at least 9 months from parturition without any detectable heat signs. Farmers of Shivanagar area kept their buffalos in traditionally built sheds adjacent to their houses.In this study, the buffaloes were maintained in their routine management except FTAI after Ovsynch and followed them upto 102 days after AI.

MATERIALS

Ultrasonography Machine

Realtime B-mode diagnostic convex ultrasound scanner (HS-1500, Honda electronics, Japan) with multi frequency (5-7.5-10.0 MHz) rectal probe (HLV-375M) was used.

Hormones

Consultan injection 10 ml (a product of ASKA Japan): Each ml of Consultan injection contains 50 microgram fertirelin and Zenoadin C 20 ml (a product of ZENOAQ, Japan): Each ml of Zenoadin C contains 0.250 mg cloprostenol.

METHODS

Case classification

Fourteen anestrous buffaloes were categorized into 2 groups as shown in (Fig. 2). On rectal palpation and per rectal ultrasonographic examinations on first day of treatment, all animals had normal tubular genitalia. Buffaloes were classified into following two types based on the structures of ovary.

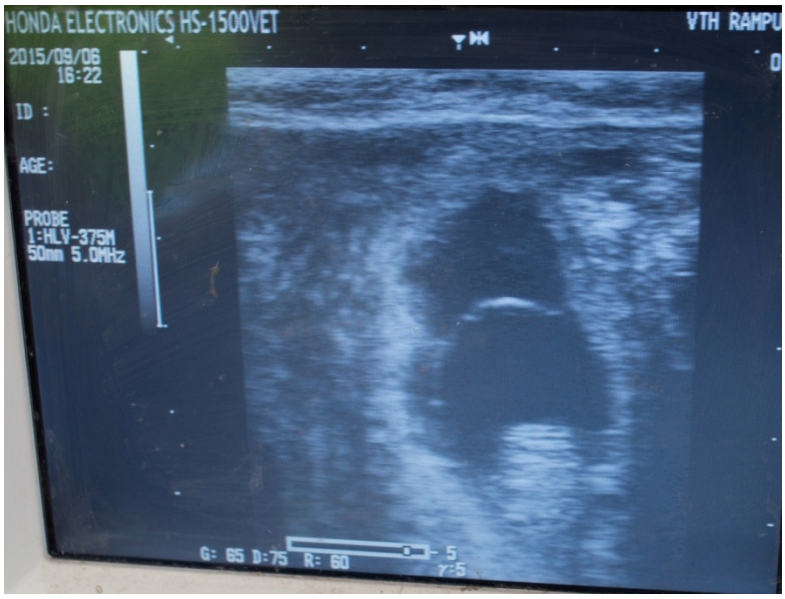

Fig 2: Embryo with amniotic and allantoic vesicles

- **True anestrus**. Buffaloes without detectable corpus luteumand follicles larger than 5 mm.
- **Subestrus (silent estrus)**. Buffaloes with large follicles and detectable CL.

Ovsynch treatment

2 ml Consultan was injected I/M on July 17 of 2015 for the first time. Zenoadin C was injected 2ml I/M on July 24. Second shot of Consultan was given 2 ml I/M on July 26.

Recording of estrus characteristics at the time of FTAI

Estrus characteristics were recorded at the time of AI based on palpation of tubular genitalia rectally, external visual observation and history taking with animal attendants.

Fixed time artificial insemination at tenth day of Ovsynch protocol

Artificial insemination was done 18 hours after second injection of Consultan using frozen semen of Murrah breed from regular government supply.

Ovulation detection based on palpable CL on ovary on tenth day of AI

On tenth day of AI, ovaries were palpated per rectal for detection of any palpable CL (Amer & Mahdi, 2008) for confirmation of ovulation. Animals were then classified as ovulated and non-ovulated.

Breeding of animals in subsequent estrus

Natural breeding was allowed in subsequent natural estruses.Pregnancy diagnosis at 41 days of AI and at 102 days of AI including breeding of subsequent natural estruses**.** Pregnancy was diagnosed at 41 days of AI at 102 days of AI by rectal palpation and ultrasonographic scanning using 7.5 MHz rectal probe.

