Reproductive Performance of Anestrus Buffaloes Treated With CIDR

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ABSTRACT

This work aimed to evaluate the efficacy of controlled internal drug release (CIDR) and its reusing in treatment of inactive ovaries in buffaloes with subsequent resumption of cyclicity and reduction of inter-calving intervals.

This study was conducted on 54 anestrus buffaloe cows suffering from ovarian inactivity. These animals were treated by new CIDRs and previously used CIDRs after disinfection for different durations (7 or 14 days) and with different combinations ($PGF_{2\alpha}$ 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 7d + i.m inj of 25 mg of $PGF_{2\alpha}$ in the 6th d + i.m inj of 10 µg GnRH in 8th d) followed by the treatment regime (CIDR 14d + i.m inj $PGF_{2\alpha}$ in the 13th d); where the EIR, PR and 1st service CR were 85.7%, 71.4% and 57.1%, respectively.

It could be concluded that using CIDR 7d + i.m inj of 25 mg of $PGF_{2\alpha}$ in the 6th d + i.m inj. of 10 µg GnRH in 8th d is the treatment of choice for ovarian inactivity in buffaloes.

INTRODUCTION

Efficiency of reproduction is the key for a profitable herd. To maximize its reproductive life, a buffalo cow must be bred within 80-90 days after parturition to produce a new calf and starts a new lactation every 13-13.5 months *(1,2)*. Moreover, longer inter-calving intervals in buffaloes are mainly due to prolonged postpartum anestrum *(3,4)* which is mainly attributed to ovarian inactivity *(5)*.

Postpartum anestrus is affected by several factors such as, nutrition plane, milk yield, body condition score (BCS) at calving, suckling, parity, calving season and other factors *(2,4,6)*.

During the last few years, several studies have been attempted to treat the prolonged postpartum anestrum in buffaloes by using a varity of hormonal treatments such as, Gonadotrophin releasing hormone (GnRH), Gonadotrophins (Gn), Estrogen, Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and Progesterone *(7-9)*.

This work aimed to evaluate the efficacy of controlled internal drug release (CIDR) and its reusing in treatment of inactive ovaries in buffaloes with subsequent resumption of cyclicity and reduction of inter-calving intervals.

MATERIAL AND METHODS

This study was conducted in 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 apparently healthy buffalo cows between the second to the fifth parity (4.5-8 years old) that had not detected in estrus since 3-9 months post partum. These animals were suffering from ovarian inactivity and were selected for this work.

The animals were kept in groups in a 50 % sheltered yard area. They were supplied daily with balanced ration and had free access to water.These buffalo cows had BCS ≥ 3 (scale 1 = thin to 5 = fat; *(10)*. Animals were milked twice (7 am and 7 pm) daily using a milking machine (Range of daily milk yield; 4-8 kg milk/head).

All animals were apparently in healthy condition and kept under strict control measures for internal and external parasitism, as they recorded a periodical deworming and

prophylactic vaccination against the endemic diseases

Buffalo cows were considered to suffer from ovarian inactivity when no corpora lutea were detected (image 1) by two rectal palpations (at 10 days interval) and two ultrasonographic examinations(simultaneously with rectal palpation) on both ovaries of each animal *(11,12)*.

The experimental animals were divided according to the treatment protocol into two groups.

Group I (CIDR group)

Including 27 buffalo cows which were assigned into four subgroups, where they were treated by Progesterone in the form of CIDR (EAZI-BREED™CIDR®; InterAg, Hamilton, New Zealand) in different combinations and durations, as the following:

Subgroup I.1 (CIDR 7d + PGF2α)

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

Subgroup I.2 (CIDR 14d + PGF2α)

In which 7 buffalo cows received CIDR for 14 days + i.m injection of 25 mg dinobrost (5 ml Lutalyse) on the 13th day of CIDR insertion for each animal.

Subgroup I.3 (CIDR 7d + PGF2α + GnRH)

In which 6 buffalo cows received CIDR for 7 days + i.m injection of 25 mg dinobrost (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 hr after CIDR removal for each animal.

