



## Improvement of conception rate in postpartum flaxseed supplemented buffalo with Ovsynch + CIDR protocol

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### ABSTRACT

The present study was conducted on lactating Murrah buffalo to assess the effect of crushed flaxseed (a source of omega-3 fatty acids) supplementation (300 g/100 kg bwt/day for 60 days), over and above the routine feed, on luteolytic signal (PGF<sub>2α</sub>), luteal function (progesterone) and conception rate. In first experiment, on day 50 post-calving, six non-supplemented buffalo were treated to synchronize time of ovulation using an Ovsynch + Controlled Internal Drug Release (CIDR) protocol followed by intravenous oxytocin treatment (OT; 100 IU) on day 15 post-ovulation. Blood samples were collected at 15 min interval, 1 h before to 4 h after OT challenge. Thereafter, the same buffalo were supplemented with flaxseed, treated to synchronize time of ovulation starting on day 35 post-supplementation using the same protocol and subjected to OT treatment and blood sampling on day 15 post-ovulation. The PGF<sub>2α</sub> response was measured as the venous concentration of 13,14-dihydro-15-keto PGF<sub>2α</sub> (PGFM). The mean hourly concentration of PGFM subsequent to flaxseed supplementation was less ( $P < 0.05$ ) than in the pre-supplementation period at all the occasions. Flaxseed supplementation did not affect plasma fatty acids and other plasma metabolites except for an increase ( $P < 0.05$ ) in plasma cholesterol and plasma alanine transaminase. In the second experiment, 31 buffalo were randomly assigned to a control ( $n = 16$ ) and flaxseed supplemented ( $n = 15$ ) group. The latter group was supplemented with flaxseed starting from day 15 post-calving. On day 50-post-calving, buffalo of both groups were treated to synchronize time of ovulation among animals as described for the first experiment followed by artificial insemination (AI). Post-AI luteal phase plasma progesterone was greater ( $P < 0.05$ ) in the supplemented group compared to controls. Conception rate on day 63 post-AI was 66.7% in supplemented and 31.2% in controls ( $P < 0.05$ ). The present study indicated the beneficial impact of dietary supplementation of crushed flaxseed on conception rate through attenuation of luteolytic signal and improvement in post-breeding luteal profile.

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## 1. Introduction

Water buffalo are highly important for small-holder resource-poor farming families of South Asia as they rear 96.4 percent of the worldwide buffalo population which contributes toward more than one third of total milk production in Asia (Cringoli et al., 2010). The greater adaptability of buffalo to a wider range of climates, resistance to diseases and ability to utilize poor quality roughages makes them ideal livestock for Asian countries. Recently, buffalo milk has received research interest and investment in several European and American countries, owing mainly to its attractive nutrient content (CNIEL, 2002).

At the same time, buffalo is alleged to have poor reproductive efficiency owing to delayed puberty and prolonged inter-calving intervals (Zicarelli, 2010). A major factor associated with the latter is occurrence of repeat breeding (5–30%; Saxena, 2004; Kumar and Singh, 2010). Hormonal aberrations are contributors toward failure of conception in 26% of repeat breeder animals (Singh et al., 2009). Moreover, a majority of the embryonic loss (30%) occurs between days 8 and 16 post-breeding in dairy cattle (Sreenan et al., 2001), while similar studies are lacking in dairy buffalo.

During the early- and mid-luteal phase, a delicate balance between the luteolytic and anti-luteolytic hormonal mechanisms is important for successful establishment of pregnancy in cattle (Binelli et al., 2001). In such a context, luteal deficiency (insufficient release of progesterone) is known as a major cause of early embryonic mortality in buffalo (Campanile et al., 2005). The presence of optimal plasma progesterone exerts a positive influence on embryo development (Mann et al., 1999). A well-developed embryo possess the capacity to release adequate interferon-tau (IFN- $\tau$ ), which in turn inhibits the luteolytic action of prostaglandin  $F_{2\alpha}$  (PGF $_{2\alpha}$ ) during the critical period (day 15–17 post-breeding) of maternal recognition of pregnancy (Mann et al., 1999).

