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Synchronization of Estrus and Ovulation Using CIDR and Prostaglandin for Improving Pregnancy Rate of Repeat Breeder Egyptian Buffaloes

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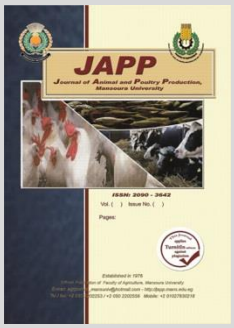

ABSTRACT

To synchronize estrus and ovulation for improving pregnancy rate (PR) of repeat-breeder buffaloes, Controlled Internal Drug Release (CIDR) + prostaglandin (PGF2α) was used before service of repeat breeder buffaloes. Total of 20 cyclic lactating buffalo-cows (4-7 years, 400-500 kg, non-pregnant up to 90-postpartum day) and 10 cyclic buffalo-heifers (2.5-4 years, 350-400 kg, not conceived after 3 services) were used in this study. In the 1st group, a CIDR was inserted for 9 days, regardless reproductive status, and then animals were intramuscularly injected with PGF2α 24 h prior to CIDR removal. In the 2nd group, control animals were used at the same interval. Animals in heat were naturally served and blood samples were collected on different days post-service for serum P4 determination. Pregnancy was diagnosed on day 25 post-service. Results showed that overall mean of PR was higher (P<0.01), while serum P4 at estrus was lower (P<0.001) in CIDR than in control, but both parameters were not affected significantly by animal type or CIDR x animal type interaction. Serum P4 at estrus was lower (P<0.05) in pregnant than in non-pregnant, regardless treatment or animal type. At the following post-service days, serum P4 showed the same trend of change, being higher (P<0.001) in CIDR than in control animals and in pregnant than in non-pregnant animals, regardless animal type. It could be concluded that the random usage of CIDR device for 9 days and prostaglandin F2α injection 24 h pre-CIDR withdrawal can be applied to improve pregnancy rate of repeat breeder Egyptian buffaloes.

***Keywords**: Buffalo, repeat breeder, CIDR, pregnancy, progesterone.*

INTRODUCTION

Reproductive efficiency is the key for a profitable herd and poor reproductive performance (long calving interval) is a main problem for buffalo breeders and farmers (Jaunadine, 1986; Singh *et al.*, 2000).

Ovarian inactivity, silent ovulation, endometritis and repeat breeding are the main reproductive disorders in Egyptian buffaloes (Ahmed *et al.*, 2010). Repeat breeding is an important reproductive disorder which causes great economic losses in farm animals. The repeat breeder animal is usually defined as sub-fertile animal which served three or more times and becomes not pregnant and continually return to service in the absence of any obvious pathological disorder in the genital tract (El-Khadrawy *et al.*, 2011). Reproductive inefficiency, without anatomical or infectious irregularities, due to repeat breeding syndrome is an expensive hitch in profitable dairy production (20-39%) as the age at first calving in heifers is delayed and the inter-calving interval is extended, thus leading to lowering of calf crop in cattle (Thakur *et al.*, 2006; Nanda and Singh, 2008) and buffaloes (Sah and Nakao, 2006; Azawi *et al.*, 2008).

Causes of repeat-breeding include reproductive hormones imbalance, ovulatory defects, poor fertilization and/or early embryonic loss (Nanda and Singh, 2008; Patel, *et al.*, 2014). In buffaloes, fertility problems are often not easily recognized; particularly studies on the repeat breeding syndrome are very few (Azawi *et al.*, 2008). In buffalo heifers, the main problem in incidence of repeat breeders was related to a disturbance in the time between onset of estrus, ovulation and insemination (Abo-Farw *et al.*, 2009).

In cattle and buffaloes, progesterone (P4) is essential for establishment of pregnancy as well as its maintenance, and embryo implantation (Kastelic, 1994). The potential beneficial effects of exogenous P4 supplementation on fertility have been acknowledged for a long time (Lonergan *et al.*, 2013). Improvements in reproductive performance in cycling cows treated with different P4 devices were reported by many authors (Walsh *et al.*, 2007; Chebel *et al.*, 2010). Many authors have been used Controlled Internal Drug Release (CIDR), GnRH, estrogen and prostaglandin to treat infertile buffaloes (Singh *et al.*, 2003; Metwelly, 2006; Azawi *et al.*, 2012). Higher fertility response in the buffaloes was obtained by using CIDR-GnRH protocol in postpartum period for the synchronization of estrus and ovulation (Ravikumar *et al.*, 2010). In cattle, use of a CIDR (PGF2α +CIDR-GnRH)-based AI protocol is a highly effective therapeutic strategy for establishing pregnancies in repeat-breeder (Honparkhe *et al.*, 2011). In addition, Kalwar *et al.* (2015) indicated that Ovsynch+CIDR protocol of estrus synchronization produces better results, and reduces the calving interval that improves the conception rate in Kundhi buffaloes in comparison with Ovsynch alone. Jiang

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et al. (2003) adopted CIDR-PGF2α to synchronize native swamp buffalo cows to 85.13%.

