

## Treatment of Repeat Breeding of Iraqi Buffaloes Using Different CIDR Protocols

### Research Article

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### ABSTRACT

The present study was undertaken to examine the efficacy of a treatment regime using a controlled internal drug release (CIDR) device in conjunction with injections of gonadotropin releasing hormone (GnRH) and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) on conception rate of repeat-breeding Iraqi buffaloes. Data were collected from 100 buffaloes with repeat breeding in Nineveh province housed at six private dairy farms. All buffalo cows were characterized as repeat breeding of more than three natural services with regular oestrous cycles without any signs of pregnancy. Any buffalo with abnormal vaginal discharge and >12% of polymorphonuclear cells in uterine smears was excluded from this study. Buffaloes were randomly assigned to four treatment groups of 25 cows each. Buffaloes in treatment 1 were injected with estradiol benzoate 1 mg and GnRH 250 µg on day 0, a CIDR in the anterior vagina from day 0 to 8, on injection with 25 mg of PGF<sub>2α</sub> on day 7 and GnRH (250 µg) on day 9. Buffaloes in treatment 2 received GnRH (250 µg) on day 0, a CIDR, from day 0 to 7 and 25 mg of PGF<sub>2α</sub> on day 7. Buffaloes in treatment 3 received a CIDR from day 0 to 7 and 25 mg of PGF<sub>2α</sub> on day 7. Treatment 4, a control group received no treatments. All buffaloes were mated during oestrus and pregnancy status was diagnosed by palpation of uterine contents at day 45. Pregnancy rates in treatments 1 to 4 were 44%, 52%, 24% and 8%, respectively. On average, Buffaloes treated with different CIDR protocols had higher pregnancy rate than control group. It could be concluded that the use of different CIDR protocols can be applied to improve fertility of repeat breeder Iraqi buffaloes.

**KEY WORDS** buffalo, CIDR, GnRH, PGF<sub>2α</sub>, repeat breeding.

### INTRODUCTION

A major problem faced by buffalo breeders and farmers include poor reproductive efficiency and prolonged intercalving intervals (Jaunadine, 1986; Singh *et al.* 2000). During the last few years, several studies have been attempted to treat infertility in buffaloes by using controlled internal drug release (CIDR), hormonal treatments such as gonadotropin releasing hormone (GnRH), gonadotropins (Gn), estrogen and prostaglandin F (PGF<sub>2α</sub>) (Metwelly, 2001;

Singh *et al.* 2003; Metwelly, 2006). Drost (2007) reported OvSynch protocol using CIDR inserted in buffaloes at the time of the first GnRH injection (day 0).

The CIDR device was removed 7 days later, at the time of PGF<sub>2α</sub> treatment (day 7). Conception rates in the control group, which did not receive progesterone, were 55.4% (36/65), and 57.5% (61/106) in the treated group which did receive progesterone (P>0.05). Ravikumar *et al.* (2010) used CIDR-GnRH protocol in postpartum period and found higher fertility response in the buffaloes. Jiang *et al.* (2003)

adopted CIDR PGF2 $\alpha$  to synchronize native swamp buffalo cows, and resulted in a synchronous oestrus rate of 85.13%. [Justolin et al. \(2010\)](#) used protocols consisted in CIDR insert + GnRH on day 10 and CIDR withdrawal + PGF2 $\alpha$  on day 3 in lactating dairy cows found an improvement of conception rate.

[Steckler et al. \(2002\)](#) concluded that the inclusion of exogenous progesterone such as CIDR during the interval between GnRH and PGF2 $\alpha$  injection prevented premature oestrus and increased conception rates in beef cows. Although much work has been done using ovsynch plus CIDR in cattle, information on their use in buffaloes especially postpartum lactating with infertility problems is limited.

Therefore, the present study was undertaken to test the efficacy of the treatment regime using a controlled internal drug release (CIDR) device in conjunction with injections of gonadotropin releasing hormone (GnRH) and prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ) on conception rate of repeat-breeding buffaloes.

## MATERIALS AND METHODS

### Animals

The study protocol was approved by the Ministry of Agriculture, Animal Resources Service General Company, Agricultural Research center. The study began in April 2011 and continued until July 2011. This study was conducted on lactating Iraqi northern buffalo cows in Nineveh province housed at six private dairy farms (latitude: 36° 20'N, longitude: 43° 8'E).

