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Efficacy of Second Prostaglandin F2 Alpha Treatment during Ovsynch Protocol on Pregnancy Rate Inseminated with Sex-sorted Semen in Buffaloes

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ABSTRACT

Background: Ovsynch is one of the most adopted estrus synchronization protocols used for synchronization of ovulation and fixed-time artificial insemination however, new corpus luteum (CL) induced by the first GnRH treatment in Ovsynch is inappropriately regressed with a single treatment with prostaglandin F2α. Inadequate CL regression reduces fertility. Considering this rationale, present research was implemented with the effect of second prostaglandin F2 alpha treatment during Ovsynch protocol on pregnancy rate inseminated with sex-sorted semen in buffaloes.

Methods: Post-partum true anestrus multiparous buffaloes (n=24) were categorized as a group I (Ovsynch; n=12) and group II (Double PG Ovsynch; n=12). Buffaloes from group I were treated with standard Ovsynch protocol (D=7; G-P-G), while additional second prostaglandin F2 alpha injection was administered on day eighth (D=7; G-P-P-G) in group II (Double PG Ovsynch). All the buffaloes were inseminated 16-20 hrs after the last GnRH injection with sex-sorted semen.

Result: The estrus signs exhibited were numerically higher in group II than in group I. The first service pregnancy rate in double PG Ovsynch was higher than the Ovsynch group. The addition of a second prostaglandin F2 alpha injection in the Ovsynch protocol improves the estrus quality and increases pregnancy rate.

Key words: Buffalo, Ovsynch, Sex-sorted semen, Synchronization.

INTRODUCTION

India continues to be highest milk-producing country globally with 198.40 million tonnes. As per the 20th livestock census-2019, the buffalo population was 109.85 million and nearly 49% of the milk production is contributed by Indigenous and non-descript buffaloes (Annual Report DAHD, 2020-21).

The main constraint in buffalo farming is low reproductive efficiency, which mainly includes seasonality of estrus, long calving interval due post-partum, weak or silent estrus, lower conception rate through artificial insemination (Kundalkar *et al.* 2017). This affects not only total milk production of buffalo but also the calf crop in her reproductive life affecting the profitability of herd.

Ovsynch is one of the most adopted estrus synchronization protocols used for synchronization of ovulation and fixed time artificial insemination at field level. However, the new corpus luteum (CL) induced by the first GnRH treatment in the Ovsynch is inappropriately regressed with a single treatment with prostaglandin F2α is the major limitation of the Ovsynch protocol. Lack of complete luteolysis of the CL to the PGF2α treatment has been observed in 10 to 25% of cows treated with the Ovsynch protocol and due to inadequate CL regression, small elevations in circulating P4 near artificial insemination (AI) was recorded which reduces fertility (Martins *et al.* 2011). Administration of the second dose of PGF2α on a subsequent day after the first PGF treatment in the Ovsynch protocol overcomes the problem with inadequate CL regression. Carvalho *et al.* (2015) found that double PGF2α injection during the Ovsynch protocol increased percentage of cows with complete CL regression (<0.4 ng/mL 56 h after PGF) after two PGF2α treatment (95.6%) compared with single $PGF_2\alpha$ treatment (84.6%) and had better reproductive outcomes.

To meet the increasing demands of milk by 2030 (266.5 million MT) to India's ever-growing population, it is necessary to substantially increase the number of elite buffalo females as replacement heifers. By widespreaad use of sex-sorted semen in spontaneous and synchronized estrus in buffaloes could achieve this. For the application of sex-sorted semen in buffaloes, it would be of great interest when used in synchronized estrus with its effect on fertility. Due to this research gap, the effect of second dose of $PGF_2\alpha$ in the Ovsynch protocol on fertility and pregnancy rate inseminated

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with sex-sorted semen in synchronized buffaloes study was implemented.

MATERIALS AND METHODS

The study was conducted on graded Murrah buffaloes maintained at dairy farms during subtropical weather conditions from July to October 2021at Akola, Maharashtra. Lactating buffaloes (n=24) weighed between 400-450 kg, were selected having second to fourth parity with the history of non-exhibition of estrus signs sixty days post-partum. These buffaloes were allocated to two groups, *viz.* Ovsynch group (group 1; n=12) and double PG Ovsynch group (group II; n=12). In group I buffaloes, busereline acetate (GnRH) 10 µg i/m on day 0 and nine and cloprostenol sodium (PGF2α) 500 µg were administered on day 7. In group II buffaloes, additional injection of cloprostenol sodium 500 µg was administered on day 8. To assess the quality of estrus, different estrus signs like frequent micturition, congestion of vaginal mucus membrane, vaginal discharge and tumification of the vulva were noted by visual observations in both groups. The buffaloes from both groups were inseminated 16 h after second GnRH injection with sex-sorted semen by depositing thawed semen in body of uterus. The pregnancy diagnoses were carried by per rectal (P/R) examination in inseminated buffaloes after the sixty days. Statistical analysis was carried out by chi-square test using Web Agri Stats Package (WASP-2), ICAR, Goa.

RESULTS AND DISCUSSION

The comparative efficacy of Ovsynch and double PG Ovsynch protocols and pregnancy rate with sex-sorted semen is shown in Table 1. The lower pregnancy rate was observed in Ovsynch synchronized buffaloes compared to double PG Ovsynch synchronized buffaloes respectively. The results are non-significant at (p<0.05) between the groups.