Based on the positive association between P4 pre-insemination level and fertilization (Abdel-Khalek *et al.*, 2018), the current study hypothesized that elevating P4 level by CIDR pre-estrus/service to increase fertilization may increase pregnancy rate in repeat-breeder buffaloes. In cattle, some attempts have been carried out to use Ovsynch+CIDR, but usage of CIDR in repeat-breeding buffaloes in Egypt is rare. Therefore, the present study aimed to synchronize estrus and ovulation for improve fertilization of repeat-breeder buffalo cows and buffalo heifers by using a CIDR+PGF2α before insemination.

MTERIALS AND METHODS

This study was conducted at Mehallet Moussa, Animal Production Research Station, Kaferelsheikh Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture during the period from April 2018 to August 2018.

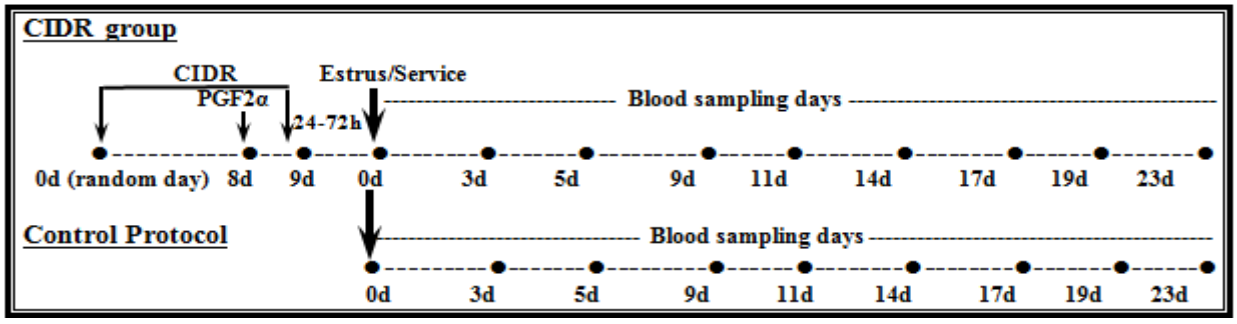
Animals:

A total of 20 cyclic lactating Egyptian buffalo cows (4-7 years old and 400-500 kg live body weight) after 90 days postpartum and 10 cyclic Egyptian buffalo heifers, 2.5 to 4 years of age and 350-400 kg live body weight, were used in this study. A number of factors were not taken into account, spanning from nutritional status, metabolic profile, experimentation season, and breed to technician skill.

All experimental buffalo and heifers used in this study normally cyclic with normal genital tract free from any diseases and disorders. All experimental animals failed to conceive after > 3 services/animal and considered to be namely repeat breeder animals. During the experimental period, all animals were housed in semi-open sheds and managed under similar feeding and managerial conditions.

Experimental design:

The experimental cows (n=20) and heifers (n=10) were allocated according body weight and age to two experimental groups (10 cows and 5 heifers in each). In the 1st group CIDR, an intra vaginal P4 device, CIDR, (1.9 g progesterone Canada) was inserted for 9 days, regardless reproductive status, and then animals were intramuscularly injected with 2.5 ml PGF2α/animal (Estrumate, Essex Animal Health Fresoythe Sedelsberger Strasse 2-4. 26169 Friesoythe, Germany) 24 h prior to CIDR removal. Each ml of Estrumate contained 250 µg cloprostenol sodium. Animals in the 2nd group (control) were left without treatment during the same interval of CIDR treatment. Animals in heat for CIDR group within 24-72 h after CIDR withdrawal as well as those in control one were naturally served by fertile buffalo bulls. The experimental protocol is summarized in the following diagram:



Blood samples and progesterone assay:

On day of estrus incidence and 3, 5, 9, 11, 14, 17, 19 and 23 day post-service, blood samples were taken from all animals in each group into sterilized glass tubes and kept at room temperature. Blood samples were centrifuged (at 3000 rpm for 15 min) within 1-2 h post-collection for serum isolation, then blood serum was stored (-20°C) till assaying progesterone profile by DirectRadioimmunoassay technique (RIA). Ready antibody coated tubes kit (Diagnosis Systems Laboratories Texas, USA) was used for P4 determination according to the procedure outlined by the manufacturer.

