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PGF2$_\alpha$ induced estrus synchronization of native buffaloes in Bangladesh

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Abstract

Buffaloes have low fertility due to silent estrus, seasonal anestrous, delayed puberty and long calving interval. This study was aimed to investigate the effect of single and double doses of prostaglandin F_{2alpha} (PGF2$_\alpha$) on synchronization of estrus in buffaloes. A total of 66 buffaloes were selected and subjected to estrus synchronization experiments: single dose PGF2$_\alpha$, and double dose PGF2$_\alpha$. In single dose PGF2$_\alpha$ experiment, buffaloes were injected with either 500 or 875 µg of cloprostenol (PGF2$_\alpha$) and all buffaloes were examined to identify corpus luteum through rectal palpation before administration of PGF2$_\alpha$. In double dose experiment, buffaloes were injected with either 500 or 875 µg of cloprostenol (PGF2$_\alpha$) on Day 0 and Day 12 without identifying corpus luteum. The buffaloes were examined for estrus by collecting cervical mucus using universal artificial insemination gun from the mid cervix and timed artificial inseminations (TAI) were done after 72 hrs of last dose of PGF2$_\alpha$ injection. The results showed that there were no significant differences in estrus rate (75±3.96% and 85±0.79%) single dose of PGF2$_\alpha$ with 500 and 875 µg of cloprostenol, respectively. However, 875 µg of cloprostenol significantly ($P<0.05$) increased estrus rate in buffaloes than double doses with 500 and 875 µg of cloprostenol (60 ± 12.72% and 63 ± 2.22%). The conception rate in buffaloes were 45±6.91% and 55±6.91% in 500 µg and 875 µg of cloprostenol in single dose of PGF2$_\alpha$ and 30±14.69% and 37±8.81% in 500 µg and 875 µg of cloprostenol in double dose of PGF2$_\alpha$. The calving rate in buffaloes were 10±5.20% and 15±0.79% in 500 µg and 875 µg of cloprostenol in single dose of PGF2$_\alpha$ and 10±8.33 and 12±6.18% in 500 µg and 875 µg of cloprostenol in double dose of PGF2$_\alpha$. However, there were no significant differences in conception and calving rates in buffaloes neither between single and double doses PGF2$_\alpha$ nor different concentrations of PGF2$_\alpha$. In conclusion, single dose of PGF2$_\alpha$ with 875 µg of cloprostenol injection efficiently increased estrus synchronization rate in buffaloes.

Keywords: Buffalo, dose, estrus, PGF2$_\alpha$, synchronization

Introduction

Buffalo (*Bubalus bubalis*) is an important livestock resource in many countries of the world, particularly in Asia, Latin America and Mediterranean region. They play a crucial role to millions of smallholder farmers in developing countries. They are known to have poor fertility in the most of the rearing systems and environmental conditions (Jainudeen and Hafez, 1993; Barile, 2005). The poor breeding is mainly manifested by late maturity, poor expression of estrus, long postpartum anoestrus intervals, poor conception rate, reduce ovarian activity and long calving intervals (Singh *et al*., 2000). In addition, a large variation in duration of estrus (4–64 hrs) and time of ovulation, the success rate of artificial insemination (AI) in buffaloes is comparatively low (Barkawi *et al*., 1993; Ohashi, 1994). This process is further compromised by the factors related to climatic condition, nutrition, management and diseases under the most smallholder production systems.

Estrus synchronizations is usually applied to optimize the estrus, improve the reproductive efficiency in mammals. It might address two well defined problems of breeding. Firstly, silent estrus in buffaloes might be managed by estrus synchronization followed by a timed insemination schedule without detection of estrus (Baruselli *et al*.,

