



## The efficacy of the recycled CIDR on estrus synchronization in postpartum anestrus in buffalo cows

N.Z. Mustafa<sup>1</sup> and M.A. Rahawy<sup>2</sup>

Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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#### Correspondence:

M.A. Rahawy

[mohammedrahawy@yahoo.com](mailto:mohammedrahawy@yahoo.com)

### Abstract

The present study aims to evaluate the efficiency of the reused controlled-internal drug-releasing devices (CIDR) compared with the hormonal method to induce estrus in the postpartum anestrus of Iraqi buffalo's cow. Thirty-two buffalo cows aged at least three years suffering from postpartum anestrus were randomly selected and divided into four groups (eight animals each). Ultrasound was used to assess the ovarian status. Animals with any reproductive defects were excluded. In the first group (G1), a new CIDR device was used for nine days, while in G2 animals, the device of the first group was reused for nine days; while, in the G3 group, the devices were further reused either for nine days (subgroup A) or 18 days (subgroup B, received PGF<sub>2</sub> $\alpha$ , at day 17; GnRH, at day 19). In the fourth group (G4), the animals received an injection of PGF<sub>2</sub> $\alpha$  (IM, 25 mg) followed by a dose of GnRH (IM, 500 mg) after 72h, without CIDR insertion. Blood samples were collected from the jugular vein pre and post CIDR insertion. Blood samples were utilized for the progesterone level assessment. For estrus and conception rates, animals were bred by fertile buffalo bulls, and pregnancy was detected at day 45 post-mating. Results showed that there was no variation in the rates of estrus induction and conception between the uses of the new CIDR device (G1) and the reused devices (G2 and G3 subgroup A); however, in the subgroup B and G4, only 50% of the treated animals demonstrated estrus. The duration, hormone, estrus, and pregnancy rate results revealed highly significant variations between groups. After the device withdrawal, the progesterone level in G2, G3, and G4 revealed a significant decline relative to G1. In addition, the duration was positively correlated to the progesterone level, estrus, and pregnancy rate. The progesterone level during the first and second services revealed a slight negative correlation with pregnancy. In conclusion, reusing the same CIDR up to three times for estrus synchronization is highly effective inducing estrus with high conception in postpartum anestrus Iraqi buffalo cows. Additionally, it is possible to reuse the CIDR device in 9 days without administration of GnRH in the lactating buffalo cows.

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### Introduction

Reproductive efficacy is a vital key point in commercial herds. To improve the productive productivity of a buffalo herd, buffalo cows should demonstrate estrus within 80-90 days after calving; thus, a calf is produced, and a new lactation period is started every 13-13.5 months (1).

Buffalo's reproduction and production are highly affected by postpartum reproductive disorders. The decreased reproductive efficiency in Iraqi buffaloes is especially due to postpartum anestrus, prolonged calving interval, and poor estrus detection (2). Early postpartum disorders manifested by increased days to first estrus, calving interval, open days, and service per conception are extended in diseased animals

more than in healthy animals (3). A survey on reproductive disorders showed that postpartum anestrus is the greatest cause of infertility in Iraqi buffalo cows, with an incidence of 12-18% (4). True anestrus due to inactive ovaries is an essential cause of infertility in buffalo cows, which leads to economic losses in buffaloe herds by declining milk and new calf production (1). The efficacy of the estrus synchronization in buffaloes by CIDR device protocol accompanied with GnRH and prostaglandin F<sub>2</sub> $\alpha$  mainly depends upon progesterone concentration in the device (5). The CIDR device protocol significantly the induction of estrus and conception rates in postpartum anestrus Iraqi buffalo cows (6). Estrus synchronization using CIDR devices plus GnRH protocol revealed high conception rates in buffalo cows (7). Because of the increased expenses of estrus synchronization programs, there is an increased demand to find other methods to decrease the cost along with the increased reproductive performance (8). The CIDR device programs are recommended for only a single use because of the risk of venereal disease transition. However, the substantial residual progesterone level in the single-used CIDR devices, made reusing more than once possible (9,10). It has been suggested that, in buffaloes, estrus synchronization by using CIDR for nine days, the blood progesterone remains detectable for the next two weeks of insertion. In addition, to decrease the costs, the previously used CIDR devices can be cleaned and re-inserted into the vagina of other buffalo cows (9). It has indicated no variation in estrus synchronizing between the reused and new devices (11,12). The use of CIDR devices in heifers and lactating cows after disinfecting and autoclaving methods before using the CIDR (13).