Subgroup I.4 (Control)

7 Buffaloes, received no treatment and considered as a control.

At the end of this group; CIDRs of each subgroup were collected separately, cleaned and disinfected *(13)*.

Group II (CIDR* reusing group)

Including 27 buffalo cows, which were divided into 4 subgroups (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.

After treatment, buffalo cows in all subgroups were left with normal fertile bull and observed for estrus detection according to *(14)*. 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.

Rectal palpation of both ovaries was performed *(15,16)*. Ultrasonographic examination was carried out *(17-19)*. The ultrasound examinations were performed using a real-time B-mode ultrasound scanner (ULTRA SCAN 50, Alliance Medical INC, 7800 côte-de-liesse, Japan) equipped with a 5-MHz transrectal linear array transducer.

The CIDR device was inserted intravaginally *(20)* 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 *(21)*.

Treatment trials were evaluated *(22,23)*; through estrus induction rate (EIR), Treatment estrus interval (TEI), Overall 1st service conception rate, (1st service CR) No. of services per conception (No. S/C) and Overall Pregnancy rate (PR).

Data were statistically analyzed according to *(24)*.

RESULTS

Reproductive performance after treatment with CIDR is summarized in Table 1.

Table 1. Reproductive performance in different treatment groups.

Treatment subgroup	Response for treatment		TEI (Mean ± SE)	No. S/C (Mean ± SE)	Overall 1st Sevice CR	No. of animals become pregnant	Overall PR
	Responded cases	EIR					
Subgroup I.1 (CIDR 7d + PGF2α) n=7	3	42.9%	77.33 ± 18.98 h	1.67 ± 0.27	14.3 %	3	42.9 %
Subgroup I.2 (CIDR 14d + PGF2α) n=7	6	85.7 %	89.33 ± 7.87 h	1.5 ± 0.31	57.1 %	5	71.4 %
Subgroup I.3 (CIDR 7d + PGF2α + GnRH) n=6	6	100 %	65.17 ± 16.47 h	1.17 ± 0.15	83.3%	6	100 %
Subgroup I.4 (Control) n=7	1	14.3 %	576 h	1	14.3%	1	14.3%
Subgroup II.1 (CIDR* 7d + PGF2α) n=7	0	0 %	---	---	0 %	0	0 %
Subgroup II.2 (CIDR* 14d + PGF2α) n=6	1	16.7 %	504 h	1	16.7 %	1	16.7 %
Subgroup II.3 (CIDR*7d + PGF2α + GnRH) n=6	1	16.7 %	72 h	2	0 %	1	16.7 %
Subgroup II.4 (Control) n=7	0	0 %	---	---	0 %	0	0 %

EIR: estrus induction rate , TEI: treatment estrus interval, CR: conception rate, PR: pregnancy rate.

In Group I, 3 out of 7 buffaloes treated with CIDR 7d + $PGF_{2\alpha}$ (subgroup I.1) exhibited estrus within 77.33 ± 18.98 h after CIDR removal. Thus, the EIR was 42.9% for this subgroup which was confirmed by TRUS (Images 2,3). Out of the 3 responded buffaloes, one buffalo conceived at the induced estrus. The remaining 2 animals, conceived at the 1st spontaneous estrus (estrus following induced estrus). All the responded animals became pregnant as confirmed by TRUS 45-60 days after last mating (Image. 4).

Six out of 7 buffaloes treated with CIDR 14d + $PGF_{2\alpha}$ (subgroup I.2) exhibited estrus within 89.33 ± 7.87 h after CIDR removal. Hence, the EIR was 85.7 % for this treatment regime. Four out of the 6 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 5 animals became pregnant.

All treated animals in subgroup I.3 (CIDR 7d + $PGF_{2\alpha}$ + GnRH) exhibited estrus within 65.17 ± 16.47 h after the end of the treatment. So, the EIR was 100% in this subgroup. Out of the 6 responded buffaloes, 5 buffaloes conceived at the induced estrus. The remaining animal, conceived at the 1st spontaneous estrus. All these animals became pregnant.

