EFFECT OF TREATMENT WITH OR WITHOUT ESTRADIOL AFTER OVSYNCH PROTOCOLS AT TIMED AI ON THE PREGNANCY RATE IN LACTATING BUFFALOES

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ABSTRACT

The present study was conducted on forty lactating Nili-Ravi buffaloes to compare the reproductive performance by treating with GnRH and $PGF_{2\alpha}$ for synchronization of estrus, ovulation and pregnancy per artificial insemination with or without supplemental estradiol. All buffaloes received 25 mg $PGF_{2\alpha}$ at 51 days in milk (DIM) for pre-synchronization of estrus cycles. After 13 days of $PGF_{2\alpha}$ injection, buffaloes were given one of four treatments; Group A (n=10) were given an intramuscular injection of 100 µg of GnRH followed by an injection of $PGF_{2\alpha}$ 7 days later and a final injection of GnRH at timed artificial insemination (AI) 48 h after the $PGF_{2\alpha}$ injection. Group B (n=10) were given the same treatment as that given to Group A buffaloes but with an injection of 1 mg of estradiol 24 h after the $PGF_{2\alpha}$ injection. Group C (n=10) were given an intramuscular injection of 100 µg of GnRH followed by an injection of $PGF_{2\alpha}$ 7 days later and a final injection of GnRH at timed artificial insemination (AI) 72 h after the $PGF_{2\alpha}$ injection. Group D (n=10) were given the same treatment as that given to Group C buffaloes but with an injection of 1 mg of estradiol 24 h after the $PGF_{2\alpha}$ injection. Blood samples (10 ml) were collected from all buffaloes 7 days before and again at the first GnRH of the BuffSynch protocols, at 48 and 72 h after the $PGF_{2\alpha}$ injection of the BuffSynch and at 7 days after the final GnRH of synchronization protocols. Estrus was observed in 65% (13/20) of the buffaloes after the BuffSynch72 (Groups C and D) compared with 45% (09/20) buffaloes in the BuffSynch48 (Groups Aand B). Of the buffaloes which were treated with estradiol, 70% (14/20) displayed estrus as compared to 40% (08/20) of the buffaloes which were not treated with estradiol. Irrespective of treatments, a preponderance of buffaloes observed in estrus was identified 72 h after injection of $PGF_{2\alpha}$. It is obvious that estrus expression increased after estradiol treatment however merely at 72 h or 48 h after estradiol injection. Among different treatment groups, pregnancy per AI at 40 and 68 days after artificial insemination did not differ. At 40 days after artificial insemination, pregnancy per AI rates were 45.0% for BuffSynch48 (Groups A and B) and BuffSynch72 (Groups C and D), whereas 45.0% for buffaloes with (Groups B and D) or without (Groups A and C) receiving estradiol treatment. It was concluded that lactating buffaloes exposed to presynchronized timed artificial insemination program with variable interval of proestrus before GnRH, in which artificial insemination was done at the time of final GnRH injection, practiced

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analogous pregnancy per artificial insemination. It is important that prolonging the period of proestrus and treating buffaloes with estradiol show estrus mainly at 72 h after $PGF_{2\alpha}$ but did not increase fertility.

Keywords: buffalo, synchronization, GnRH, estradiol

INTRODUCTION

The reproductive efficiency of farm animals, which denotes the maximum effective use of their reproductive capacity, is of considerable practical and economic significance to farmers. Buffalo reproduction is an important issue, which has been the focus of many studies. Reproductive management of buffaloes has always been a challenge. Silent estrus is perhaps the most important factor leading to poor reproductive efficiency in buffaloes (Kanai and Shimizu, 1983). As the signs of estrus in buffaloes are less obvious than in cattle, the estrus detection accuracy is one of the major problems limiting the use of artificial insemination (AI) in this species.

Various estrus synchronization protocols have been tried among many other reproductive technologies for improving the fertility of buffaloes. A novel synchronization protocol named Ovsynch was developed in cows; this requires a three-injection schedule (GnRH-$PGF_{2\alpha}$-GnRH) for synchronization of ovulation. The technique was successfully carried out in cycling buffaloes (Paul and Prakash, 2005) for synchronization of ovulation and fixed timed artificial insemination (AI). Ovsynch is a timed artificial insemination (AI) program that reduces days open and increases pregnancy rates by allowing for control of first and subsequent inseminations. It has now been a trend to administer GnRH and/ or $PGF_{2\alpha}$ in early postpartum cows and buffaloes in order to hasten early resumption of cyclic ovarian activity and thereby to increase the reproductive efficiency. The gonadotropin releasing hormone (GnRH) and prostaglandin ($PGF_{2\alpha}$) method of estrous synchronization has proven to be very successful in synchronizing estrus in cattle and buffaloes. Studies by Pursley *et al.* (1995) verified that, administration of GnRH after $PGF_{2\alpha}$ injection increases the rate of synchronized ovulation in cattle. It has been observed that when $PGF_{2\alpha}$ is administered on palpation of functional carpus leutum, about 60-70% of treated animals, were detected in estrus within 4 days post $PGF_{2\alpha}$ injection.