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2013). Secondly, the anestrus postpartum buffaloes might be bred, thus achieving yearly calving by reducing days open for breeding (Kumar *et al*., 2012). A number of protocols have been developed synchronization of estrus in buffaloes in last few decades. Prostaglandin ($PGF2_{\alpha}$); ovsynch (gonadotropin-releasing hormone (GnRH)+$PGF2_{\alpha}$); controlled internal drug release device (CIDR), progesterone (P_4)-estradiol benzoate (EB), P_4-GnRH, P_4-equine chorionic gonadotropin (eCG), P_4-human chorionic gonadotropin (hCG) are applied for synchronization of estrus in buffaloes (Ahmad and Arshad, 2020). Tervit *et al*. (1973) reported the induction of estrus in cattle by intramuscular injection of $PGF2_{\alpha}$ analogue. Later it was reported that neither $PGF2_{\alpha}$ analogue nor $PGF2_{\alpha}$ induce estrus in cattle by a single dose of $PGF2_{\alpha}$ analogue until all animals were treated at 5 days before the onset of estrus cycle (Lauderdale, 1972; Liehr *et al*., 1972; Hill *et al*., 1973; Cooper and Furr, 1974). To overcome this problem Cooper and Furr (1974) reported a valuable method that could be used to increase fertility of domestic animals. They stated that two doses of $PGF2_{\alpha}$ analogue given 10–12 days apart induced estrus in all animals irrespective of their natural estrus cycle schedule. Few reports have been demonstrated the $PGF2_{\alpha}$ induced estrus synchronization in buffaloes (Chohan, 1998; Warriach *et al*., 2008). Usually the protocol developed for cattle are being used for synchronization of buffaloes by the farmers. Thus, the success rate of estrus synchronization is lower in buffaloes. However, so far the protocol for estrus synchronization of buffaloes using $PGF2_{\alpha}$ has not been investigated under smallholder production system. The study was conducted to compare the potentialities of single and double dose of $PGF2_{\alpha}$ for estrus synchronization in buffaloes.

Materials and Methods

Animal Selection and Management

A total 66 of female indigenous buffaloes with the body weight of 300-500 kg were used to investigate the efficiency of single and double doses of $PGF2_{\alpha}$ on estrus synchronization in buffaloes from smallholder farmers. The age of these animals were 6-8 years. The buffaloes were identified with ear tags. Anthelmintic drugs were administered to deworm the animals and vaccinated against common infectious diseases. The buffaloes were supplied with a ration using commonly available feedstuff to fulfill their nutrient requirements (Terramoccia *et al*., 2005). The effects of $PGF2_{\alpha}$ single and double dose on estrus synchronization were examined by intramuscular injection of $PGF2_{\alpha}$ (cloprostenol; Bayer New Zealand Limited, Auckland, New Zealand). The detailed experimental designs and procedures are shown in Figure 1. Briefly, in single dose $PGF2_{\alpha}$ experiment, estrus was synchronized by injecting either 2 ml (500 µg cloprostenol) or 3.5 ml (875 µg cloprostenol) of Ovoprost ($PGF2_{\alpha}$). Before administering $PGF2_{\alpha}$ all buffaloes were examined to identify corpus luteum through rectal palpation. Buffaloes with palpable corpus luteum were selected for single dose of $PGF2_{\alpha}$ experiment. In double dose $PGF2_{\alpha}$ experiment, estrus was synchronized by injecting either 2 ml (500 µg cloprostenol) or 3.5 ml (875 µg cloprostenol) of Ovoprost ($PGF2_{\alpha}$) without rectal palpation and identification of corpus luteum. The day of first $PGF2_{\alpha}$ injection was calculated as Day 0. A second round of $PGF2_{\alpha}$ injection with the same dose was given to the buffaloes on Day 12.

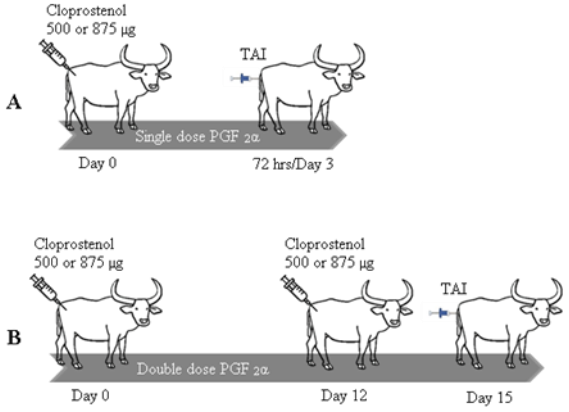

Figure 1: Estrus synchronization of buffaloes using single and double doses of $PGF2_{\alpha}$. In single dose of $PGF2_{\alpha}$ treatment timed artificial insemination (TAI) was conducted at 72 hrs after $PGF2_{\alpha}$ injection (A). Similarly, in case of $PGF2_{\alpha}$ double dose treatments TAI was done at 72 hrs after the second dose of $PGF2_{\alpha}$ injection (B).