One of the seven control animals in subgroup 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 to Group II, only one case in subgroup II.2 lost the CIDR at the 12 d and subsequently it was excluded from this subgroup. Thus the overall retention rate of CIDR in group II was 95% (19/20).

None of the 7 treated buffalo-cows in subgroup II.1 (CIDR* 7d + $PGF_{2\alpha}$), respond to the treatment. Out of the 6 treated buffalo-cows in subgroup II.2 (CIDR* 14d + $PGF_{2\alpha}$),one (16.7 %) buffalo cow resumed cyclicity and exhibited heat after 504 h from

CIDR removal and conceived at this heat.Out of the 6 treated buffalo-cows in subgroup II.3 (CIDR* 7d + $PGF_{2\alpha}$ + 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 subgroup II.4 came into heat during the experimental procedures.

From table 1; it is clear the highest EIR, PR and 1st service conception rate among treatment subgroups were achieved in subgroup I.3 (CIDR 7d + $PGF_{2\alpha}$ + GnRH) followed by subgroup I.2 (CIDR 14d + $PGF_{2\alpha}$).

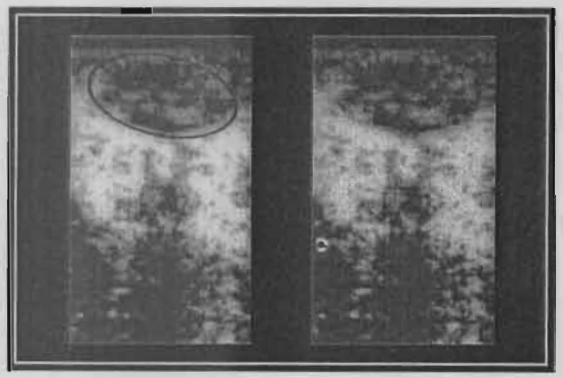

Image 1. Buffalo cow's ovary; inactive ovary

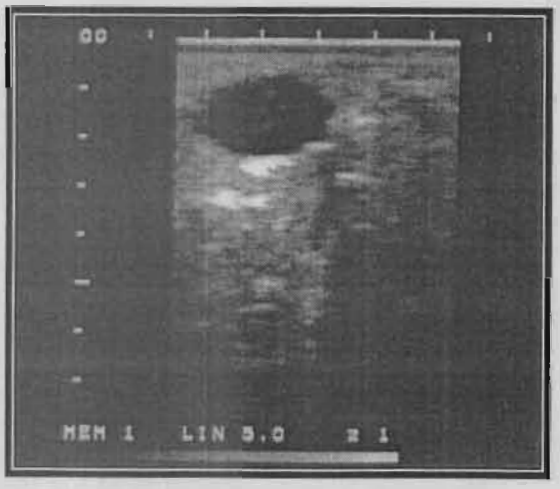

Image 2. Buffalo cow's ovary, showing amature graffian follide

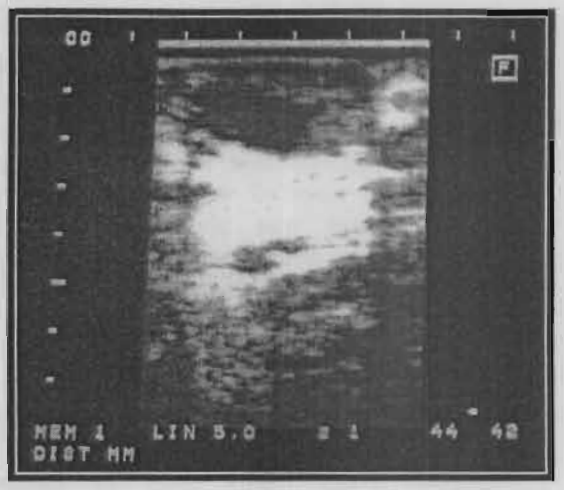

Image 3. Buffalo cow's ovary, showing corpus luteum.