Pregnancy diagnosis:

Ultrasonoghrphy examination (Digital ultrasonic diagnostic imaging System, Model Dp-30 Vet. 50/60 HZ, SHENZHEN ,MINDRAY BIO-MEDICAL. ELECTRONICS, CO. LTD) at 7.5 MHz Linear array transducer and Depth 4.3 cm was used for pregnancy diagnosis on day 25 post-service. On day 50 post-service, pregnancy was indicated by rectal palpation of animals non-returned to estrus. Then pregnancy rate was calculated based on number of conceived animals relative to served animals.

Statistical analysis:

Data of P4 level at estrus and pregnancy rate as well as P4 level during each sampling interval were statistically analyzed by ANOVA as a factorial design using SAS program (SAS, 2004) to test the effect of protocol, animal type and their interaction (2 x 2) for P4 at each sampling time and pregnancy rate. The T-test analysis was done for determination the differences in P4 concentration between pregnant and non-pregnant.

RESULTS AND DISCUSSION

Results

Pregnancy rate and P4 level at estrus:

Pregnancy rate (PR) was significantly ($P<0.01$) higher, while progesterone (P4) concentration at estrus significantly ($P<0.001$) decreased in CIDR than in control group. Both PR and P4 concentration at estrus were nearly similar in cows and heifers. However, the effect of interaction between treatment and animal type was not significant (Table 1). The determined insignificant interaction between CIDR treatment and parity on PR and P4 level reflected higher PR with lower P4 level at estrus in CIDR treatment than in control either in buffalo cows or heifers (Figs. 1 and 2, respectively).

Table 1. Pregnancy rate and serum P4 concentration at estrus in repeat breeder buffaloes as affected by CIDR treatment, parity and their interaction.

Item	Treatment (T)				Parity (P)				Interaction
	Control	CIDR	SEM	P-value	Cows	Heifers	SEM	P-value	(T*P)
Pregnancy rate	10	60	0.122	0.008**	40	30	0.120	0.569	0.486
P4 at estrus (ng/ml)	0.465	0.190	0.023	0.000***	0.325	0.330	0.022	0.878	0.446

**** Significant at P<0.01. *** Significant at P<0.001.**

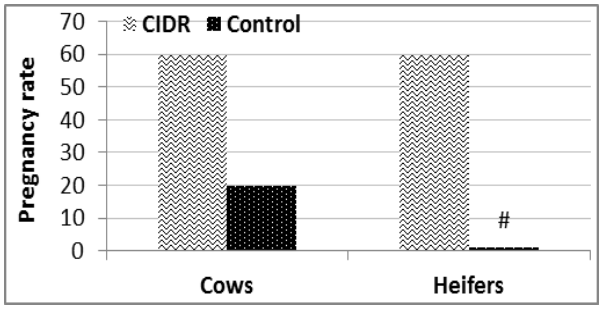


Fig. 1. Pregnancy rate of treated and control buffalo cows and heifers. (# pregnancy rate = 0)

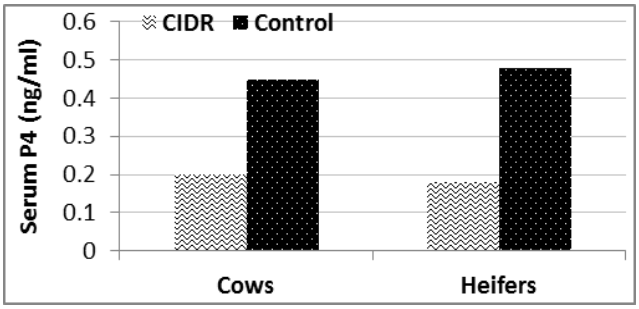


Fig. 2. Serum P4 level at estrus in treated and control buffalo cows and heifers.

Progesterone concentration at estrus in pregnant and non-pregnant animals:

Concentration of P4 at estrus significantly (P<0.05) increased in non-pregnant than in pregnant for CIDR and control groups, cows and heifers (Table 2) and in each animal type of CIDR and control groups (Fig. 3).

Table 2. Serum P4 level at estrus/service for pregnant and non-pregnant as affected by treatment and parity.

Item	Treatment		Parity	
	Control	CIDR	Cows	Heifers
Pregnant	0.340±0.050	0.155±0.024	0.213±0.041	0.133±0.033
Non-pregnant	0.476±0.018	0.250±0.018	0.400±0.026	0.414±0.029
P-value	-	0.011*	0.021*	0.047*