Estrus Detection, Timed Artificial Insemination (TAI) and Pregnancy Diagnosis

Frequent urination, mucus discharge and swollen vulva are the symptoms of estrus in buffaloes. Therefore, cervical mucus was sucked from the buffaloes to detect estrus as described by Joshi *et al*. (2017). Briefly, cervical mucus was aspirated from

cervix through recto-vaginal method using blue sterile sheath and universal artificial insemination gun (Figure 2). Timed artificial inseminations (TAI) were carried out at 72 hrs after PGF2$_\alpha$ injection in single dose experiment and at 72 hrs after second dose of PGF2$_\alpha$ injection in the double dose experiment. Pregnancy diagnosis was carried out at 60 days after TAI by transrectal palpation.

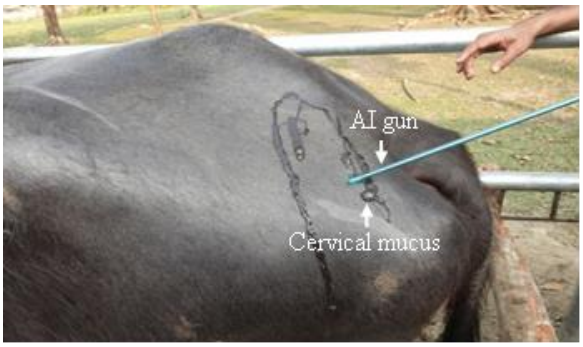

Figure 2: Observation of cervical mucus for estrus detection in buffaloes. Cervical mucus was sucked from mid cervix by using universal artificial insemination gun. Watery state of mucus indicated the estrus in buffaloes.

Statistical Analyses

Data were analyzed using one-way ANOVA followed by Duncan's test (IBM SPSS Statistics, version 22). Differences at $P < 0.05$ were considered statistically significant.

Results and Discussion

In the present study, buffaloes were injected with either 500 or 875 µg of cloprostenol for estrus synchronization in both single and double dose protocols of PGF2$_\alpha$ treatments. The effects of single and double doses of PGF2$_\alpha$ injection on estrus, conception and calving rate are shown in Figure 3.

There were no significant differences in estrus rate between 500 or 875 µg of cloprostenol treated groups in the single dose of PGF2$_\alpha$ experiment. Single dose of PGF2$_\alpha$ induced estrus synchronization in cattle when it was administrated at a particular day of their reproductive (estrus) cycle. Whereas rest of the day of estrus cycle were not effective for estrus synchronization by PGF2$_\alpha$ single dose (Lauderdale, 1972; Liehr *et al*., 1972; Rowson *et al*., 1972). Similarly, administration of PGF2$_\alpha$ at selective day of estrus cycle induced estrus synchronization in buffaloes (Chohan, 1998; Warriach *et al*., 2008).

The estrus synchronization was highly variable, predominantly due to the presence or absence of corpus luteum in single dose of PGF2$_\alpha$ administration, thus the double dose of PGF2$_\alpha$ administration was developed (Dhaliwal *et al*., 1987).

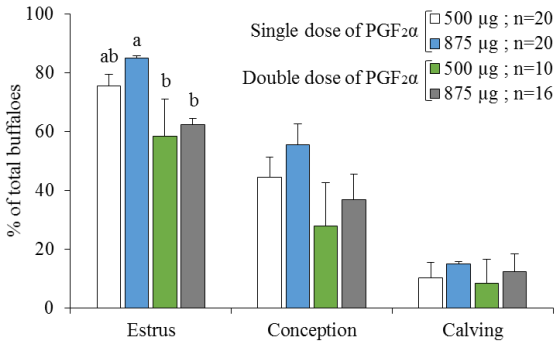

Figure 3: Effect of single and double doses of PGF2$_\alpha$ on estrus synchronization, conception and calving rate of buffaloes. Bars with different letters (a,b) differed significantly ($P < 0.05$).

