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# Comparative analysis of two protocols (Ovsynch and double synch) in postpartum cyclical and anoestrus Buffaloes

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#### Abstract

Present study was conducted to compare the efficacy of Ovsynch and double synch protocols on postpartum cyclic and anoestrus buffaloes during the October-January month. Buffaloes belonging farmers in nearby dairy farm and local region of Bharatpur district. 30 buffaloes with parity of 2<sup>nd</sup> to 5<sup>th</sup> were selected to induce synchronized estrus/estrus using ovsynch and double synch protocols. These animals were randomly divided into two groups. Group I buffaloes (n=15) were treated with Ovsynch protocol on day0 and day9, 10µg Buserelin acetate), day7, 500µg cloprostenol sodium (PGF2a) i/m and Group II: In double synch protocol (n=15), buffaloes were treated with 500µg cloprostenol sodium  $(PGF2\alpha)$  on Day 0, 10µg Buserelin acetate (GnRH) on Day 2, a second PGF2 $\alpha$  on Day 9, and a second GnRH on Day 11. Timed artificial insemination (TAI) was carried out 16-20 hours following the second GnRH in both treatment groups. Buffaloes were routinely observed through rectal examination. Samples of cervico-vaginal mucous were taken directly from the animal's cervix by using cytobrush at the time of artificial insemination. Under a microscope, the cervical mucus of buffaloes was examined for either a typical or atypical fern pattern. A higher chance of conception has been observed in animals with typical fern patterns. Visible behavioral changes and estrus signs i.e. bellowing, mounting, quality and consistency of cervical mucus discharge, pinkish vaginal mucosa and duration of estrus was observed in both groups of buffaloes. During the breeding season, there was a greater estrus response (100%) in double-synch treated buffaloes compared to ovsynch protocol (86.66%) in both farm and field conditions. Behavioral changes and estrus signs were observed in 26.66% (4/15) and 40% (6/15) of buffaloes in Group I and Group II respectively. Group II had a greater conception rate of 60% (9/15) than Group I had a lower rate of 33.3% (5/15). Our findings corroborate and bolster earlier reports that the Double synch treatment increases the Conception rates in postpartum cyclical and anoestrus buffaloes. Additionally, our data show that the Double synch approach increases conception rates. These data thus imply that, independent of the period of the estrous cycle, the Double synch protocol can be employed to achieve satisfactory conception rates following TAI in both anestrous and cycling in buffaloes.

Keywords: Anoestrus buffaloes, Ovsynch, Double synch, Timed artificial insemination (TAI), Fern pattern

## Introduction

In India, buffaloes are important to the economy and livestock production; they produce more than half of the country's milk. However, their low reproductive efficiency limits their output. Buffaloes have reproductive problems such as delayed puberty, older first calving ages, postpartum anoestrous, silent heat, seasonal breeding, and low conception rates (Terzano *et al.* 2012)<sup>[7]</sup>. The success rate of artificial insemination (AI) in buffaloes is limited by variations in the duration of the estrus cycle, which range from 17 to 26 days with a mean of approximately 21 days (Thakur *et al.*, 2013)<sup>[27]</sup> and variable duration of estrus from 4 to 64 hours (Baruselli *et al.*, 2001)<sup>[28]</sup>. Additionally, the challenge of accurately predicting the time of ovulation is another factor. The most common, challenging and difficult problem that field veterinarians deal with in buffaloes are postpartum anoestrous which significantly reduces the animal's capacity for reproduction at the field level (Das and Khan, 2010)<sup>[5]</sup>. It has been discovered that different forms of anoestrous in buffaloes are related to lower levels of nutrients, such as macro and micro minerals (Abou-Zeina *et al.*, 2009)<sup>[1]</sup>.