*** Significant at P<0.05.**

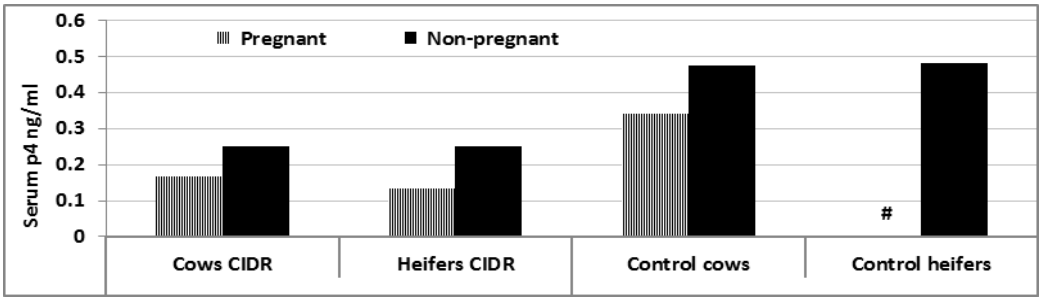


Fig. 3. Serum P4 level at estrus/service for pregnant and non-pregnant as affected by treatment and parity. (# None of heifers in control were pregnant)

Progesterone profile during post-service period:

Although overall mean of serum P4 concentration was significantly (P<0.001) lower in CIDR than in control at estrus, P4 concentration was significantly (P<0.001) higher in CIDR than in control at the following post-service days thereafter. Serum P4 concentration showed sharp increase in CIDR and slight increase in control group by advancing post-service day (Fig. 4). Overall mean of serum P4 concentrations were nearly similar in cows and heifers at estrus and the following post-service days with the same trend of increase by advancing post-service day in both parties (Fig. 5). The insignificant interaction between treatment and parity reflected higher serum P4 concentration at post-service days in CIDR than in control for either cows or heifers (Fig. 6).

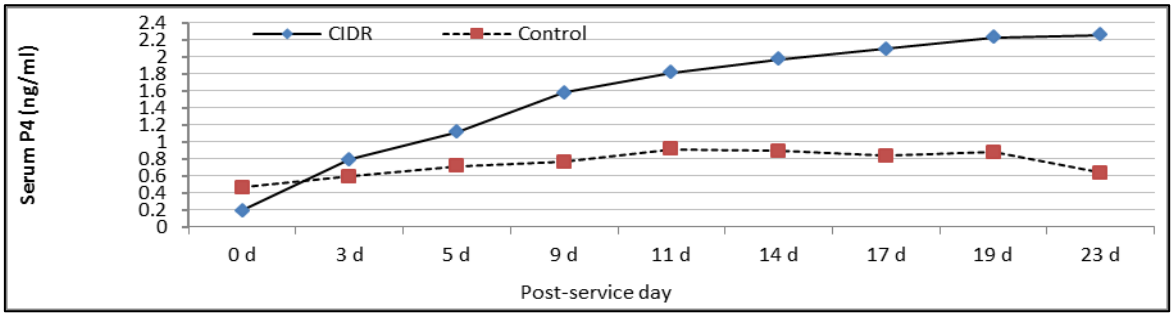


Fig. 4. Serum P4 level at estrus and successive post-service days of animals in CIDR and control groups

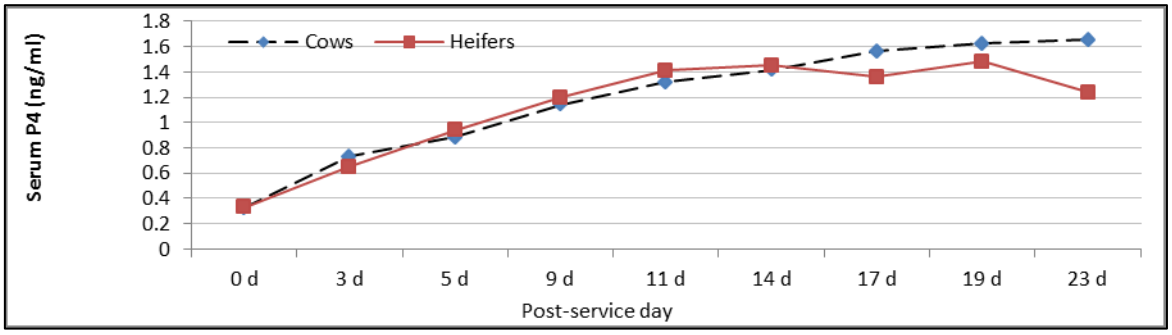


Fig. 5. Serum P4 level at estrus and successive post-service days of buffalo cows and heifers.

