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Reproductive performance of anestrous buffaloes treated with CIDR

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Abstract

The aim of this study was to evaluate the efficacy of controlled internal drug release (CIDR) and its reuse in treatment of inactive ovaries in buffaloes with subsequent resumption of cyclicity and reduction of inter-calving intervals. This study was conducted on 54 anestrous buffaloes suffering from ovarian inactivity. The animals were treated by new CIDRs and disinfected second used CIDRs for 7 or 14 days and $PGF_{2\alpha}$ with or without GnRH. The highest estrus induction rate (EIR; 100%), pregnancy rate (PR; 100%) and 1st service conception rate (1st service CR; 83.3%) were achieved with the treatment regime (CIDR 7 days plus i.m. injection of 25 mg of $PGF_{2\alpha}$ in the 6th day plus i.m. injection of 10 µg GnRH in 8th) day followed by the treatment regime (CIDR 14 days plus i.m. injection of $PGF_{2\alpha}$ in the 13th day); where the EIR, PR and 1st service CR were 85.7, 71.4 and 57.1%, respectively. It could be concluded that the use of CIDR 7 days + i.m. injection of 25 mg of $PGF_{2\alpha}$ in the 6th day + i.m. injection of 10 µg GnRH in 8th day is an alternative to restart the ovarian activity in buffalo cows.

Keywords: anestrous, buffaloes, CIDR, GnRH, inactive ovaries.

Introduction

Efficiency of reproduction is the key for a profitable herd. To maximize the productive life of a buffalo cow, it should be bred within 80-90 days after parturition to produce a calf and start a new lactation every 13-13.5 months (Abdalla, 2003; El-Wishy, 2007). Moreover, longer inter-calving intervals in buffaloes are mainly due to prolonged postpartum anestrum (Barile, 2005a, b) which is mainly attributed to ovarian inactivity (Hattab and Osman, 2000).

Postpartum anestrous is affected by several factors such as nutrition plane, milk yield, body condition score (BCS) at calving, suckling, parity, calving season and other factors as documented by Shah *et al*. (1986), Barile (2005b) and El-Wishy (2007).

During the last few years, several studies have been attempted to treat the prolonged postpartum anestrum in buffaloes by using hormonal treatments such as gonadotropin releasing hormone (GnRH), gonadotropins (Gn), estrogen, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and progesterone (Metwelly, 2001; Singh *et al*., 2003; Metwelly, 2006).

The aim of this study was to evaluate the efficacy of progesterone as controlled internal drug release (CIDR) and its reuse in buffalo cows on its subsequent resumption of cyclicity and reduction of intercalving intervals.

Materials and Methods

This study was conducted on a dairy buffalo farm at El-Max, Alexandria, Egypt (Latitude: 9'31"N; Longitude: 51'13"E) in the period from June 2005 to August 2007.

Fifty four healthy buffalo cows between the second to the fifth parity (4.5-8 years old) that had not been detected in estrus since 3-9 months postpartum were used. These animals were suffering from ovarian inactivity and were selected for this study.

The animals were kept in groups with a shelter that corresponds to 50% of the total area. They were supplied daily with a balanced ration and water *ad libitum*. These buffalo cows had BCS ≥3 (scale 1 = thin to 5 = fat; Bhalaru *et al*., 1987). Animals were milked twice (7 am and 7 pm) daily by using a milking machine (range of daily milk yield 4-8 kg milk/head).

All animals were in healthy condition and kept under strict control measures for internal and external parasitism, as they undergo a periodical deworming and prophylactic vaccination against the endemic diseases.

Buffalo cows were considered to suffer from ovarian inactivity when neither corpora lutea nor follicles were detected at 10 day intervals by two ultrasonographic examinations (simultaneously with rectal palpation) on both ovaries of each animal (Bakr and Ramoun, 2000; Ramoun and Darwish, 2006).

Rectal palpation of both ovaries was performed according to Roberts (1986) and Noakes *et al*. (2001). Ultrasonographic examination was carried out according to Singh (1998), Dahiya *et al*. (2003), and Terzano (2005) using a real-time B-mode ultrasound scanner (ULTRA SCAN 50, Alliance Medical INC, 7800 côte-de-liesse, Canada) equipped with a 5-MHz transrectal linear array transducer.

The animals were distributed according to the treatment protocol into two groups:

Group I (CIDR first use)

Group I included 27 buffalo cows which were assigned into four treatment where they were treated by

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Progesterone in the form of CIDR (EAZI-BREED™CIDR®; InterAg, Hamilton, New Zealand) in different combinations and durations, as the following:

Treatment I.1 (CIDR 7 days + PGF2α)

Seven buffalo cows received CIDR for 7 days + i.m. injection of 25 mg dinoprost (5 ml Lutalyase™; Pharmacia N.V./S.A. Purus, Belgium) on the 6th day of CIDR insertion for each animal.