Buffaloes tend to have weak estrus expression during the summer, when breeding conditions are unfavorable, and enhanced sexual activity during the winter, when breeding conditions are most favorable (Madan et al., 1996; Singh et al., 2000) [8, 9]. Different techniques for synchronizing ovulation have been devised that can precisely synchronize ovulation within a specific period of time, eliminating the need for heat detection that can lead to variable conception rates. To synchronize follicular development, luteal regression, and ovulation, the Ovsynch protocol was developed in 1995. This milestone protocol for estrus synchronization occupies a sequence of GnRH-PGF2a-GnRH injections following this, the AI can be performed at a fixed time without the need for estrus detection (Pursley et al., 1995)<sup>[11]</sup>. A lower pregnancy rate was seen when the ovsynch protocol was used between days 13 and 17 or early in the estrous cycle (days 2-4) (Moreira et al., 2000 and Vasconcelos *et al.*, 1999) [12, 13]. The stage of the estrous cycle at which the Ovsynch protocol is initiated largely affects the protocol's success rate (Galina and Orihuela, 2007)<sup>[14]</sup>. A novel estrus synchronization technique called Double synch has been proposed to overcome this problem. It involves administering an additional PGF2a injection 48 hours prior to the start of Ovsynch protocol, which has the benefit of being applicable regardless of the stage of the estrous cycle (Cirit et al., 2007)<sup>[15]</sup>. The aim of the current study was to evaluate the effectiveness of the Double PG and Ovsynch protocols in anoestrous buffaloes.

## **Materials and Methods**

The present study was conducted on buffaloes located nearby dairy farm and local region of Bharatpur district. 30 buffaloes with parity of 2<sup>nd</sup> to 5<sup>th</sup> were selected to induce synchronized estrus/estrus using ovsynch and double synch protocols. 60-90 days postpartum cyclical anoestrous buffaloes were selected and divided into two groups randomly based on their ovarian status. Group I buffaloes (n=15) were treated with ovsynch protocol on day0 and day 9, 10µg Buserelin acetate (GnRH), day 7, 500µg cloprostenol sodium (PGF2a) intramuscularly and Group II: In double synch protocol (n=15), buffaloes were treated with 500µg cloprostenol sodium (PGF2a) on Day 0, 10µg Buserelin acetate on Day 2, a second PGF2α on Day 9, and a second GnRH on Day 11. Timed artificial insemination (TAI) was carried out 16-20 hours following the second GnRH in both treatment groups. Buffaloes were routinely observed for rectal examination. Under a microscope, the cervical mucus of buffaloes was examined for either a typical or atypical fern pattern. Visible behavioral changes and estrus signs i.e. bellowing, mounting, quality and consistency of cervical mucus discharge, pinkish vaginal mucosa and duration of estrus was observed in both groups of buffaloes. During the breeding season, there was a greater estrus response (100%) in double-synch treated buffaloes compared to Ovsynch protocol (86.66%) in both farm and field conditions. Behavioral and estrus signs were observed in 26.66% (4/15) and 40% (6/15) of buffaloes in Group I and Group II respectively. Conception rates were higher in Group II of 60% (9/15) than in Group I of 33.33% (5/15) with fern pattern. Our findings corroborate and bolster earlier reports that the Double synch treatment increases the Conception rates in postpartum cyclical and anoestrus buffaloes. Additionally, our data show that the Double synch approach increases conception rates. These data thus imply that, independent of the period of the estrous cycle, the Double synch protocol can be employed to achieve satisfactory

conception rates following TAI in both anestrous and cycling in buffaloes.

 Table 1: Treatment protocol for postpartum cyclical and anestrous buffaloes

Treatment group	Dairy farm and field	Number of buffaloes
	Dairy farm	6
Ovsynch (Group I)	In village	9
	Total	15
Double synch (Group II)	Dairy farm	8
	In village	7
	Total	15

 
 Table 1: Parameters which were observed in group I and group II of buffaloes

S. No.	Particular		Percentage of animals	
			Group I	Group II
1.	Behavioral and estrus signs		26.66%	40%
2.	Quality & Consistency of cervical mucus	Hanging & transparent	75%	83%
		Slightly watery & translucent	25%	17%
4.	Estrus Response rate		86.66%	100%
5.	Conception rate		40%	60%