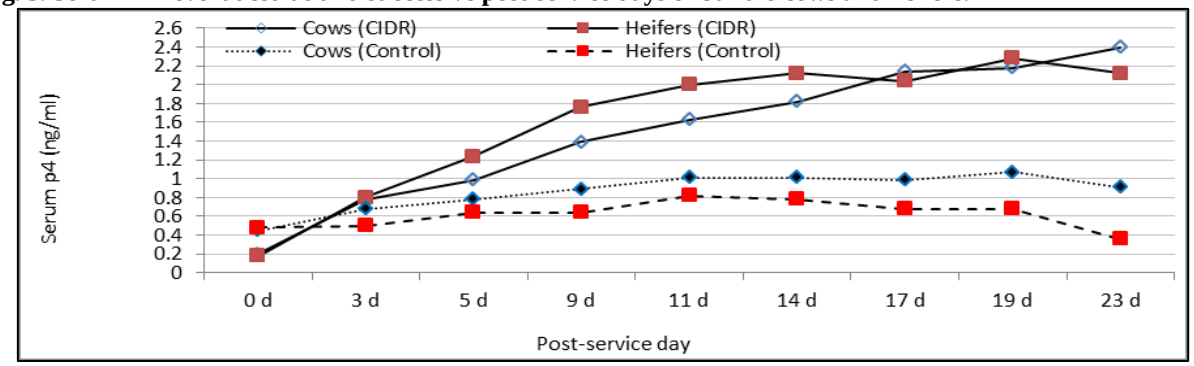


Fig. 6. Serum P4 level at estrus and successive post-service days of buffalo cows and heifers in control and CIDR treatment groups.

Progesterone profile during post-service period in pregnant and non-pregnant:

Overall mean of serum P4 concentration was significantly (P<0.05) lower at estrus and significantly (P<0.05) higher on the following post-service days in CIDR than in control group. In pregnant animals, P4 level was lower in CIDR than in control animals at estrus, while an opposite trend was observed on the following post-service days. In non-pregnant animals, P4 level was nearly similar on all post-service days in CIDR and control groups (Fig. 7). Similar trend of overall mean of P4 level was observed in pregnant and non-pregnant cows and heifers (Fig. 8) and for P4 level in pregnant and non-pregnant cows or heifers (Fig. 9).

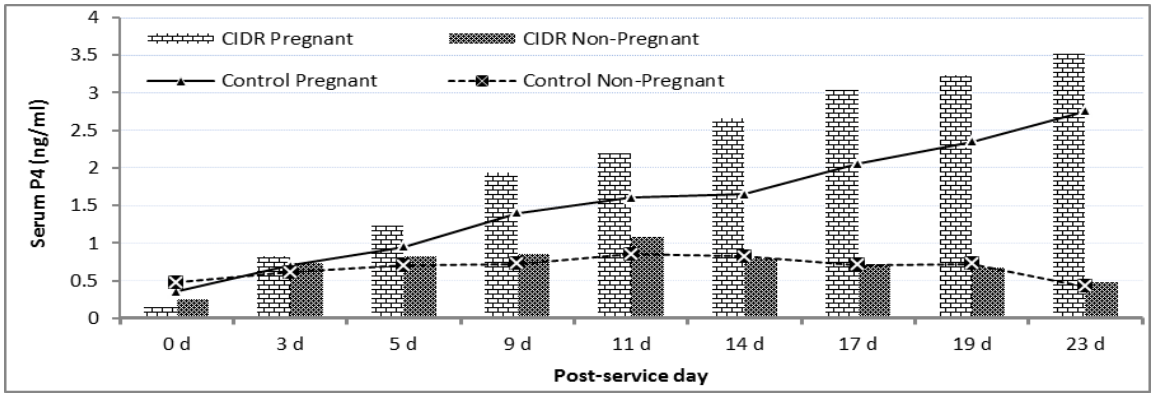


Fig. 7. Serum P4 level at estrus and post-service days of pregnant and non-pregnant animals in CIDR and control groups.

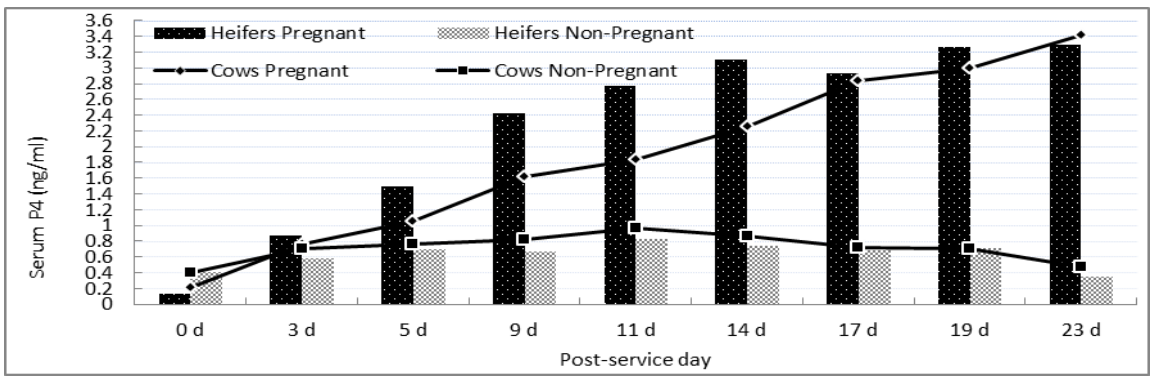


Fig. 8. Serum P4 level at estrus and post-service days of pregnant and non-pregnant buffalo cows and heifers.