Therefore, the present study aimed to evaluate the effectiveness of the reused (1-3 times) CIDR device on the reproductive efficiency in postpartum anestrus Iraqi buffalo cows.

## **Materials and methods**

### **Animals**

This study was conducted on Iraqi buffalo cows in Mosul city- Iraq (latitude: 36° 20'N, longitude: 43° 8'E) from the period extended from September 2022 to February 2023. Thirty-two postpartum lactating Iraqi buffalo cows aged 4.52 $\pm$ 0.262 years old with body weight 378.2 $\pm$ 2.72 kg were included. The buffalo cows were kept under the same management and nutritional conditions. To eliminate any conflicting influence, animals with a history of cesarean operation, luteal cystic ovaries, pyometra, or other diseases were excluded from the study. Data were recorded for each buffalo cow including 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.

### **Clinical examinations**

Rectal palpation and trans-rectal ultrasonography examination of the female genital system were performed for each buffalo cow, where any buffalo cow with abnormal ovaries, uterus was excluded. Buffalo cows suffering from postpartum anestrus were included at least three months before the last calving (14).

### **Protocols of treatment**

Buffalo cows (n=32) aged at least three years suffering from anestrus at first three months from last calving were randomly chosen and divided into four groups (8 buffalo cows each). Ultrasound was used to assess the ovarian status for the presence of the persistent corpus luteum or inactive ovaries. In addition, it has been used to evaluate the oviducts and uterus to exclude any defect. A new CIDR device (progesterone 1.9g) was used in the first group (G1) for nine days, while in (G2) the devices of the first group were reused for other buffaloe cows for the same period. Animals in the G3 group were subdivided into two groups (4 buffalo cows in each subgroup); subgroup-A received double reused devices for nine days while subgroup-B received PGF<sub>2</sub> $\alpha$  (IM, 25 mg) 24h before the day of CIDR withdrawal (18 day) and 24h later animals were injected with GnRH (IM, 500 mg). In the fourth group (G4), buffalo cows (n=8) received an injection of PGF<sub>2</sub> $\alpha$  (IM, 25 mg) followed by a dose of GnRH (IM, 500 mg) after three days of PGF<sub>2</sub> $\alpha$  injection without the insertion of CIDR. Pre- and post-insertion of the CIDR devices (day 0 of insertion and the ninth day after withdrawal, respectively), blood samples were collected from the jugular vein to determine the blood progesterone level (15,16). Buffalo cows were inseminated naturally with fertile buffalo bulls. Pregnancy diagnosis was applied on day 45 post-mating to estimate the conception rate (17,18).

## **Results**

Results of the estrus synchronization by the reused CIDR device in postpartum anestrus buffaloes are presented in table 1. The analyzed data (estrus and pregnancy rate) between the studied groups exhibited highly significant variation ( $P \leq 0.001$ ) in estrus and pregnancy rate after a natural mating in the G1, G2, and G3A groups 100%, each. At the same time, estrus and pregnancy rates declined in G3-B and G4 (50% each).

Regarding the 1<sup>st</sup> and second services, the analyzed pregnancy rate data between the studied groups exhibited highly significant variation ( $P \leq 0.001$ ). The chi-square test demonstrated significant variations between groups in estrus duration, hormonal level, estrus, and pregnancy rates ( $P$  value 0.000, 0.000, 0.015, and 0.000, respectively) with  $\chi^2$  values 64.00, 32.00, 12.308, and 21.333, respectively, among the studied groups. Meanwhile, compared to the G1 and G2, the pregnancy rate from the first insemination declined in both the G3 and G4 groups (Figure 1).