Following picture showed fern pattern during estrus in buffaloes

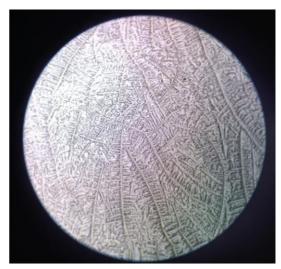


Fig 1: Typical fern pattern

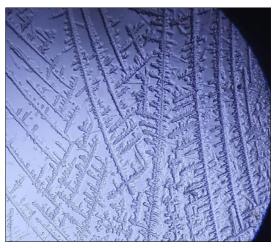


Fig 2: Atypical fern pattern

### **Results and Discussion**

During the breeding season, a greater estrus response (100%) in double-synch treated buffaloes compared to Ovsynch protocol (86.66%) in both farm and field conditions was observed which agreement with the findings of (Negalia et al. 2003; Bhoraniya et al., 2012; Ali et al., 2012)<sup>[21, 3, 2]</sup>. In farm buffaloes, they observed a corresponding estrus response rate (88.00%). During the breeding season, ovsynch treated buffaloes (100%) showed a stronger estrus response in both farm and field conditions, according to some recent research (Mujawar et al., 2019)<sup>[22]</sup>. Behavioral and estrus signs were observed in 26.66% (4/15) and 40% (6/15) of buffaloes in Group I and Group II respectively. Quality & Consistency were noted Hanging & transparent and slightly watery & translucent during estrus. Buffaloes with Hanging & transparent cervical mucus were observed 75% and 83% in group I and group II respectively. Animals with transparent mucus have a higher possibility of conception than those with translucent mucus. Cervical discharge has been described as nearly transparent or somewhat translucent, occurring between two days prior to estrus and one day following estrus (Hamana et al., 1971)<sup>[23]</sup> and as opaque during luteal phase (Elstein, 1974)<sup>[24]</sup>. Under a microscope, the cervical mucus of buffaloes was examined for either a typical or atypical fern pattern. A higher chance of conception has been observed in animals with typical fern patterns. Either late proestrus or early estrus was represented by the characteristic fern patterns. In order to increase the chance of conception, AI is typically recommended 12 to 18 hours following the onset of estrus in buffaloes (Hamid, M., 2019)<sup>[25]</sup>. At the peak of estrus, a larger percentage of buffaloes have been observed to attain a typical or complete fern pattern (Noonan et al., 1975) <sup>[26]</sup>. Conception rates were higher in Group II of 60% (9/15) than in Group I of 33.33% (5/15) in field condition. In low breeding season under field conditions, gonadotropin releasing hormone (GnRH), prostaglandins (PGF2a), and their many synthetic analogues have been utilized to synchronize estrus in cattle buffaloes (Khumran et al., 2012) <sup>[6]</sup>. This may result from differences in the monitoring of feeding and observation of heat-detection. Ovsynch treatment has been successfully used in swamp buffaloes for improved ovulation with acceptable fertility rates, In order to improve ovulation and achieve acceptable fertility rates in swamp buffaloes, ovsynch protocol have been utilized with success (Chaikhun et al., 2010)<sup>[4]</sup>. Mammals' reproductive success is greatly influenced by the cervix and its secretions (Hafez and Kanagawa, 1972; Matner, 1973)<sup>[16, 17]</sup>. The secretory cells of the endocervix continuously generate cervical mucus (CM), and its quantity and quality change according to the gonadal hormonal status of the estrus cycle (Elthohamy et al., 1990; Noonan et al., 1995; Tsiligianni et al., 2001) [18, 19, 20]. In the current study, buffaloes treated with the Double synch protocol had a greater conception rate in breeding season when compared to those treated with the Ovsynch protocol. This may have been caused by the PGF2 $\alpha$  injection, which was given 48 hours prior to the first GnRH. It may have worked alone or in collaboration with GnRH to partially or significantly overcome the factors that were responsible for the decline in ovulation and/or pregnancy rates in anestrous cows (Ozturk et al., 2010)<sup>[10]</sup>. The current study concludes that double synch is better protocol than ovsynch protocol in postpartum cyclical and anoestrous buffaloes.

#### Conclusion

The current study concludes that double synch is better protocol than Ovsynch protocol in postpartum cyclical and anoestrous buffaloes.

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