The results of the present study for estrus exhibition or synchronization after the Ovsynch treatment (Group-I) are in agreement with results obtained by Navarange *et al.* (2011) Nakrani *et al*. (2014) and Savani *et al.* (2017) reported 100% estrus response with the Ovsynch protocol in buffaloes. The results of the present study for estrus exhibition or synchronization after double PG Ovsynch treatment (Group-II) are in concurrence with results obtained by Carvalho *et al.* (2015), Wiltbank *et al.* (2015), Borchardt *et al.* (2018), Nowicki *et al.* (2019), Rheinberger *et al.* (2020) and Tippenhauer *et al.* (2021) reported 100% estrus exhibition after double PG Ovsynch synchronization in buffaloes. The variation in the estrus response could be due to differences in parity, management practices, estrus detection methods, body score of animals, cyclic stage of animals and dose of agonist used in the synchronization protocols. The low estrus response and ovulation observed by some authors may be due to implementation of the protocol in non-breeding season or during summer season.

Estrus signs exhibited in Ovsynch and double PG Ovsynch protocols are shown in Table 2. Congestion of the vaginal mucus membrane was predominant sign shown by buffaloes in the Ovsynch and double PG Ovsynch groups.

In present study, the frequent micturition and congestion of vaginal mucus membrane signs shown were lesser than those reported by Sawant (2021) which might be due to the high milk production of buffaloes and signs reported in synchronized estrus. The variation in the estrual signs shown by buffaloes may be due to season, management practices of buffaloes, frequency of estrus detection, spontaneous or induced estrus and milk yield of animals.

In present study, a higher pregnancy rate was observed in double PG Ovsynch compared to the Ovsynch protocol. The results are non-significant (p<0.05) between the groups (Table 1). The pregnancy rate for sex-sorted semen in Ovsynch synchronization was in harmony with results reported by Shinde *et al.* (2022) as 40% pregnancy rate in sex-sorted and 50% in conventional groups treated with Ovsynch synchronization protocol in Gir cows. Carvalho *et al.* (2015) reported 32% and 37% pregnancy rates per artificial insemination (P/AI) in Ovsynch and double PG and reported cows treated with double PGF2á injections with lower P4 concentration on the day of TAI had better reproductive outcomes. Wiltbank *et al.* (2015) reported increased pregnancies (36.1 vs. 33.3%) per artificial insemination compared with cows with a single dose of PGF. They concluded second PGF treatment in the Ovsynch

Table 1: Comparative efficacy and pregnancy rate in Ovsynch and double PG Ovsynch protocols in buffaloes.

Groups	No. of buffaloes synchronized	No. of buffaloes exhibited estrus	No. of buffaloes became pregnant	Pregnancy rate (%)	Chi square value
Group-I (Ovsynch protocol)	12	12 (100%)	4	33.33^{NS}	0.178
Group-II (Double PG Ovsynch protocol)	12	12 (100%)	5	41.66^{NS}	

NS- Non-significant difference in pregnancy rate between the groups (P<0.05).

Table 2: Estrus signs exhibited in Ovsynch and double PG Ovsynch synchronization protocols in buffaloes.

Groups	No. of buffaloes exhibited estrus	Estrus signs exhibited by buffaloes			
		Tummification of vulval lips	Congestion of vaginal mucous membrane	Vaginal discharge	Frequent micturation
Group-I (Ovsynch)	12	4 (33.33%)	6 (50.00%)	3 (25.00%)	3 (25.00%)
Group-II (Double PG Ovsynch)	12	5 (41.66%)	7 (58.33%)	4 (33.33%)	4 (33.33%)

protocols could increase pregnancy success by about 10%, which is in accordance with present research findings.

Borchardt *et al.* (2018) observed that there was a clear benefit of an additional PGF2α treatment during the Ovsynch protocol on luteal regression (+11.6 percentage units) and on P/AI (+4.6 percentage units). Nowickiv *et al*. (2019) reported the pregnancy loss rate was lower in cows receiving two PGF2α treatments than in the control animals. Rheinberger *et al*. (2020) reported cconception rates that were 7% (95% CI 2%-12%) greater in double Ovsynch protocol than cows receiving the original Ovsynch program. After adjusting for age, days in milk at mating start date and herd, the odds of conception to FTAI was significantly greater for modified Ovsynch cows (OR = 1.36, 95% CI 1.12-1.66) compared with original Ovsynch cows. Borchardt *et al.* (2021) reported there was a clear benefit of an additional PGF2α treatment during the Ovsynch protocol on pregnancy per insemination despite additional costs for hormones and labor. This additional treatment with PGF2α on day eight was more profitable in 95% of all scenarios because of the associated increase in fertility, which is accordance with present result findings. Tippenhauer *et al.* (2021) observed pregnancy per artificial insemination (P/AI) was 31.8% (n=888) in the Ovsynch protocol and 40.3% (n=871) in GPPG protocol respectively and concluded that the addition of a second PGF treatment on day eight during seven day Ovsynch protocol increased P/AI compared to Ovsynch. The variation in the pregnancy rate may be a difference in parity, site of semen deposition, no. of sperms in frozen semen dose, AI technician, season, body condition score, managemental and nutritional conditions, exogenous supplementation of hormones after insemination and spontaneous or induced estrus.

CONCLUSION

The results acquired supported the hypothesis that double PG Ovsynch protocol showed better fertility and sex-sorted semen can be used in synchronized buffaloes with optimum pregnancy rate and should used at the field level.

Conflict of interest: None.

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