Table 1: Results of CIDR application

Groups	No. Animals	Duration of insert CIDR days	PGF <sub>2</sub> α	GnRH	Estrus rate	Pregnancy rate
G1	8	9	+ve	-ve	100%**	100**
G2	8	9	+ve	-ve	100%**	100**
G3-A	4	9	+ve	-ve	100%**	100**
G3-B	4	18	+ve	+ve	50%	50
G4	8	-	+ve	+ve	50%	50

\*\* high significant at the P≤0.001 level.

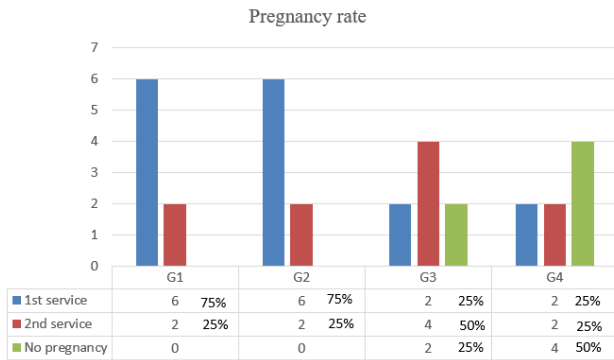


Figure 1: The pregnancy rates among groups according to the required insemination.

Before CIDR insertion, the results showed that the progesterone level significantly declined in the G2, G3, and G4 relative to the G1 group (P value 0.00). Similarly, after the device withdrawal, the G2, G3, and G4 revealed a significant decline in the progesterone level relative to its level in G1 (P value 0.024) (Figure 2).

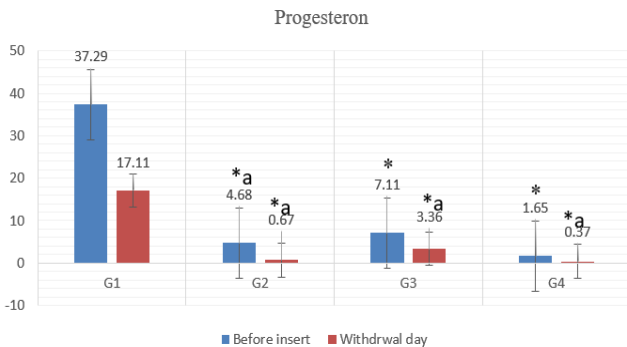


Figure 2: Variation in progesterone levels among experimental groups.

The Pearson correlation test showed that the duration was positively correlated to the hormonal level (r value 0.942), estrus (r value 0.584), and the pregnancy rate (r value 0.590, P value 0.000). The hormonal level was also positively correlated (P value 0.000) to estrus and pregnancy (r value 0.620 and 0.745, respectively). In addition, the estrus was

positively correlated to pregnancy rate (P value 0.000 with r value 0.832), as in table 2. The progesterone level during the first and second inseminations revealed a slight negative correlation with pregnancy (P value 0.038 with r value -0.368), as in table 3.

Table 2: Pearson Correlation Sig. (2-tailed) between the studied parameters in the treatment groups

Correlations	Duration	Hormone	Estrus	Pregnancy
Duration	1			
Hormone	0.942**	1		
Estrus	0.584**	0.620**	1	
Pregnancy	0.590**	0.745**	0.832**	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

Table 3: Pearson Correlation Sig. (2-tailed) of progesterone level with pregnancy rate

Correlations	Progesterone	Pregnancy
Progesterone	1	
Pregnancy	-0.368*	1

\* Correlation is significant at the 0.05 level (2-tailed).

### Discussion

The current study revealed that Iraqi buffalo cows suffering from postpartum anestrus and treated with a new or previously used CIDR device expressed similar reproductive performance, including estrus and pregnancy. A corpus luteum on the ovary causes negative effects on follicular development, and removing the exogenous progesterone hormone source (CIDR) promotes ovarian activity (19). On the other hand, in the postpartum period, the administration of PGF<sub>2</sub>α causes a sharp decline in progesterone levels, resulting in degeneration of the active luteal tissue; thereby, it blocks the negative feedback of progesterone on the pituitary hormones. In addition, following the withdrawal of

CIDR devices, the rapid drop in the circulating progesterone level would promote the release of GnRH from the hypothalamus. Consequently, the pituitary FSH and LH are released, followed by the resumption of ovarian cyclicity (20). Therefore, releasing GnRH after CIDR device removal can effectively stimulate the pituitary gonadotropins with consequent estrus induction in buffalo cow's postpartum anestrus (21).