These herds consisted of 30 to 150 buffaloes (average 65 buffaloes; 1 buffalo bull to 25 buffalo cows). The animals were kept outdoors near the rivers for wallowing and milked twice daily. A balanced nutritional diet including green fodder and concentrate mixture were fed to these animals.

Animals with a history of caesarean operation, metritis, endometritis, lameness, abdominal disorders or other underrcurrent diseases were excluded from the study.

Following data was recorded for each buffalo cow included in the present study: name of buffalo, breed, number of parturitions, obstetrical problems if present, type of last parturition, retained placenta, vaginal prolapse, uterine prolapse, abortion, number of services, and milk production.

All buffalo cows were characterized as repeat breeding of more than three natural services with regular estrous cycles without any signs of pregnancy. Lactating buffaloes included in this study had a mean age 7.6 $\pm$ 0.5 y, mean body weight 320 $\pm$ 7 kg, mean body condition score 3.4 $\pm$ 0.03 (which is a subjective, visual assessment based on rib visibility, rump-fat thickness and fat thickness around the hooks and pins).

### Clinical examinations

The general health status, appetite, rectal temperature, pulse rate, respiration rate and other clinical signs such as arched back, colic, pain, presence of fresh discharge on the vulva, perineum or tail.

Any buffalo with abnormal vaginal discharge was excluded from the beginning of the study. A uterine fluid aliquot was aspirated from the uterine lumen using sterile uterine catheter and transferred into sterile tubes, then transported to the laboratory at 4 °C for the determination of the percentage of polymorphonuclear cells. Smears were prepared from the uterine fluid and fixed with absolute methyl alcohol and stained by Wright-Giemsa stain. Two stained smears per sample were used and the average of the two readings of >12% was excluded from this study to exclude cases of subclinical endometritis which may hinder pregnancy ([Dubuc et al. 2010](#)).

### Treatment protocols

Buffaloes were randomly assigned to four treatment groups of 25 cows each. Buffaloes in treatment 1 were injected with estradiol benzoate 1 mg and GnRH 250  $\mu$ g (Cystorelin, Ceva Sante Animale, La Ballastiere-33501, Libourne Cedex, France) on day 0, a CIDR in the anterior vagina from day 0 to 8 (to give 48 h for the PGF2 $\alpha$  action for the luteolysis of corpus luteum before GnRH injection), on injection with 25 mg of PGF2 $\alpha$  (Intervet, B.V., Boxmer, Holland) on day 7 and GnRH (250  $\mu$ g) on day 9. Buffaloes in treatment 2 received GnRH (250  $\mu$ g) on day 0, a CIDR from day 0 to 7 and 25 mg of PGF2 $\alpha$  on day 7. Buffaloes in treatment 3 received a CIDR from day 0 to 7 and 25 mg of PGF2 $\alpha$  on day 7. Treatment 4, a control group received no treatments.

To reduce the possibility of CIDR removal by pen mates or other buffaloes, CIDR tails were clipped to be appearing on vulva lips. Oestrus was detected on farm by expert personnel using visual estrus detection every 3 hours (8 times daily) as the method described by [Senger \(1994\)](#).

According to [Suthar and Dhami \(2010\)](#) acceptance of the male is considered as the most reliable estrus indicator in buffalo as well as frequent urination, bellowing, swelling of the vulva and clear, shiny, stringy odorless mucus discharge.

All buffaloes were mated naturally at least two times after oestrus detection with buffalo bulls of proven fertility. Pregnancy status was diagnosed by palpation of uterine contents 45 days after mating. Close observations by owners to record any buffalo retained to estrous from mating to time of pregnancy diagnosis.

### Statistical analysis

Pregnancy rates of buffaloes in different treatment groups and control were compared using  $\chi^2$  test.

The level of significance was observed at the 5% level. Statistical analyses were performed with the software Sigma Stat (Jandel scientific software V2.0 2004; Richmond, CA, USA).

## RESULTS AND DISCUSSION

None of the CIDR devices were lost during the experiment. Buffaloes in the different CIDR protocols exhibited oestrus signs at 70±1 h (treatment 1), 74±1 h (treatment 2) and 96±1 h (treatment 3) after CIDR withdrawal. Pregnancy results are shown in Table 1.