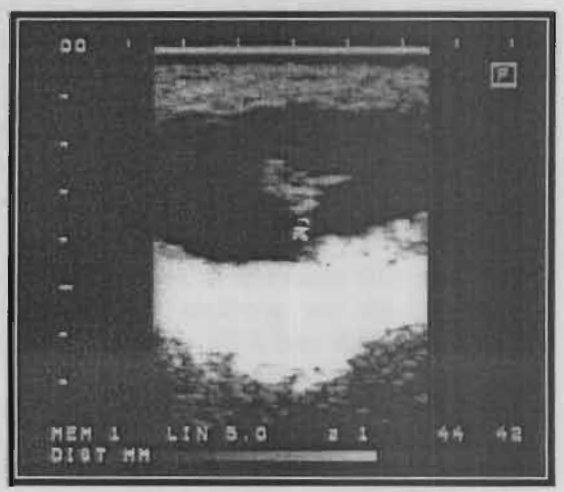

Image 4. Buffalo cow's pregnant horn 60d.

DISCUSSION

Several lines of evidence suggested that dysfunction of the hypothalamic gonadotropin releasing hormone (GnRH) and pituitary gonadotropin (FSH and LH) secretion are the contributing factors in the etiology of inactive ovaries *(25,26)*.

CIDR has recently come to the forefront in various countries throughout the world for estrus synchronization, increased pregnancy rates and the treatment of postpartum anestrum in cattle *(27)*. CIDR has been effectively used to treat anestrus buffaloes *(28-32)*.

The exhibition of estrus with subsequent ovulation (indicated by presence of CL) in the responded buffaloes after CIDR removal in group I, suggests that P_4 had been released from CIDR inserted intra-vaginally in these buffaloes and was absorbed through the vaginal mucus membrane into the circulation ***(30-32)***. This increased circulatory concentration of P_4 exerted -ve feed back 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 -ve feed back of P_4 was abolished, followed by FSH and LH release with subsequent resumption of ovarian cyclicity ***(33)***. Also, that increased circulatory concentration of P_4 have sensitized hypothalamic-pituitary system ***(30,31)***. Likewise, P_4 increased hypothalamus sensivity to estrogen with subsequent increase in the intensity of heat ***(34)***.

The EIR observed in subgroup I.1 and I.2 agrees with the previous report that which indicated 33% and 83% of anestrus buffaloes with smooth ovaries treated with CIDR 8d + $PGF_{2\alpha}$ in the 6^{th} day of CIDR insertion and CIDR 14d + $PGF_{2\alpha}$ in the 12^{th} day of CIDR insertion responded to treatment within 48-120 h and 72-96 h , respectively***(30)***.

The EIR in subgroup I.2 is higher than that obtained in India ***(30,31)*** which achieved a 70% (7/10) EIR, after 14 days insertion period of CIDR without PGF2α inj in anestrus buffaloes with smooth ovaries. Using CIDR in combination with i.m inj of $PGF_{2\alpha}$ was more effective than CIDR alone in terms of exhibition of estrus and conception rate ***(30)***. This can be explained by the fact that, PGF2α increases pituitary responsiveness to GnRH in the postpartum cow ***(35)*** Hence, the released GnRH after CIDR removal effectively stimulate the pituitary gonadotrophins with subsequent estrus induction in anestrus buffaloes.

In this study, it is clear that; an insertion period of 14 days of CIDR was superior than 7 days insertion period in resumption of estrus cyclicity in anestrus buffalo cows. This observation is in harmony with previously reported data which 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 ***(28,30)***. Possibly inadequate duration of CIDR insertion results in insufficient synthesis and storage of GnRH and pituitary gonadotrophis in buffaloes for induction of follicular development and ovulation ***(18)***. This may give a reason to the poor response of anestrus buffaloes subjected to the CIDR 7d + $PGF_{2\alpha}$ treatment regieme.

The treatment regime in subgroup I.3 (CIDR 7d + $PGF_{2\alpha}$ + GnRH) gave encouraging results in inducing cyclicity (100%) in anestrus buffaloes within 89.33 ± 7.87 h. This may be attributed to the supplemental i.m inj of GnRH (buserelene 10 µg) which may cover the insufficient GnRH released after 7 day CIDR insertion period. In respect, GnRH injection 24 h after end of treatment protocol (before mating) may induce ovulation at the appropriate time relative to natural mating and to stimulate luteinisation, thereby improving the chances of successful fertilization and embryo survival ***(36-39)***.