Treatment I.2 (CIDR 14 days + PGF2α)

Seven buffalo cows received CIDR for 14 days + i.m. injection of 25 mg dinoprost (5 ml Lutalyse) on the 13th day of CIDR insertion for each animal.

Treatment I.3 (CIDR 7 days + PGF2α + GnRH)

Six buffalo cows received CIDR for 7 days + i.m. injection of 25 mg dinoprost (5 ml Lutalyse) on the 6th day of CIDR insertion + i.m injection of 10 µg of Buserelene (2.5ml Receptal®; Intervet international B.V., Boxmeer, Holland) 24 h after CIDR removal for each animal.

Treatment I.4 (Control)

Seven non treated buffaloes were considered as a control.

At the end of the experiment, the CIDR devices from each treatment were collected separately, cleaned and disinfected. The CIDRs devices were reused according to Martinez *et al*., (2007) for further use in group II.

Group II (CIDR reuse group)*

Group II included 27 buffalo cows, which were distributed into four treatments (II.1, II.2, II.3, II.4) where they were treated by the previously used CIDRs (after disinfection) by the same regime sequence of group I.

Buffalo cows from all treatments were observed for estrus detection according to Srivastava and Sahni (2003) and naturally bred by a fertile bull. Rectal palpation and ultrasonographic examination were performed weekly for each buffalo cow post treatment for detection of resumption of cyclicity. Pregnancy diagnosis was conducted at 45-60 days after the last service by rectal palpation and ultrasonographic examination.

The CIDR device was inserted intra-vaginally as described by Macmillan *et al*. (1991) and the nylon filament attached to the CIDR was cut to be even with the vulva lips to prevent other buffaloes from removing the inserted device (Hill *et al*., 1992).

Treatment trials were evaluated according to Hafez (2000) and El-Bawab and Metwelly (1999) through estrus induction rate (EIR), treatment estrus interval (TEI), overall 1st service conception rate, number of services per conception (No. S/C) and overall pregnancy rate (PR).

Data were analyzed according to Norman and Bailey (1997). Student-T test or ANOVA with LSD were performed whenever needed.

Results

Reproductive performance after treatment with CIDR is illustrated in Fig. 1.

In Group I, three out of seven buffaloes received CIDR 7 days + $PGF_{2\alpha}$ (treatment I.1) exhibited estrus within 77.3 ± 19.0 h after CIDR removal. Thus, the EIR was 42.9% for this treatment which was confirmed by ultrasonographic examination. Out of the three buffaloes that responded, one buffalo conceived at the induced estrus. The remaining two animals conceived at the 1st spontaneous estrus (estrus following induced estrus). All animals that showed estrus became pregnant as confirmed by ultrasonographic examination 45-60 days after last mating.

Six out of seven buffaloes treated with CIDR 14 days + $PGF_{2\alpha}$ (treatment I.2) exhibited estrus within 89.3 ± 7.9 h after CIDR removal. Hence, the EIR was 85.7% for this treatment regime. Four out of the six recovered buffaloes conceived at the induced estrus, one conceived at 1st spontaneous estrus and the last animal serviced 3 successive times without conception (considered repeat breeder). So, finally five animals became pregnant.

All treated animals in treatment I.3 (CIDR 7 days + $PGF_{2\alpha}$ + GnRH) exhibited estrus within 65.2 ± 16.5 h after the end of the treatment. So, the EIR was 100% in this treatment. Out of the six buffaloes that responded, five buffaloes conceived at the induced estrus. The remaining animal conceived at the 1st spontaneous estrus. All of these animals became pregnant.

One of the seven control animals in treatment I.4 came into heat after 576 h from the beginning of treatment protocol. None of the animals lost the CIDR during the insertion period. Thus, the overall retention rate of CIDR in Group I was 100%.

Regarding Group II, only one case in treatment II.2 lost the CIDR at the 12 days and subsequently it was excluded from this reproductive performance evaluation of this treatment. Thus the overall retention rate of CIDR in Group II was 95% (19/20).