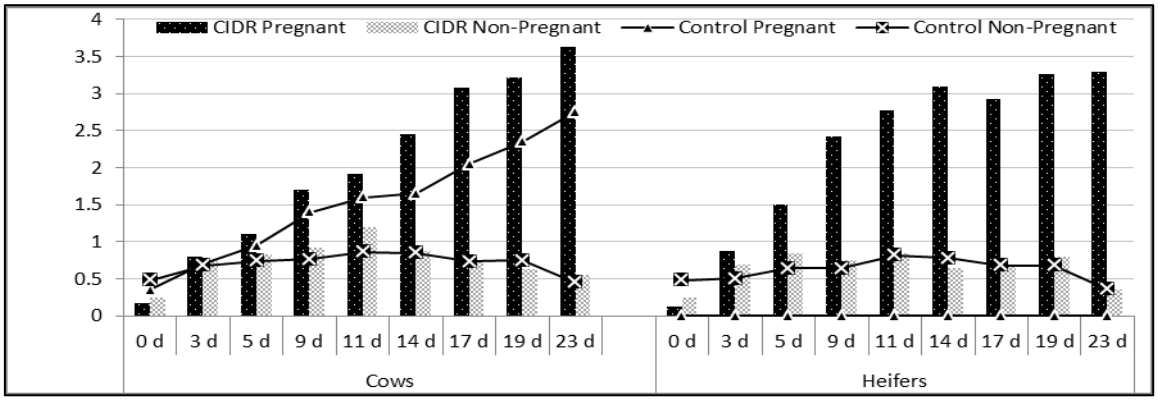


Fig. 9. Serum P4 level at estrus and post-service days of pregnant and non-pregnant buffalo cows and heifers in control and CIDR groups.

Disscusion

For increasing the reproductive efficiency of repeat breaded buffaloes (cows and heifers), the present study aimed to improve fertilization by using a Controlled Internal Drug Release (CIDR) for 9 days with PGF2α injection 24 h pre-CIDR withdrawal and animals in heat were served. In Iraqi buffaloes, Azawi *et al.* (2012) concluded that the use of different CIDR protocols can be applied to improve fertility of repeat breeder animals. The obtained results indicated higher pregnancy rate in CIDR than in control animals (60 vs. 10%, P<0.01). Efficacy of CIDR treatment on pregnancy rate was in parallel with pronounced reduction in serum P4 level at estrus as compared to control (0.190 vs. 0.465 ng/ml). It is of interest to indicate that P4 level at estrus ranged between ≤0.1 and <0.3 ng/ml in CIDR treated animals versus ≥0.3 to 0.6 ng/ml in controls. In CIDR treated animals, insertion of CIDR for 9 days lead to synchronization of the reproductive status of all animals within the luteal phase pre- PGF2α injection. In fact, Meneghetti *et al.* (2009) observed that when a PGF2α treatment was administered 48 h before CIDR withdrawal verified a positive effect on pregnancy rate to timing-artificial insemination (TAI) compared to the same treatment immediately after CIDR withdraw. Alnimer 2009, indicated that the CIDR device improved synchronization to return to estrus and increased PR to first AI during high temperature months by reducing embryonic losses in dairy heifers.

The noticed decrease serum P4 level observed at estrus in CIDR treated animals was mainly due to the action of PGF2α injection (day 8 of CIDR insertion) on CLs regression in all animals, regardless CIDR withdrawal on day 9. In our study, all treated animals came to estrus within 24-72 h after CIDR removal, which may indicate complete regression of CLs in all animals due to PGF2α treatment. Muth-Spurlock *et al.* (2016) reported that incidence of estrus in all animals treated with CIDR within 24-72 h of CIDR withdrawal may indicate higher level of P4 more than 1 ng/ml pre-CIDR treatment (cyclic animals). The exhibition of estrus in the responded buffaloes after CIDR removal, suggests that P4 had been released from CIDR inserted intra-vaginally in these buffaloes and was absorbed through the vaginal wall into the circulation (Singh, 2003 a,b,c). The repeat-breeder cattle exhibited estrus between 48–96 h after CIDR removal. Prolonged-treatment with P4 device increases the ability of the hypothalamo-pituitary-gonadal axis to generate estrus/LH surge in response to an increase in endogenous estradiol (Todoroki and Kaneko, 2006).

