



Effect of oestradiol benzoate on oestrus intensity and pregnancy rate in CIDR treated anoestrus nulliparous and multiparous buffalo

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ABSTRACT

The objective of the present study was to examine if administration of oestradiol benzoate (OEB) after removal of control internal drug releasing device (CIDR) could enhance oestrous intensity and pregnancy rate in anovular nulliparous and multiparous Nili-Ravi buffalo. For this, a total of 298 nulliparous ($n=91$) and multiparous ($n=207$) buffaloes received a CIDR on Day 0, and were administered PGF_{2α} on Day 6 followed by removal of CIDR on Day 7. At Day 8, OEB was administered in approximately half of nulliparous ($n=45/91$) and multiparous ($n=100/207$) buffalo. All animals were fixed time inseminated 48 and 60 h after CIDR removal, respectively. The results showed that administration of OEB but not the parity, improved oestrous intensity (3.15 ± 0.05 vs 2.99 ± 0.05 ; $P=0.0026$) compared to those not received OEB, respectively. However, OEB did not affect (46.2 vs 44.1 ; $P=0.8$) pregnancy per AI (P/AI). In addition, P/AI was greater (50.7 vs 39.6 ; $P=0.036$) in multiparous compared to nulliparous buffalo, respectively. The oestrous intensity ($P=0.025$) and response to OEB ($P=0.0002$) was greater in buffalo having a greater body condition (>3.0). Though, non significant, timing of ovulation was more synchronous (62.9 ± 1.8 vs 72.4 ± 3.6 h; $P>0.05$) and ovulation rate was greater (91% vs 64% ; $P>0.05$) in buffalo after OEB administration. It is concluded that administration of OEB in conjunction with the CIDR improves oestrous intensity without affecting P/AI in nulliparous and multiparous anovular buffalo.

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1. Introduction

Buffalo have an integral role in strengthening the agricultural economy, and improving food security to farmers in many Asian countries. They are excellent converters of low quality roughages and well adapted to harsh weather (Gordon, 1996). Despite these qualities, some of the main

reproductive problems include delayed puberty in heifers, poor oestrous expression, anoestrus, long calving intervals and seasonality in breeding (Singh et al., 2000). Accumulated evidence suggests that these reproductive problems are associated with smaller ovaries and its structures, which release less estradiol and progesterone concentrations, eventually limiting breeding values of buffalo by late puberty, ovarian hypo-function and pathologies (Warriach et al., 2008; Purohit, 2014). Therefore, in an attempt to overcome such problems hormonal treatments have been used for optimization of ovarian functions.

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Most of the oestrous synchronization protocols in buffalo are empirically based on those developed for cattle by inducing premature luteolysis using prostaglandins (Kumaratillake et al., 1977; Pathiraja et al., 1979) or prolonging the luteal phase using progestagens (Martinez et al., 1997; Martinez et al., 2000, 2002) which ultimately resulted in variable fertility. Among many of these oestrous synchronization protocols the use of CIDR device for reproductive management in buffalo is most prevalent, as few previous studies achieved comparable pregnancy rates in anoestrous buffalo during breeding or non-breeding seasons (Murugavel et al., 2009; Ahmad et al., 2010; Naseer et al., 2011; Carvalho et al., 2013; Barlie et al., 2015). The use of the CIDR has resulted in a resumption of oestrous cycles in anovular cows (Rhodes et al., 2002) and Holstein heifers (Yusuf et al., 2010). However, the effectiveness of these oestrous synchronization protocols appears to be limited in buffalo because farmers have to rely on visual methods of oestrous detection, which is not efficient in most of the buffalo herds or commercial farms. To overcome this, administration of OEB after removal of CIDR allows for a timed artificial insemination (TAI) program with optimal pregnancy rates (~60%) in a limited number of buffalo (Naseer et al., 2011). This approach induced acceptable ovulation rates (~80%) with greater synchrony of behavioral oestrus (Sales et al., 2012). Perhaps the addition of OEB in CIDR-treated buffalo optimizes the secretions of oestradiol which are at suboptimal concentrations due to either size of the mature follicle or insufficiency of neural mechanism controlling oestrous expression and hormone synthesis. It is likely, that anoestrous buffalo would have a similar hormonal status. Therefore, in the present study, oestrous intensity, ovulation synchrony and pregnancy rate were assessed following OEB administration in CIDR-treated nulliparous and multiparous buffalo and associations with body condition score (BCS) were assessed.