A previous study on dairy buffalo cows revealed a positive impact of the exogenous progesterone embedded in the CIDR device on estrus induction and follicular development (22). The unused CIDR device contains progesterone in a concentration of 1.92g, while the residual progesterone concentration after the single use may reach  $1.31 \pm 0.01$  g, and the estimated progesterone release is 0.61g; in the same way, the received second and third used CIDR devices contain residual progesterone of 1.38 and 1.05 ng/ml, respectively (23,24).

In the present study, the ovaries of the presynchronized animals demonstrated insufficient follicular growth and absence of mature follicles with the presence of mature corpora lutea; however, after being synchronized with a new or reused CIDR devices, estrus was detected within 2-3 days (25). In addition, the estrus and pregnancy rates were 100% in the treated groups (G1-G3A). Therefore, high significance of a CIDR device appears to be the presence of a corpus luteum at the end of the estrus synchronization program without the need to complete the program by administration of GnRH hormone at day 11, when the presence of dominant follicle development in the final stage of the program (12). Similar observations of ovarian responses and pregnancy rates were recorded in buffalo cows treated with new or reused CIDR devices (25,26). These results suggested that treating buffalo cows with a new or used CIDR device during the postpartum period provides adequate regulation of follicular growth and improves the final follicular growth by reducing the progesterone level. Moreover, injection of PGF2 $\alpha$  before the CIDR removal in estrus synchronization programs improves fertility and pregnancy rates (12,27,28).

This study found that CIDR can be reused effectively up to three times (9 days each) to induce estrus and increase pregnancy rates. So that the progesterone released from both the new or reused CIDR is adequate to induce estrus, this might be attributed to the reduced level of progesterone released from reused CIDR devices, which can induce estrus following the removal of the reused CIDR (13). In this study, after the CIDR removal, the onset of estrus was earlier in buffalo cows treated with the reused CIDR (1-3 times) than in animals with a new CIDR. It has been suggested that the low progesterone released from the reused CIDR might efficiently control ovarian follicular growth, where a similar effect on the induction of estrus and pregnancy rates of the postpartum anestrus buffalo cows was revealed (25,29).

There was a significant variation in the progesterone level between the day of CIDR insertion and the withdrawal

possibly due to the administration of PGF2 $\alpha$  on the eighth day. The response of postpartum anestrus buffaloes to the new or reused CIDR protocols might predict a high response to estrus and pregnancy (30-32). Furthermore, buffalo cows that responded to CIDR protocols during the postpartum period had progesterone hormone levels  $\geq 2.0$  ng/ml at the time of CIDR insertion (33,34). After the PGF2 $\alpha$  injection, the decreased progesterone level (less than 2.0 ng/ml) might be considered a positive responsive sign for the treatment (35,36). However, with low progesterone, the growing follicles might undergo persistent follicle and during the LH surge, the follicle growth will be promoted toward ovulation (13,37).

Using PGF2 $\alpha$ - GnRH protocol for estrus synchronization in buffalo cows can promote luteolysis, decrease the progesterone level, and induce estrus (38). However, another study in buffalo cows noted that using GnRH-PGF2 $\alpha$  protocol for estrus synchronization revealed higher estrous observation than other treatments (39). In the present study, administration of PGF2 $\alpha$  in buffalo cows with the presence of corpus luteum caused a decline of corpus luteum diameter, progesterone levels, and a rise in the follicle diameter, suggesting the initiation of a new estrous within 56-72hr. However, in the G4, only 50% of buffalo cows failed to ovulate after administering PGF2 $\alpha$ , possibly due to the declined serum progesterone level in the first 24-48 hr. This decline did not reach  $>1.0$  ng/mL after administration, where this result agrees with a previous study (7,40).

## Conclusions

The present study verified that the reused CIDR, up to three times, can effectively increase the reproductive performance in the postpartum anestrus buffalo cows by inducing estrus and increasing the pregnancy rates. Notably, following this protocol can halve the expenses of postpartum anestrus in buffaloes.