These findings may suggest the association of pregnancy (fertilization) with lowering serum P4 level at estrus, regardless animal typ. Also, Leonardia *et al.* (2012) found that PGF2α treatment was associated with ovulation in pre-pubertal heifers, regardless of whether exogenous P4 was used as a pre-treatment, and the hypothesis that PGF2α will induce ovulation by a luteolysis-independent mechanism was supported. Although PGF2α (and its analogues) are primarily used as luteolysins, they have also been reported to affect ovulation, implantation, pregnancy maintenance, and postpartum physiology (Weems *et al.*, 2006). Apparently, PGF2α increases pituitary responsiveness to GnRH, thereby enhancing the release of LH in a process leading to ovulation in postpartum cows and heifers (Randel *et al.*, 1996).

In accordance with the present results, Raj *et al.* (2016) mentioned that blood P4 concentration at the time of AI and pregnancy status was negatively correlated indicating that when P4 level was <0.3 ng/ml (basal level) at the time of AI, the chances of the animal becoming pregnant were more. The pregnancy rate was higher (65.90%) in buffaloes that had <0.3 ng/ml P4 concentration compared to buffaloes that had P4 concentration between 0.3–1.0 ng/ml (38.88%) and >1 ng/ml (0%) at the time of AI. Sankar and Vijayarajan (2014) recorded higher conception rate of buffaloes treated with CIDR and PGF2α than in controls (79.46 vs. 37.50%). In Iraqi buffaloes, Azawi *et al.* (2012) significantly increased pregnancy rate from 8% (control group) to 40% by insertion of CIDR. De Silva *et al.* (1981) reported that higher P4 level at the time of estrus might affected sperm and ovum transport, as well as the fertilization process and subsequent embryo passage to the uterus. In addition, repeat-breeder cattle that failed to conceive had active (P<0.05) luteal profile (plasma progesterone: 0.55±0.1 ng/ml) at the time of AI in comparison to their conceiving counterparts (plasma progesterone, 0.18±0.0 ng/ml). Suprabasal plasma progesterone (>0.3 ng/ml) around the period of LH surge is known to interfere with nuclear and cytoplasmic maturation of the oocytes by affecting LH surge parameters, and hence, subsequent embryo development (Duchens *et al.*, 1996). Out of these, about 70% record

suprabasal progesterone (0.35 ng/ml) at estrus, thus, leading to delayed ovulation of 76% follicles due to improper LH surge (Bage *et al.* 2002) because inadequate luteolysis prior to estrus might have resulted in higher circulating P4 level near AI which subsequently resulted in anovulation (Wiltbank *et al.,* 2002). Also, Duchens *et al.* (1995) reported that supra-basal progesterone level will delay the ovulation and lead to retention of Graafian follicle for an extended period and cause damage of the oocyte to such an extent that even inseminating close to the time of ovulation may not ensure fertilization.

Based on these findings, the present study may suggest somewhat modification of ovulation time and fertilization in repeat breeder animals in CIDR group resulting in elevating the pregnancy rate of animal in this group. In buffaloes, Raj *et al.* (2016) found that the ovulation rate for the buffaloes that had <0.3 ng/ml and >0.3-1.0 ng/ml was 100 and 90.90%, respectively, indicating that all the buffaloes that had <1 ng/ml of P4 at the time of first AI were ovulated. The ovulation rate was lower in buffaloes that had serum P4 value of >1.0 ng/ml compared to buffaloes that had serum P4 concentration of < 0.3 ng/ml (P<0.01). In the same line, using the shorter 9-day CIDR-PG protocol resulted in similar follicular dynamics at time points of interest and offered potential improvements in estrous response rate, synchrony of estrus, and TAI pregnancy rates (Thomas *et al.,* 2016). The inhibitory mechanism of CIDR on LH surge release and estrus requires more than 1 ng/mL of P4 circulation in the blood, which is considered the threshold level for CIDR device administration (Muth-Spurlock *et al.,* 2016). In this respect, Mantovani *et al.* (2010) suggested that the presence of corpus luteum during synchronization protocols is the main factor responsible for the increase in the plasma P4 concentrations and inhibition of dominant follicles growth. In good agreement with the present results, Sankar and Vijayarajan (2014) found that conception rate increased in buffalo cows or heifers treated with CIDR and PGF2α compared with controls. In this contest, Takkar *et al.* (1982) observed that P4 levels were 0.360±0.062 and 0.334±0.066 ng/ml on the day of estrus in buffalo-heifers and buffalo-cows, respectively. In contrast to the present results, Chacher *et al.* (2017) observed that blood P4 concentration and conception rate were lower for lactating cows than those for heifers.