Several embryo-independent strategies like intra-uterine infusion of bovine IFN- $\tau$  or feeding of flunixin granules (a PGF $_{2\alpha}$ -inhibitor) around the critical period of maternal recognition of pregnancy have been proposed to suppress the luteolytic signal by the dam (Binelli et al., 2001). Another strategy is aimed at the decrease in endometrial synthesis of PGF $_{2\alpha}$  through prolonged dietary supplementation of omega-3 fatty acids, viz.  $\alpha$ -linolenic acid (Ambrose et al., 2006). Alpha-linolenic acid (C18:3n-3) is a precursor to eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). Both EPA and DHA interfere with the conversion of arachidonic acid to PGF $_{2\alpha}$  (Binelli et al., 2001). To assess the impact of omega-3 fatty acid diet on endometrial PGF $_{2\alpha}$ , intravenous oxytocin treatment around day 15 or 16 of the estrous cycle is an established test that stimulates the release of PGF $_{2\alpha}$  from uterine glands (Mattos et al., 2002; Malik et al., 2011a).

Flaxseed (also known as alsin or linseed) is a major source of  $\alpha$ -linolenic acid (Petit et al., 2004), whereas, fish products (meal/oil) are a predominant source of EPA and DHA (Heravi et al., 2007). Supplementation of whole flaxseed or fish products (for 40–60 days) in dairy cattle diet leads to suppression of PGF $_{2\alpha}$  release, improves luteal profile, and ultimately increases conception rate (Wamsley et al., 2005;

Heravi et al., 2007). Moreover, supplementing lactating heifers with flaxseed at 12.6% dry matter intake increases plasma cholesterol (Gonthier et al., 2005), without any alteration in plasma glucose and triglycerides (Ambrose et al., 2006; Ponter et al., 2006).

In a preliminary study in buffalo, fish meal supplementation was beneficial with regard to PGF $_{2\alpha}$  release and conception rate (Malik et al., 2011a, b). However, fish meal supplement has disadvantages such as poor palatability as a consequence of strong fish odor and lack of availability of good quality fish meal/oil. On the contrary, flaxseed is one of the greatest non-fish sources of omega-3 fatty acids. Flaxseed offers high  $\alpha$ -linolenic acid content (58% of the total fatty acids) compared to fish meal (45%). In addition, flaxseed contains about 23% protein and 28% dietary fiber, thus making it an energy dense replacement compared to other costly feed ingredients (Erasmus, 1993).

Applying estrus/ovulation synchronization protocols along with fixed-time breeding in buffalo provides a potential alternative for increasing the productive period (Ghuman et al., 2012). However, the protocols used have achieved variable success with respect to conception rate in buffalo (De Rensis et al., 2005; Ghuman et al., 2012). This justifies the need to develop measures for improving the conception rate of buffalo subjected to fixed-time breeding protocols.

Considering the economic potential of water buffalo, the issue of improving (re)productive efficiency is of considerable relevance. Hence, the present study aimed at improvement in conception rate of postpartum flaxseed-supplemented buffalo with utilization of a fixed-time breeding protocol (Ovsynch+Controlled Internal Drug Release, CIDR) through: (1) attenuation of PGF $_{2\alpha}$  release during the post-breeding period when pregnancy recognition occurs and, (2) improvement in post-breeding luteal phase plasma progesterone.

## 2. Materials and methods

### 2.1. Animals and diets

This study was conducted using 37 Murrah buffalo that did not have calving difficulty at the time of the previous parturition (parity: 1–5, age:  $59.0 \pm 1.9$  months, body weight:  $493.7 \pm 15.9$  kg, daily milk yield:  $7.1 \pm 0.2$  kg) in the District Ludhiana (latitude:  $30^{\circ}56'N$ , longitude:  $75^{\circ}52'E$ ), India, during the winter-spring months from October to April (with maximum ambient temperatures and relative humidity ranging from  $13$  to  $27^{\circ}C$  and  $44$  to  $74\%$ , respectively). Using an ultrasonic scanner, all the buffalo were subjected to gynecological examination before inclusion in the study, and the buffalo diagnosed with any apparent pathological condition of the reproductive tract were not included.

Buffalo were confined for the entire period of study to a barn with access to an open sheltered space. The daily feed of the buffalo consisted of 40 kg oat fodder (30% dry matter, DM) and 2 kg concentrate mixture (maize and wheat bran 30 parts each, soybean meal and

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