Synchronization of ovulation with 1 mg of estradiol cypionate increased pregnancy per artificial insemination (AI) compared with artificial insemination (AI) after a synchronized estrus (Cerri *et al.,* 2004). It is practical to suggest that methods that increase estradiol concentrations and expression of estrus in timed artificial insemination (AI) protocols might improve fertility of dairy cows. This may be particularly important for the high-producing dairy cow, which seems to lack sufficient blood concentrations of estradiol to induce estrus, ovulation, and uterine priming because of inherently high metabolism and clearance of steroid hormones. No such studies have been undertaken in buffalo.

It is hypothesized that extending the period of proestrus and delaying the time of artificial insemination (AI) from 48 to 72 h after induced luteolysis would increase plasma concentrations of estradiol, expression of estrus, and fertility of buffalo. It is also hypothesized that supplemental estradiol would further enhance these responses, primarily in buffaloes receiving timed artificial

insemination (AI) at 48 h after induced luteolysis. The present study was therefore conducted to compare the reproductive performance of lactating Nili-Ravi buffalos treated with GnRH and $PGF_{2\alpha}$ for synchronization of estrus, ovulation and pregnancy per artificial insemination with or without supplemental estradiol.

MATERIALS AND METHODS

The study was carried out on lactating Nili-Ravi buffaloes at the Buffalo Research Institute, Pattoki, District Kasur. Forty Nili-Ravi buffaloes of above 50 days post-partum were divided into four (n=10) groups (A, B, C, D). All buffaloes received 25 mg $PGF_{2\alpha}$ at 51 days in milk (DIM) for pre-synchronization of estrus cycles. Thirteen days after the $PGF_{2\alpha}$ injection, buffaloes were given one of four treatments;

BuffSynch48

Group A (n=10) were given an intramuscular injection of 100 µg of GnRH followed by an injection of $PGF_{2\alpha}$ 7 days later and a final injection of GnRH at timed artificial insemination (AI) 48 h after the $PGF_{2\alpha}$ injection.

Group B (n=10) were given the same treatment as that given to Group A buffaloes but with an injection of 1 mg of estradiol 24 h after the $PGF_{2\alpha}$ injection.

BuffSynch72

Group C (n=10) were given an intramuscular injection of 100 µg of GnRH followed by an injection of $PGF_{2\alpha}$ 7 days later and a final injection of GnRH at timed artificial insemination (AI) 72 h after the $PGF_{2\alpha}$ injection.

Group D (n=10) were given the same treatment as that given to Group C buffaloes but with an injection of 1 mg of estradiol 24 h after the $PGF_{2\alpha}$ injection.

A buffalo which stood to be mounted by a penile deviated bull was considered to be in estrus. The artificial insemination (AI) was done once in the morning after the $PGF_{2\alpha}$ injection

Blood Sampling for Progesterone Analysis

Blood samples (10 ml) were collected from all buffaloes 7 days before and again at the first GnRH of the BuffSynch protocols, at 48 and 72 h after the $PGF_{2\alpha}$ injection of the BuffSynch and at 7 days after the final GnRH of synchronization protocols, respectively. Blood samples were collected at 48 and 72 h after the $PGF_{2\alpha}$ injection of the BuffSynch protocols (Group A, B, C, D), which corresponded to 24 and 48 h after treatment with estradiol, respectively. Samples were immediately transported to the laboratory within 1 h of collection. Progesterone was determined through ELISA by using a commercially available kit (BioCheck, Inc, Lot. RN-34859).

Pregnancy Diagnosis

All buffaloes were examined for pregnancy by rectal palpation of the uterus and its contents for detection of an embryonic vesicle at 40 ± 1 day after artificial insemination (AI), and pregnant buffaloes were reexamined 4 week later at 68 ± 1 days. Pregnancy per artificial insemination (AI) is defined as the number of pregnant buffaloes divided by the number of inseminated buffaloes within each treatment at 40 and 68 days after artificial insemination (AI).