Statistical analyses

Independent-Samples T test assuming equal variances and Fisher's exact test in IBM SPSS Statistics version 21 were utilized to compare means of follicles in different group of animals and for testing independence of qualitative responses to factors affecting them respectively. Microsoft Office Excel was used for making bar diagrams.

RESULTS

Overall response of Ovsynch protocol

Response rates in true and silent estrus animals are summarized in (Table 1). Statistically significant difference was not found in responses of true and silent anestrous buffaloes.

Table 1: Overall response rate of Ovsynch protocol

Response indicator	Response rate (%)		P value	Overall Response rate (%)
	True anestrus	Silent anestrus		
Estrus Expression rate	55.6	40.0	1.00	50.0
Ovulation rate	77.8	100.0	0.50	85.7
Pregnancy rate at 41 days	22.2	40.0	0.58	28.6
Pregnancy rate at 102 days	66.7	60.0	1.00	64.3

Estrus characteristics

Since primary signs of estrus were absent and most of secondary signs were also not intense enough to describe, we may say that there was absence of characteristic estrus signs or estrus signs were subnormal in response to Ovsynch treatment. However, most frequent sign found were uterine tonicity and alertness of the animal. Other signs observed

include mucus discharge on internal genital manipulation during rectal palpation, swelling of vulva, teat engorgement, frequent urination, red color of vulva, and off-feed condition.

Although we did not find any statistically significant different response between groups of animals, the tendency of higher estrus response was observed in buffaloes having 10 months or shorter anestrous length (P value 0.08).

Ovulation rate

Ovulation rate was 85.7%. Ovulation response was not significantly different between groups of animals. Result is summarized in (Table 2).

Table 2: Ovulation rate as confirmed by detection of CL on tenth day of FTAI

Basis of categorization	Category	Number of animal	Ovulation rate (%)	P value
Type of anestrous condition	True	9	77.8	0.50
	Silent	5	100.0	
Length of anestrous period	Upto 10 months	5	100.0	1.00
	Above 10 months	6	83.3	
BCS	< 3	6	100.0	0.47
	≥3	8	75.0	
Milking status	Lactating	8	100.0	0.17
	Non-lactating	6	66.7	
Parity	Heifer	3	66.7	0.40
	Parous	11	90.9	
Age	Young	9	88.9	1.00
	Old	5	80.0	
Management condition	University mgmt.	6	83.3	1.00
	Farmers mgmt.	8	87.5	
Total		14	85.7	

Pregnancy rate

Pregnancy rate to FTAI checked at day 41 of FTAI was 28.6% without statistically significant difference between groups of animals. Pregnancy rate to FTAI and subsequent breeding diagnosed at day 102 of FTAI was found to be 64.3% without statistically significant difference between groups of animals. Among the ovulated buffaloes, 33.3 Ü were pregnant to FTAI. Result is summarized in (Table 3).

DISCUSSION

Results of this study showed the effect of Ovsynch/FTAI treatment in anestrous buffaloes having at least 8 months of anestrous period. As Neglia *et al*., (2003) and Devkota and Bohora (2009) mentioned, intense estrus signs were rare in the present study too. Estrus signs in 55.6% and 40% of true anestrous and silent estrous buffaloes in the present study are comparable with 40% result of Malik *et al*., (2011) in true anestrous animals and 30% estrus expression rate found by Ullah (2013) in silent buffaloes.

Table 3: Pregnancy rate on 41 and 102 days of FTAI

Basis of categorization	Category	Number of Animal	Pregnancy rate			
			41 day		102 day	
			%	P value	%	P value
Type of anestrous condition	True	9	22.2	0.58	66.7	1.00
	Silent	5	40.0		60.0	
Length of anestrous period	Upto 10 months	5	20.0	1.00	80.0	0.55
	Above 10 months	6	33.3		50.0	
BCS	< 3	6	33.3	1.00	50.0	0.58
	≥3	8	25		75.0	
Milking status	Lactating	8	37.5	0.58	75	0.58
	Non-lactating	6	16.7		50	
Parity	Heifer	3	33.3	1.00	66.7	1.00
	Parous	11	27.3		63.6	
Age	Young	9	33.3	1.00	77.8	0.27
	Old	5	20.0		40.0	
Management condition	University mgmt.	6	16.7	0.58	50.0	0.58
	Farmers mgmt.	8	37.5		75.0	
Follicle count	<12	7	14.3	0.56	85.7	0.27
	≥12	7	42.9		42.9	
Site of semen deposition	Body of uterus	9	33.3	1.00	66.7	1.
	Cervix	5	20.0		60.0	
Total		14	28.6		64.3	