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## Conflict of interest

The author has no financial or personal ties to groups or individuals who might have improperly influenced the paper's content.

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## فعالية إعادة تدوير استخدام اللولب المهبلي لتوحيد الشبق في إناث الجاموس التي تعاني من انعدام الشبق بعد الولادة

نور جان مصطفى زينل و محمد عبد الاله رحاوي

فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### الخلاصة

تهدف الدراسة الحالية الى تقييم كفاءة إعادة استخدام اللولب المهبلي ومقارنة مع الطريقة الهرمونية في إحداث الشبق إناث الجاموس العراقي التي تعاني من انعدام الشبق بعد الولادة. اثنان وثلاثون من إناث الجاموس بعمر لا يقل عن ثلاث سنوات تعاني من انعدام الشبق بعد الولادة اختيرت عشوائيا وقسمت الى أربعة مجاميع (كل مجموعة مكونة من ثمانية إناث). فحصت حالة المبايض والرحم باستخدام جهاز الأمواج فوق الصوتية لاستبعاد أي عيوب تناسلية. المجموعة الأولى تم فيها إدخال اللولب المهبلي الجديد لمدة ٩ أيام بينما المجموعة الثانية تم إعادة استخدام نفس اللولب المهبلي المستخدم بالمجموعة الأولى و لمدة ٩ أيام، أما المجموعة الثالثة تم إعادة استخدام اللولب المهبلي المستخدم لمرة واحدة وقسمت هذه المجموعة الى تحت المجموعة ١ و لمدة ٩ أيام ومجموعة الثالثة ب و لمدة ١٨ يوما حيث تم حقن البروستوكلاندين يوم ١٧ وحقن هرمون المحرر للقتد يوم ١٩ أما المجموعة الرابعة فهي مجموعة استخدم البرنامج الهرموني حيث تم حقن البروستوكلاندين بجرعة ٢٥ ملغرام بالعضل وبعد ٧٢ ساعة هرمون المحرر للقتد بجرعة ٥٠٠ ملغرام بالعضل بدون استخدام اللولب المهبلي، تم سحب عينات الدم من الوريد الوداجي من أجل تقدير مستوى هرمون البروجستيرون قبل وبعد إدخال اللولب المهبلي، تسجيل حدوث الشبق و معدل الحمل بعد اطلاق ثور الجاموس الخصب لتقدير إناث الجاموس وفحص الحمل بعد ٤٥ يوم بعد التسفيد. أظهرت نتائج عدم وجود فرق معنوي بين مجموعة استخدام اللولب المهبلي الجديد ومجاميع إعادة استخدام اللولب المهبلي لمرة و مرتين ولمدة ٩ أيام من حيث إحداث الشبق ومعدل حصول الحمل فيها من دون الحاجة لحقن الهرمون المحفز للقتد في اليوم ١١ ، بينما سجل فرق معنوي عالي في المجموعة الثالثة ب والمجموعة الرابعة لتصل نسب إحداث الشبق ومعدل الحمل فيها الى ٥٠%، كما سجل وجود فرق معنوي عالي في مستوى هرمون البروجستيرون إذ انخفض بعد سحب اللولب المهبلي في المجاميع الثانية والثالثة، ب والرابعة مقارنة مع المجموعة الأولى، بالإضافة الى وجود علاقة ارتباط موجبة بين فترة بقاء اللولب ومستوى هرمون البروجستيرون وإحداث الشبق ومعدل الحمل، فيما لوحظ وجود علاقة ارتباط سلبية بين حصول الحمل من التسفيد الأول والتسفيد الثاني مقارنة مع معدل حصول الحمل. نستنتج أن توحيد الشبق بإعادة نفس استخدام اللولب المهبلي ولثلاث مرات يكون بكفاءة عالية في إحداث الشبق وينسب حمل مرتفعة في إناث الجاموس العراقي التي تعاني من انعدام الشبق بعد الولادة، إضافة الى إمكانية إعادة استخدام اللولب لمدة ٩ أيام ودون الحاجة لحقن الهرمون المحفز للقتد في اليوم ١١ من برنامج توحيد الشبق في إناث الجاموس المرضعة.