**Table 1** Pregnancy rate of buffaloes with repeat breeding treated using different treatment protocols

Treatment protocols	No. of animals	No. of buffaloes pregnant	Pregnancy rate (%)
Treatment 1 (E <sub>2</sub> +GnRH+CIDR— <sup>8days</sup> —PGF <sub>2</sub> α+GnRH)	25	11	44 <sup>a</sup>
Treatment 2 (GnRH+CIDR— <sup>7 days</sup> — PGF <sub>2</sub> α)	25	13	52 <sup>a</sup>
Treatment 3 (CIDR— <sup>7 days</sup> — PGF <sub>2</sub> α)	25	6	24 <sup>a</sup>
Control	25	2	8 <sup>b</sup>

The means within the same column with at least one common letter, do not have significant difference (P>0.01).

Overall, buffaloes that were treated with different CIDR protocols had a significant higher pregnancy rate (40% (30/75) than control group (8% (2/25); P<0.05).

Separately, the CIDR treatment protocols used in the present study did not significantly different in pregnancy rate. Controlled internal drug release (CIDR) has recently come to the forefront in various countries throughout the world for oestrus synchronization, increased pregnancy rates and the treatment of postpartum infertility in cattle (MacMillan and Peterson, 1993).

CIDR has been effectively used to treat infertility problems in buffaloes (Metwelly, 2001; Singh, 2003; Singh *et al.* 2003; Metwelly, 2006; Drost, 2007). In the current study, repeat breeder buffaloes (n=75) received progesterone application through CIDR in the various stages of the oestrus cycles using different protocols.

A total of 48 out of 75 buffaloes became pregnant (64%) in the different treatment protocols. Results of the present study indicated no significant differences between the different CIDR protocols used in this study.

Although, treatments incorporated a GnRH injection at the day of CIDR insertion seemed to result in a higher pregnancy rate 44% (treatment 1) and 52% (treatment 2) compared to treatment 3 (24%) where no GnRH was injected.

GnRH injection at the day of CIDR insertion increases the level of progesterone by providing luteal support (Drost, 2007), which exerts negative feedback on hypothalamus and anterior pituitary, favoring GnRH, FSH and LH storage (Zaabel *et al.* 2009).

As a consequence, the drop in circulatory concentration of progesterone after CIDR withdrawal promotes the release of GnRH, followed by FSH and LH release with subsequent resumption of ovarian cyclicity (Zerbe *et al.* 1999).

Also, GnRH injection on luteal phase (most buffaloes were in this stage) could decrease the estradiol concentration which is high during this period by luteinizing dominant follicle and suppress the rise of oxytocin receptor and secretion of PGF<sub>2</sub>α in uterus (Ryan *et al.* 1994).

The increased circulatory concentration of progesterone has sensitized the hypothalamic-pituitary system (Singh, 2003).

Likewise, progesterone increased hypothalamus sensitivity to estrogen with subsequent increase in the intensity of heat (Fabre-Nys and Martin, 1991). This is in agreement with Singh (2003) who pointed out that using CIDR + GnRH in combination with intramuscular injection of PGF<sub>2</sub>α after CIDR withdrawal was more effective than CIDR alone in terms of exhibition of oestrus and conception rate. This can be explained by the fact that PGF<sub>2</sub>α increases pituitary responsiveness to GnRH in the postpartum cow (Randel *et al.* 1996). Hence, the released GnRH after CIDR removal effectively stimulates the pituitary gonadotropins with subsequent oestrus induction in buffaloes. This may give a reason for the poor response of repeat breeder buffaloes subjected to the CIDR 7 days + PGF<sub>2</sub>α treatment regime. The present study using estradiol benzoate +GnRH+CIDR—8days—PGF<sub>2</sub>α+GnRH on day 9 for treating repeat breeding in buffaloes demonstrated that there was no beneficial effect of using estradiol benzoate in combination with GnRH at 9 day of the treatment protocol. In the present study, injecting estradiol benzoate to milking buffaloes resulted in a sudden decrease in milk production as complained by buffalo's owners. From these data, it could be concluded that the use of different CIDR protocols can be applied to improve fertility of repeat breeder Iraqi buffaloes.

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