2. Materials and methods

2.1. Animals

The experiment was conducted at commercial dairy farms in the breeding season (September to December) in years 2011–2013 in the suburb of District Lahore, Pakistan. Anoestrous, adult (multiparous) Nili-Ravi buffalo ($n=207$) 4 to 7 (5.8 ± 1.3) years of age and with body weights (BW) of 450 to 550 kg, and a BCS of 3.25 ± 0.38 were managed and fed under optimal conditions. Similarly, buffalo heifers ($n=91$, nulliparous) 3.1 ± 1.4 years of age with a BW of more than 350 kg (BCS, 3.25 ± 0.44), and with no history of any oestrous signs were selected and fed under optimal

conditions. Each buffalo was scored for body condition as described elsewhere (Alapati et al., 2010). For further confirmation of the anoestrus ovarian ultrasonography (Honda HS-1500V with 10 MHz probe) was conducted twice within a 14-day interval to ascertain whether there was a functional CL present or not. At the start of experiment, both uterine horns were assessed using ultrasonography for any unexpected pregnancies or any uterine abnormalities.

2.2. Oestrous synchronization protocol

Briefly, CIDR devices (1.38 g progesterone; Eazi-breed®, New Zealand) were placed in the anterior vagina of all selected buffalo and this date was designated as Day 0. On Day 6, all animals received a single dose of PGF2 α (0.150 mg; Dalmazine, cloprostenol, Fatro®, Italy; 2 ml; i.m.) and after 24 h (Day 7) the CIDR was removed. To compare efficacy of OEB, approximately half of multiparous (100 of 207) and nulliparous (45 of 91) buffalo were randomly injected with 4 ml of OEB, 24 h after CIDR removal (0.4 mg; Oestradiol benzoate; Sigma®, St. Louis, MO, USA). Ovarian ultrasonography on Day 7 (CIDR removal day), Day 8, Day 9 (morning and evening) and Day 10 was conducted to measure the diameter of the largest follicle and to confirm ovulation.

2.3. Measurement of oestrous intensity

All the animals were examined for the oestrous intensity with the scale of 1 to 5 in ascending order (1 = poor and 5 = excellent), after slight modifications as reported earlier by Van Eerdenberg et al. (1996) (Table 1).

2.4. Trans-rectal ultrasonography, AI and pregnancy diagnosis

The follicular dynamics was monitored using the Honda HS-1500 V ultrasonography machine having a trans-rectal probe (10 MHz). The follicular diameter was measured twice a day from the day of CIDR removal until disappearance of the largest follicle (>9 mm, apparent ovulation). Each animal was artificially inseminated twice using frozen–thawed semen from a single bull (Semen Production Unit, Qadirabad, Punjab, Pakistan) of known fertility at approximately 48 and 60 h after removal of the CIDR. On Day 40 after insemination all the buffalo were screened for pregnancy by using trans-rectal ultrasonography. The pregnancy was based on the presence of fetal heart beat and amniotic fluid.

Table 1
Oestrous intensity score based on the animal's characteristics.

Oestrous intensity score	Grade	Characteristics
1	Poor	No uterine tone with no behavioral signs
2	Satisfactory	Mild uterine tone, slight mucus discharge, some restlessness
3	Good	Intermediate uterine tone, mucus discharge, restlessness, nervousness
4	Very good	Good tone, stand to be mounted, vulvar swelling, thick mucus discharge, restlessness
5	Excellent	High tone, stand to be mounted, thick mucus discharge, restless

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