None of the seven treated buffalo cows in treatment II.1 (CIDR* 7 days + $PGF_{2\alpha}$) responded to the treatment. Out of the six treated buffalo cows in treatment II.2 (CIDR* 14 days + $PGF_{2\alpha}$), one (16.7%) buffalo cow resumed cyclicity and exhibited heat after 504 h from CIDR removal and conceived at this heat. Out of the six treated buffalo cows in treatment II.3 (CIDR* 7 days + $PGF_{2\alpha}$ + GnRH), 1 (16.7%) buffalo cow displayed estrus with formation of CL. The responded case came into heat 72 h after end of treatment. None of the seven control animals in treatment II.4 came into heat during the experimental procedures.

Figure 1 shows that the highest EIR, PR and 1st service conception rate among treatment were achieved in treatment I.3 (CIDR 7 days + $PGF_{2\alpha}$ + GnRH) followed by treatment I.2 (CIDR 14 days + $PGF_{2\alpha}$).

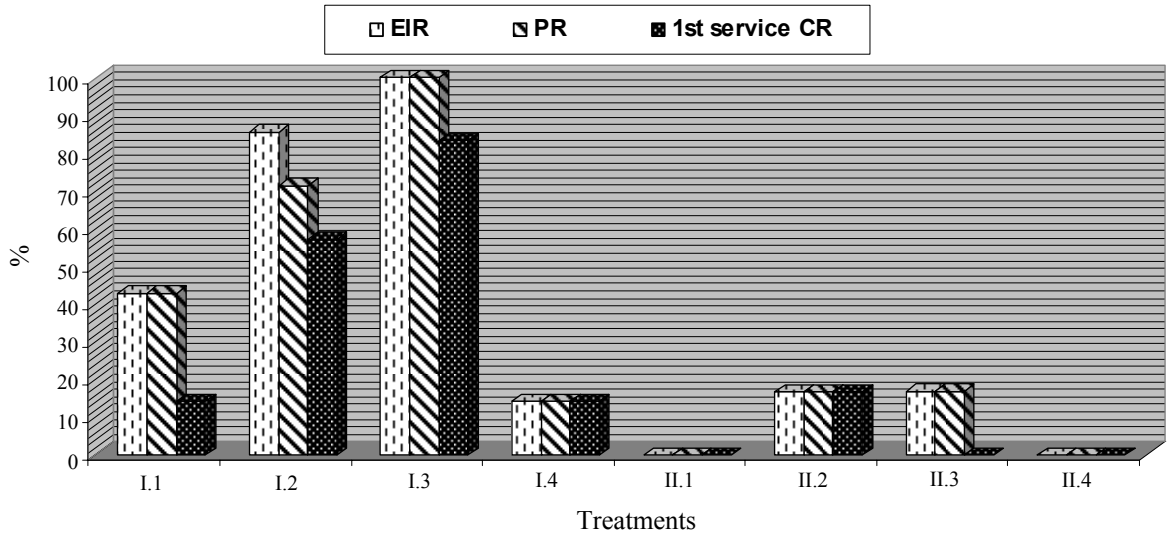

Fig 1. Reproductive performance of anestrous buffaloes after different treatments with CIDR.

Discussion

Several lines of evidence suggest that dysfunction of the hypothalamic gonadotropin releasing hormone (GnRH) and pituitary gonadotropins (FSH and LH) secretion are the contributing factors in the etiology of inactive ovaries (Aboul-Ela *et al*., 1985; Gordon, 1996).

CIDR has recently come to the forefront in various countries throughout the world for estrus synchronization, increased pregnancy rates and the treatment of postpartum anestrous in cattle (Macmillan and Peterson, 1993). CIDR has been effectively used to treat anestrous buffaloes (Andukar and Kadu, 1995; Andukar *et al*., 1997; Singh, 2003a).

The exhibition of estrus with subsequent ovulation (indicated by presence of CL) in the responded buffaloes after CIDR removal in group I, suggests that progesterone (P_4) had been released from CIDR inserted intra-vaginally in these buffaloes and was absorbed through the vaginal wall into the circulation (Singh, 2003a, b, c). This increased circulatory concentration of P_4 exerted negative feedback on hypothalamus and anterior pituitary. Hence, favoring GnRH, FSH and LH storage. Following termination of P_4 therapy (after CIDR withdrawal by the day 7 or 14 after insertion), the rapid drop in circulatory concentration of P_4 promotes the release of GnRH as the negative feedback of P_4 was abolished, followed by FSH an LH release with subsequent resumption of ovarian cyclicity (Zerbe *et al*., 1999). Also, the increased circulatory concentration of P_4 has sensitized the hypothalamic-pituitary system (Singh 2003a, b). Likewise, P_4 increased hypothalamus sensitivity to estrogen with subsequent increase in the intensity of heat (Fabre-Nys and Martin, 1991).