In the present study, double doses of cloprostenol were injected 12 days apart. Significant differences were not found in estrus rates between 500 and 875 µg of cloprostenol groups in the double doses of PGF2$_\alpha$ experiment. Significantly ($P < 0.05$) higher rates of estrus were recorded in 875 µg single dose of cloprostenol treated group than double doses of cloprostenol (Figure 3). There were no significant differences in conceptions and calving rates among different treatment groups.

PGF2$_\alpha$ were injected twice at a 10-12 day interval in cattle because it was effective only at 0-5 day of the estrous cycle (Lauderdale *et al*., 1981). The PGF2$_\alpha$ reduced progesterone concentrations in pseudo pregnant rats and ended pseudo pregnancy as a luteolytic hormone (Pharriss and Wyngarden, 1969). Several initial reports recognized luteolytic actions of PGF2$_\alpha$ in cattle (Liehr *et al*., 1972; Rowson *et al*., 1972). Therefore, potent prostaglandin analogues were developed subsequently for use as luteolytic agent in multiple species including buffaloes. Intrauterine administration of PGF2$_\alpha$ reduced the diameter of corpus luteum (Louis *et al*., 1973). Later, Lauderdale *et al*. (1974) used the palpation method to detect corpus luteum and succeeded in the single dose of PGF2$_\alpha$ treatment for estrus synchronization. In the present study, PGF2$_\alpha$ single

dose was treated only in buffaloes with palpable corpus luteum because $PGF2_{\alpha}$ single dose induced luteolytic action. On the other hand, treatment of double dose at a 12 day interval was conducted due to the acyclicity and anestrus nature of buffaloes. The present study revealed that single dose of $PGF2_{\alpha}$ injection increased estrus rate in buffaloes. Our results also showed that single and double doses of $PGF2_{\alpha}$ did not show any significant differences in pregnancy and calving rates in buffaloes. It was reported that administration of $PGF2_{\alpha}$ did not increase pregnancy outcomes in Italian buffaloes (Neglia *et al*., 2012). Similarly, applications of estradiol benzoate (EB) and GnRH along with $PGF2_{\alpha}$ did not improve pregnancy rates in buffaloes (Carvalho *et al*., 2017).

Estradiol injection during progesterone (P4) withdrawal induced ovulation in a synchronization/timed-AI (TAI) protocol in buffaloes (Carvalho *et al*., 2020). The administration of equine chorionic gonadotropin (eCG) to an estrus synchronization and TAI could not improve pregnancy rates of buffaloes under tropical condition (Murugavel *et al*., 2009). The diameter of ovulatory follicle is an important factor for TAI procedure as it is directly associated with the size of the succeeding corpus luteum in buffalo (Vecchio *et al*., 2012; Carvalho *et al*., 2013; Monteiro *et al*., 2016) and cattle (Dadarwal *et al*., 2013). A larger corpus luteum secretes more P4 (Pfeifer *et al*., 2009; Dadarwal *et al*., 2013), which upsurges the probability of preserving a pregnancy and increases fertility in buffalo (Vecchio *et al*., 2012) and cattle (Binelli *et al*., 2001; Inskeep, 2004; Baruselli *et al*., 2009; Lonergan, 2011). However, cloprostenol is not efficient to increase the blood flow of pre-ovulatory follicle and corpus luteum as well as unable to increase the size of ovulatory follicle and corpus luteum, and secretion of progesterone in Italian Mediterranean buffaloes (Neglia *et al*., 2012). In the present study, pregnancy rates did not differ among the treatment groups which might be associated with the smaller size of ovulatory follicles and corpus luteum and also concentrations of progesterone in buffaloes.

Conclusion

In conclusion, single dose of $PGF2_{\alpha}$ administration effectively synchronized estrus in buffaloes. Meanwhile, the conception and calving rate did not significantly differ among the doses of $PGF2_{\alpha}$ in buffaloes. Therefore, estrus synchronization using the single dose of $PGF2_{\alpha}$ administration might be applied for overcoming the silent estrus and seasonal anoestrus in the native buffaloes.

Conflict of interest: None.

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