In accordance with decreasing P4 level at estrus in pregnant than in non-pregnant buffaloes in the current study, Raj *et al.* (2016) found that the mean values of serum P4 in pregnant and no pregnant buffaloes were 0.895±0.134 and 1.429±0.235 ng/ml, respectively. It is of interest to note that increasing P4 concentration on post-service days in pregnant than in non-pregnant animals may be suggest that the delay in the normal rise in P4 concentrations between days 4 and 5 post-ovulation and low systemic P4 concentrations during the subsequent di-estrus reduce pregnancy rates and result in lower conception rates (Shams-Esfanabadi and Shirazi, 2006).

CONCLUSION

The CIDR and PGF2α -treatment increased the synchronization rate of estrus and ovulation in repeat breeder buffaloes compared with untreated group (Sankar and Vijayarajan, 2014). Ovsynch+CIDR protocol of estrus synchronization produces better results, and reduces the calving interval that improves the conception rate in Kundhi buffaloes in comparison with Ovsynch alone (Kalwar *et al.*,2015). Injection of PGF2α 24 h pre-CIDR withdrawal was associated with ovulation by a luteolysis-independent mechanism (Leonardia *et al.* (2012).

Based on the foregoing results, it is concluded that the pregnancy rate obtained was significantly higher in animals either buffalo-cows or buffalo-heifers treated with CIDR and PGF2α under field condition as compared to those in untreated groups. Both buffalo-cows and buffalo-heifers showed similar pregnancies for the CIDR and PGF2α treatment. By CIDR and PGF2ᾱ treatment the overall pregnancy rate improved to 60% as compared to 10% in controls. Accordingly, it could be concluded that the random usage of CIDR device for 9 days and 2.5 ml PGF2α/h injection 24 h pre-CIDR withdrawal can be applied to improve pregnancy rate of repeat breeder Egyptian buffaloes.

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التزامن الشبقي والتبويض باستخدام البروجيستيرون (CIDR) و البروستاجلاندين لتحسين معدلات الحمل في الجاموس المصري متكرر التلقيح

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تهدف هذه الدراسة لإحداث التزامن الشبقي والتبويض لتحسين معدل الحمل في الجاموس متكرر التلقيح باستخدام اجهزة القطع البلاستيكية المشبعة بالبروجيستيرون (CIDR) + البروستاجلاندين قبل تلقيح الجاموس متكرر التلقيح.أستخدم في هذه الدراسة عدد 20 من اناث الجاموس الحلاب متكرر التلقيح بعد 90 يوم من الولادة و تتراوح اعمارهم (4- 7 سنوات)، و اوزانهم (100- 500كجم)، وعدد 10 عجلات جاموسي تتراوح اعمارهم (2.5- 4 سنوات) ، أوزانهم (350 - 400كجم) ، وأخزت ثلاثة تلقيحات ولم تحرز.في المجموعة الاولى ، تم وضع السيدر بالمهبل (مصدر للبروجيستيرون الخارجي) لمدة 9 ايام بغض النظر عن الحالة التناسلية للحيوان وتم حقن الحيوانات بالبروستاجلانين قبل ازالة السيدر من المهبل ب 24 ساعة ، وفى المجموعة الثانية (كنترول) كانت الحيوانات بدون أي معاملة خلال نفس الفترة التجريبية للمعاملة الاولى . أظهرت الحيوانات شياع و لقحت طبيعيا بطلائق جاموسي مختبرة ، تم جمع عينات الدم في أيام مختلفة بعد التلقيح لتقدير مستوى هرمون البروجيستيرون في سيرم الدم، و تم تشخيص الحمل في اليوم ال 25 بعد التلقيح لكل حيوان.وكانت اهم النتائج المتحصل عليها:زاد المتوسط العام لمعدل الحمل معنويا ($P<0.01$)، بينما انخفض تركيز مستوى هرمون البروجيستيرون اثناء الشياع ($P<0.01$) في مجموعة السيدر عنه في مجموعة الكنترول، لكن لم يتأثر كل من معدل الحمل ومستوى هرمون البروجيستيرون معنويا ($P\geq0.05$) بنوع الحيوان او التداخل بين نوع الحيوان و السيدر. انخفض تركيز مستوى هرمون البروجيستيرون في الدم أثناء الشياع ($P<0.05$) في الحيوانات العشار عن الغير عشار، بغض النظر عن معاملة السيدر او نوع الحيوان، وخلال ايام ما بعد التلقيح زاد المتوسط العام لمستوي هرمون البروجيستيرون في سيرم الدم أخذ نفس اتجاه التغير ($P<0.001$) في مجموعة السيدر عنه في مجموعة الكنترول وفى الحيوانات العشار عنه في الحيوانات الغير عشار بغض النظر عن نوع الحيوان.يوصى البحث باستخدام بروتوكول السيدر (جهاز CIDR) لمدة 9 ايام و حقن البروستاجلاندين ($PGF2\alpha$) قبل ازالة السيدر ب 24 ساعة لتحسين معدل الحمل في الجاموس المصري متكرر التلقيح.