The EIR observed in treatment I.1 and I.2 agrees with the results of Singh (2003a), who reported that 33 and 83% of anestrous buffaloes with smooth ovaries treated with CIDR 8 days + $PGF_{2\alpha}$ in the 6th day of CIDR insertion and CIDR 14 days + $PGF_{2\alpha}$ in the 12th day of CIDR insertion responded to treatment within 48-120 h and 72-96 h, respectively.

The EIR in treatment I.2 is higher than that obtained by Singh (2003b) who achieved 70% (7/10) EIR after 14 day period of CIDR without $PGF_{2\alpha}$ injection in anestrous buffaloes with smooth ovaries. This is in agreement with Singh (2003a) who pointed out that using CIDR in combination with i.m. injection of $PGF_{2\alpha}$ was more effective than CIDR alone in terms of exhibition of estrus and conception rate. This can be explained by the fact that $PGF_{2\alpha}$ increases pituitary responsiveness to GnRH in the postpartum cow (Randel *et al*., 1996). Hence, the released GnRH after CIDR removal effectively stimulate the pituitary gonadotropins with subsequent estrus induction in anestrous buffaloes.

In this study, it is clear that a period of 14 days of CIDR was superior to 7 days period in resumption of estrus cyclicity in anestrous buffalo cows. This observation is also demonstrated by Andurkar and Kadu (1995) and Singh (2003a). The latter author postulated that elevation of P_4 for at least 10 days (10-14 days) was sufficient to sensitize the hypothalamo-hypophyseal and gonadal system of buffalo for resumption of estrus cyclicity. Possibly inadequate release of P_4 by CIDR results in insufficient synthesis and storage of GnRH and pituitary gonadotropins in buffaloes to induce follicular development and ovulation (Dahiya *et al*., 2003). This may give a reason for the poor response of anestrous buffaloes subjected to the CIDR 7 days + $PGF_{2\alpha}$ treatment regime.

Treatment I.3 (CIDR 7 days + $PGF_{2\alpha}$ + GnRH) produced encouraging results in inducing cyclicity

(100%) in anestrous buffaloes within 89.3 ± 7.9 h. This may be attributed to the supplemental i.m. injection of GnRH (buserelene 10 µg) which may cover the insufficient GnRH released after 7 day CIDR insertion period. Moreover, GnRH injection 24 h after the end of treatment (before mating) may induce ovulation at the appropriate time relative to natural mating and to stimulate luteinization, thereby improving the chances of successful fertilization and embryo survival (Pineda, 2003; Herbert and Trigg, 2005; Pawson and McNeilly, 2005; Peters, 2005).

Although a single use of CIDR is recommended by the manufacturer, the residual P_4 content after a 7-day insertion period of the 1.38 g CIDR in cattle is 0.72 g (Rathbone *et al*., 2002), thus having the potential for reutilization. Reutilization of CIDR device had been widely reported (Stevenson *et al*., 2003; Colazo *et al*., 2004).

There are apparently no reports about reutilization of CIDR or the residual P_4 in CIDRs after the initial use for different periods in buffaloes. The results of CIDR reutilization in this work were disappointing in comparison to other studies on CIDR reutilization for estrus synchronization in cows with different approaches having been used to clean, disinfect or sterilize implants. The very poor EIR in group II suggests that the residual P_4 in the previously used CIDR is not sufficient to favor gonadotropin storage. This may be attributed to the extended exposure of CIDR to the disinfectant solution, which increases the P_4 loss from the device (Zuluaga and Williams, 2008).

Only one animal had lost (treatment II.2) the CIDR at the 12th day of insertion. Thus the overall retention rate of CIDR in group I was 100%. Likewise, Baruselli *et al*. (2002) reported 89.6% (86/96) retention rate. However, low retention rates were reported (62.5%, 15/24; Hill *et al*., 1992). Indeed, the latter authors recommended cutting off the plastic tail to prevent other buffaloes from removing the inserted device and that was what carried out in the present study. The overall retention rate of reused CIDR was 95% (19/20), this may be explained by the fact that the reused CIDR did not press as tightly against the vaginal wall as new CIDR (Colazo *et al*., 2004).

From these data, it could be concluded that the use of CIDR 7 days + i.m. injection of 2 mg of $PGF_{2\alpha}$ in the 6th day + i.m. injection of 10 µg GnRH in the 8th day can be applied to buffaloes in order to restart ovarian activity. Further investigations on the residual P_4 content after a 7-day and 14-day insertion period of the 1.38 g CIDR in buffaloes are needed to explain the poor response of anestrous buffaloes to CIDR reuse.

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