Statistical analysis

The data thus obtained were analyzed statistically by using the Z-test. (Steel and Torrie,

1980).

RESULTS

By postponing the time of artificial insemination from 48 to 72 h, the estrus expression increased. Similarly by treating buffaloes with estradiol increased the estrus expression. Estrus was observed in 65% (13/20) of the buffaloes after the BuffSynch72 (Groups C and D) compared with 45% (09/20) the buffaloes in the BuffSynch48 (Groups A and B). Of the buffaloes which were treated with estradiol, 70% (14/20) displayed estrus as compared to 40% (08/20) of those which were not treated with estradiol. Irrespective of treatment, a preponderance of the buffaloes observed in estrus was identified 72 h after injection of $PGF_{2\alpha}$. It is obvious that estrus expression increased after estradiol treatment however merely at 72 h or 48 h after estradiol injection. Short-cycling buffaloes were not affected by BuffSynch or estradiol treatment (Table 1). Among different treatment groups, pregnancy per AI at 40 and 68 days after artificial insemination did not differ. At 40 days after artificial insemination, pregnancy per AI rates were 45.0% for BuffSynch48 (Groups A and B) and BuffSynch72 (Groups C and D), whereas pregnancy per AI rates were 45.0% for buffaloes receiving (Groups B and D) or not receiving (Groups A and C) estradiol treatment (Table 1). Between 40 and 68 days of gestation, none of the buffaloes lost pregnancy.

DISCUSSION

Scheduled artificial insemination programs are commonly used aimed at reproductive management of dairy cattle to confirm insemination of suitable cows (Caraviello *et al.,* 2006). In lactating dairy cows, Pursley *et al.* (1998) evaluated the effect of the interval between GnRH and the time of artificial insemination. The pregnancy rate per artificial insemination was decreased in cows

Table 1. Effect of BuffSynch and estradiol treatment on reproductive responses of Nili-Ravi buffaloes.

Items	Group A	Group B	Group C	Group D
Cyclic	80.0 (8/10)	70.0 (7/10)	70.0 (7/10)	70.0 (7/10)
Detected estrus[a]	30.0 (3/10)	60.0 (6/10)	50.0 (5/10)	80.0 (8/10)
Short-cycling	5.8 (14/240)	6.6 (16/243)	3.3 (8/245)	5.4 (13/239)
Pregnancy per AI (day 40)	40.0 (4/10)	50.0 (5/10)	50.0 (5/10)	40.0 (4/10)
Pregnancy per AI (day 68)	40.0 (4/10)	50.0 (5/10)	50.0 (5/10)	50.0 (5/10)
AI interval(days)	25.6±1.1	25.2±1.1	27.0±1.1	27.0±1.0

[a]Buffaloes were classified as cyclic when progesterone concentration in plasma was ≥1 ng/ml in at least 1 of 2 sampling days at 57 and 64 days in milk.

inseminated about 32 h after GnRH administration while in the Ovsynch program, a high proportion of cows come to be pregnant and more calvings were attained once artificial insemination was completed 16 h after the final injection of GnRH (Pursley *et al.,* 1998). Modifications in the Ovsynch program were evaluated, and it was observed that number of calvings increased when cows received the final GnRH injection and timed artificial insemination at 72 h as compared with GnRH and timed artificial insemination 48 h after the $PGF_{2\alpha}$ (Portaluppi and Stevenson 2005). Portaluppi and Stevenson (2005) further found that pregnancy loss was reduced in cows which were given a GnRH injection and timed artificial insemination at 72 h in comparison with cows given GnRH and timed artificial insemination at 48 h. When dairy cows were exposed to a presynchronized estrus synchronization program and artificially inseminated at detected estrus, the estrus distribution after induced luteolysis gave rise to mean and median intervals of 3.2±1.0 and 3.0 days, respectively (Cerri *et al*., 2004). Out of 258 cows detected in estrus, the percentage of cows showing estrus on day 1, 2, 3, 4 and 5 after $PGF_{2\alpha}$ injection were correspondingly 2.4, 12.2, 52.4, 22.8 and 7.1 % (Cerri *et al.,* 2004). Cerri *et al.* (2004) further found that distribution of estrus at day 1, 2 and 3 after $PGF_{2\alpha}$ injection was 7.1, 26.5 and 66.5 % in cows that were given estradiol cypionate injection 24 h after $PGF_{2\alpha}$. Thus, 3 days after the estrus synchronization program is the time when most of the animals are observed in estrus. Likewise, in our study, the majority of the buffaloes were observed in estrus at 72 h after $PGF_{2\alpha}$ injection. Walker *et al.* (1996) reported that after a preliminary show of estrus, the time of ovulation is about 28 h, and this is comparable in those cows in which ovulation was induced by GnRH in a synchronization program (Pursley *et al.,* 1995; Stevenson *et al.,* 2004).