Ovulation rate was 85.7% as determined by detection of palpable CL on rectal palpation at least in any one ovary on tenth day of FTAI. Ninety percent ovulation rate in the study of Paul and Prakash (2005) with 10 non-lactating buffaloes is higher than 66.7% in non-lactating group of this study. This may be because of cyclicity in all buffaloes in their study. 93.3% ovulation rate in the study of De Araujo et al. in 2002 is closer to result of this study. In Heifers, Ghuman *et al*., (2009) reported 100% ovulation rate which is higher than our finding of 66.7% in heifers. In the present study a heifer aged 2 years was unable to ovulate among 3 heifers included in the study. So, ovulation rate decreased to be 66.7% in the present result. 87.5% ovulation rate obtained by Jabeen *et al*., (2013) is almost similar to 90.9% ovulation rate of parous buffaloes in this study.

Pregnancy rate of buffalo was found to be 28.6% at FTAI after Ovsynch while scanned by ultrasonography at day 41 of FTAI. Thirty six percent conception rate obtained by Neglia *et al*., (2003) is closer to our study. 17% conception rate found in a study of De Rensis *et al*., (2005) with 21 non cyclic buffaloes, and 20% in the study of Malik *et al*., (2011) with 15 true anestrus buffaloes is closer to our result 22.2% of our true anestrous buffaloes.

Pregnancy rate of silent buffaloes under this study was 40% and was equal to that obtained by Ullah (2013) and was congruent with 43.7% of De Rensis *et al., (2005)*Lower pregnancy rate of 14.28% obtained by Sah (2000) may be due to lower dose of fertirelin used.Forty percent pregnancy rate in lactating buffaloes obtained by Devkota *et al.,*(2009) is similar to 37.5% in lactating buffaloes of this study.

In the study including heifers and parous buffaloes to find out effect of parity on conception rates were higher (62.5%)in heifers as per the findings of present study (33.3%)conception rate of heifers was in the study by Derar *et al*.,(2012). Selection of cyclic heifers in their study might have increased the conception rates. Furthermore there might be some effect of sample size as this study had only 3 heifers included. Conception rate of 22.7% in parous buffaloes in the study of Derar *et al*.,(2012) which was similar to the findings of present study (27.3%).

Pregnancy rate was found to be 64.3% as examined at 102 days of FTAI which was the result of FTAI and subsequent natural breeding. Pregnancy rate at 102 days of FTAI including subsequent breeding in present study was found to be 60% in silent estrous buffaloes which is comparable with pregnacy rate of 66.7% obtained in the study of Brar and Nanda (2010). Sixty percent pregnancy rates of upto third AI in true anestrus animals reported by Malik *et al*., (2011) is also closer to the result of 66.7% in true anestrus animals. Pregnancy rates of 20% at first AI and 60% up to third AI as obtained by Malik *et al*., (2011) in anestrous buffaloes are like the results of this study.

CONCLUSION

Estrus signs expressed at the day of FTAI were mild. Major estrus signs were uterine tonicity, alertness, mucus discharge on rectal manipulation of internal genitalia, swelling of vulva and teat engorgement. Tendency of estrus expression was higher in buffaloes with 10 months or shorter anestrous period compared to those having longer anestrous period. Ovulation induction in 85.7% buffaloes indicates potential use of protocol for fixed time insemination. Conception rate of forty percent in silent estrus buffaloes are closer to national figures of conception rate to conventional breeding method. Ovsynch protocol with FTAI can be used in silent estrus buffaloes. Based on pregnancy rates of 66.7% in anestrous buffaloes within 102 days of FTAI, it may be concluded that the protocol causes good resumption of cyclicity in true anestrous buffaloes.

There was no statistically significant difference in the response rates of the protocol in heifers and parous buffaloes. This showed the prospects of using the protocol in heifers to shorten the age at first calving. So, it is recommended to further evaluate Ovsynch protocol in heifers with appropriate sample size as only 3 heifers were included in this study.

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