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 ***(40)***, thus having the potential for reutilization of CIDR inserts had been widely reported ***(41,42)***.

There are apparently no reports about re-utilization of CIDR or the residual P_4 in CIDRs after the initial use for different periods in buffaloes. But the result of CIDR reutilization in this work were disappointing with other studies on CIDR re-utilization for estrus synchronization in cows with different approaches have been used to clean, disinfect or sterilize inserts in these studies. The very poor EIR in the group II; suggests that the residual P_4 in the previously used CIDR is not sufficient to favor gonadotrophins storage.

This may be attributed to the extended exposure of CIDR to the disinfectant solution, which increase the P_4 loss from the device *(43)*.

None of the animals in group I & II lost the CIDR during the insertion period, except one case in subgroup II.2 lost the CIDR at the 12 d of insertion. Thus the overall retention rate of CIDR in group I was 100%. Likewise, it has been reported that 89.6% (86/96) retention rate *(44)*. However, a low retention rates were reported 62.5% (15/24) *(21)*. Indeed, the later authors recommended to cut off plastic tail to prevent other buffaloes from removing the inserted device and that what was carried out in the present study. The overall retention rate of reused CIDR was 95% (19/20), this may be explained by that the reused CIDR not press as tightly against the vaginal wall as new CIDR *(42)*.

From these data, it could be concluded that using CIDR 7d + i.m inj of 25mg of $PGF_{2\alpha}$ in the 6^{th} d + i.m inj. of 10 µg GnRH in 8^{th} d is the treatment of choice for ovarian inactivity in buffaloes. 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 anestrus buffaloes to CIDR reusing .

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الملخص العربي

الأداء التناسلي لإناث الجاموس التي تعاني من عدم الشياع بعد علاجها بالفتائل المهبلية المشبعة بالبروجسترون

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قسم التوليد و التناسل و التلقيح الاصطناعي - كلية الطب البيطري – جامعة المنصورة

تم إجراء هذه الدراسة على عدد (54) أربع و خمسون من إناث الجاموس(لم تظهر عليها علامات الشياع لاكثر من ثلاثة أشهر بعد الولادة) في مزرعة خاصة بمدينة المكس- محافظة الإسكندرية- مصر. و كانت هذه الإناث تعاني من خمول المبايض (كما اظهر التشخيص باستخدام الجس عبر المستقيم و الموجات فوق الصوتية). حيث تم استخدام هرمون البروجستيرون على هيئة فتائل مهبلية لمدد مختلفة (٧ أو ١٤ يوم) و بمصاحبة هرمونات مختلفة [البروستاجلاندين مع أو بدون الهرمون المحرر للحاثة المنسلية (المنشط للاقناد)]. بالإضافة إلى تقييم الفتائل المهبلية التي سبق استخدامها من خلال إعادة استعمالها مرة أخرى بعد تطهيرها.

داخل المجموعات المختلفة أظهرت النتائج أن أعلى معدل استجابة(١٠٠%) و نسبة حمل (١٠٠%) و نسبة إخصاب من التلقيحة الأولى (٨٣,٣%) كانت مصاحبة للنظام العلاجي (وضع الفتيلة المهبلية لمدة ٧ أيام داخل مهبل الحيوان + حقن ٢٥ مجم من البروستاجلاندين عضليا في اليوم السادس من وضع الفتيلة المهبلية + الحقن العضلي بالهرمون المحرر للحاثة المنسلية في اليوم التالي لنزع الفتية المهبلية) تلاه النظام العلاجي (وضع الفتيلة المهبلية لمدة ١٤ يوم داخل مهبل الحيوان + حقن ٢٥ مجم من البروستاجلاندين عضليا في اليوم الثالث عشر من وضع الفتية المهبلية) حيث كان معدل الاستجابة و نسبة الحمل و نسبة الإخصاب من التلقيحة الأولى؛ ٨٥,٧% و ٧١,٤% و ٥٧,١% على التوالي.