Our assumption was that prolonging the time of induced ovulation and artificial insemination from 48 to 72 h in a timed artificial insemination program will increase pregnancy per artificial insemination because of prolonged proestrus subsequently prolonged exposure to increased concentrations of estradiol and display of estrus. The findings of present study are inagreement with Portaluppi and Stevenson (2005). These authors reported similar pregnancy per artificial insemination in cows given GnRH injections and timed artificial insemination at either 48 or 72 h after luteolysis. As observed in the current study, many authors (Sterry *et al.,* 2007; Brusveen *et al.,* 2008) have reported non-existence of alteration in pregnancy per artificial insemination to first and following inseminations while cows were programmed for a 48 or 72 h synchronization protocol.

An important logic for prolonging proestrus was to enhance synchronization of ovulation with the anticipated time when the maximum number of buffaloes show estrus after luteolysis. This may be significant when buffaloes are induced to ovulate and inseminated at the same time. Peters and Pursley (2003) reported that in the Ovsynch program, restricting proestrus to below 36 h resulted in ovulation of smaller follicles and lower pregnancy per artificial insemination in dairy cows.

Earlier studies (Pancarci *et al.,* 2002) indicated that in a timed artificial insemination program, no effect on pregnancy per artificial insemination was observed if the final GnRH injection is replaced by 1 mg estradiol cypionate. In contrast, Cerri *et al.* (2004) reported that when ovulation was induced with 1 mg estradiol cypionate, pregnancy per artificial insemination was increased in comparison with cows which were inseminated

after a synchronized estrus. Enhancements in pregnancy per artificial insemination were proposed by increased contact to estradiol during proestrus, which might have upgraded oocyte fertilization or well prepared the uterus for the succeeding luteal phase, which is significant for induction of endometrial progesterone receptors (Zelinsky and Stormshak, 1981). It was suggested that in high producing cows, there is a deficiency of adequate concentrations of estradiol in the blood to induce estrus, ovulation and uterine priming on account of their characteristically wide-ranging steroid metabolism and clearance (Wiltbank *et al.,* 2006). Sartori *et al.* (2002) reported that heifers had advanced peak estradiol levels as compared to lactating cows, nevertheless cows had larger ovulatory follicles. Hence, it is likely that supplementation with estradiol during proestrus might benefit fertility of buffaloes, principally in synchronization programs which may restrict exposure to follicular estradiol.

Previously, Sellars *et al.* (2006) verified the assumption that supplemental estradiol might advantage fertility of dairy cows at timed artificial insemination programs. Cows were given 0.25 mg estradiol cypionate at the time of final GnRH injection in the Ovsynch program, and even though serum estradiol concentrations increased in cows treated with estradiol, pregnancy per artificial insemination persisted unchanged. In cows on the Ovsynch programgiven 0.5 mg estradiol cypionate 24 h before the final GnRH injection, a decrease in the percentages of oocytes and embryos was observed with no effect on fertilization (Cerri *et al.,* 2004). Similarly, in buffaloes with 48 h of proestrus, it becomes doubtful that supplemental estradiol improves fertility. The findings of current study are in agreement with Sellars *et al.* (2006).

Actually, in the current study, it was observed that synchronized buffaloes and those having higher concentrations of progesterone at 7 days after artificial insemination were more likely to be pregnant at 40 and 68 days after artificial insemination, representing the significance of increased progesterone concentrations for pregnancy.

CONCLUSION

Lactating buffaloes exposed to presynchronized timed artificial insemination program with variable intervals of proestrus before GnRH, in which artificial insemination was done at the time of the final GnRH injection, practiced analogous pregnancy per artificial insemination. It is important that prolonging the period of proestrus and treating buffaloes with estradiol show estrus mainly at 72 h after $PGF_{2\alpha}$ but did not increase fertility. Buffaloes that showed estrus had higher concentrations of progesterone 7 days after the time artificial insemination. These interventions caused buffaloes which showed estrus are more likely to become pregnant than those not expressing estrus. The findings of current study established that timed artificial insemination protocols should be taken into consideration to increase displayed estrus either on the day of timed artificial insemination or after as it is related to increased fertility.

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