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TIMED ARTIFICIAL INSEMINATION (TAI) BASED ON CIDR FIRST, SECOND OR THIRD USE IN *Bos indicus* COWS

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RESUMO: O estudo objetivou verificar os efeitos de dispositivos intravaginais com progesterona (CIDR) de primeiro, segundo e terceiro uso e a taxa de prenhes (TP) na inseminação artificial em tempo fixo (IATF). Quinhentas e quarenta e sete vacas Nelore foram distribuídas: CIDR 1= CIDR de primeiro uso (n=196); CIDR 2= segundo uso (n=188) e CIDR 3 terceiro uso (n=163). Todos os animais foram submetidos ao mesmo protocolo, exceto ao número de usos do dispositivo: dia zero (d0), 2mg BE+CIDR1, CIDR2, CIDR3 respectivamente; no d9 remoção do CIDR + PGF2α + 0,6mg de ECP, + remoção do bezerro (48 horas); d11, IATF e retorno dos bezerros. A taxa de prenhes (TP) resultou em 63,8, 53,7 e 27,0% para CIDR1, CIDR2 e CIDR3, respectivamente. Houve diferenças da TP entre os grupos: CIDR1 e CDR2 (P=0,04); CIDR1 e CDR3 (P=0,0001) e CDR2 e CDR3 (P0,0001). Foram observadas diferenças (P<0,05) relativas aos dias abertos pós parto x escore da condição corporal (ECC) entre os grupos. Houve correlação entre dias abertos no CIDR 1 e 2 perante o ECC, assim como entre ECC do CIDR1 e 3 com a TP. Concluiu-se que o CIDR1 mostrou-se o mais eficiente que o CIDR2 ou o CIDR3; vacas submetidas ao protocolo para a IATF mais tarde após o parto tiveram melhor TP que as com menor período de dias abertos; o ECC de 3,1 resultou em maior TP do que 3,5 e 3,7.
Palavras-chave: IATF, Reuso CIDR, *Bos indicus*

ABSTRACT: The study aimed to verify the effects of CIDR (P4) first, second or third use on PR in protocols for TAI. Five hundred and forty-seven Nelore cows were divided: CIDR1 (CIDR first use; n=196); CIDR2 (second use; n=188) and CIDR3 (third use; n=163). All animals were submitted to the same protocol: day zero(d0), 2mg EB+CIDR1, CIDR2 or CIDR3, according the CIDR use; d9, CIDR removal + 12.5 dinoprost thrometamin+0.6mg ECP + 48 hours calf removal; d11, TAI + return of the calves to dams. The overall pregnancy rate (PR) was 63.8, 53.7 and 27.0% for CIDR1, CIDR2, and CIDR3, respectively. Differences in the PR were observed between: CIDR1 and CIDR2 (P=0.04); CIDR1 and CIDR3 (P=0.0001); and CIDR2 and CIDR3 (P=0.0001). The open days (OD) in *post p*artum and body condition score (BCS) resulted in differences (P<0.05) among groups. There was a correlation between open days in CIDR 1 and 2 versus BCS, as well as between BCS of the CIDR 1 and 3 against PR In conclusion, CIDR1 showed better reproductive efficiency than CIDR2 and CIDR3 use. Differences were observed between the groups in regard to DO, and by cows submitted to CIDR earlier in the postpartum period having a lower PR than those submitted at a later time. A BCS of 3.1 resulted in a higher PR than those with 3.5 and 3.7.
Key Words: TAI; Reused; CIDR; *Bos indicus*

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INTRODUCTION

In recent years, Brazil has seen an increase in the production, slaughter, and export of beef cattle meat (IBGE, 2012), surpassed only by the United States (USDA, 2012). Several factors have influenced this increase, including technical advancements in animal reproduction biotechnologies. Besides the expansion of conventional artificial insemination (AI, after estrus observation) (Baruselli *et al.*, 2012), other reproductive biotechnology—fixed time artificial insemination (TAI)—was developed (Pursley *et al.*, 1995), increasing its use among farmers.

The TAI reduces the period of postpartum anestrus and eliminates the deficiencies in correct detection of estrus caused by low skilled labor (Marques *et al.*, 2012). Moreover, the TAI enables animals to become pregnant without the estrus observation, and the AI is performed on pre-fixed days and times, resulting in reduced animal handling (Lamb, 2012).

In recent years, many hormonal TAI protocols have been proposed for beef (Demeterco *et al.,* 2014) and for dairy cattle (Macmillan, 2002). One of the limitations to further expansion of TAI in Brazil is the cost associated with the hormonal protocols (hormones) required for the controlled progesterone intravaginal device (CIDR). Rodgers *et al.* (2012) report real savings in terms of cost-effectiveness following the adoption of TAI protocols.

Several studies investigating CIDR re-use as a means of reducing the costs associated with the TAI protocols have been performed in cattle (Melo *et al.*, 2012) and goats (Vilarino *et al.,* 2011). Furthermore, Sudano *et al.* (2011) employed the reuse of ear implants with progesteron in protocols for cattle superovulation. Several studies have verified the efficiency of the re-use of CIDR or norgestomet ear implants in cattle, without compromising the pregnancy rates (PR) (Melo *et al.*, 2012). These authors reported that the re-use of the device did not affect the PR when compared with the use of new devices. Schneider *et al.* (2009), used the CIDR twice in beef cows reporting a lower PR using TAI protocols. Sudano *et al.* (2011) used P4 implants twice for embryo transfer and observed a lower rate of follicular stimulation of the ovaries (superovulation) in Nelore cows. Moreover, other studies have reported conflicting evidence regarding the re-use of the CIDR in TAI protocols.

To address the previously reported discrepancies, the present study aimed to compare the effectiveness of CIDR devices used for the first time (new) with those used a second and third time for estrus synchronization and fixed time artificial insemination (TAI) in a commercial herd of Nelore (*Bos indicus*) cows in cycling or in postpartum anestrus.

MATERIALS AND METHODS

The present study used 547 lactating multiparous Nelore cows (suckling calf) from a commercial herd. The animals were kept on extensive pasture (*Brachiaria brizantha*) and received water and mineral salt ad libitum. The cows were assigned to three groups as follows: CIDR1, CIDR device of first use (n = 196); CIDR2, CIDR device of second use (n = 188); and CIDR3, CIDR device of third use (n = 163). The body condition score (BCS) was 3.1 (range 2.5–4.0), 3.5 (2.5–4.0), and 3.7 (2.5–4.0) for CIDR1, CIDR2, and CIDR3, respectively (1 = emaciated, 5 = obese) (Hougton *et al.*, 1990). The animals were subjected to the TAI protocol at 55.7 (44–67), 47.0 (37–66), and 45.6 (39–49) days after delivery. The CIDRs of second and third use were washed with a povidone-iodine

detergent solution (PVP degermante, VANSIN LTDA, São Paulo, Brazil), washed in running water, rinsed with clear water, dried (in the oven), and finally stored. All animals were subjected to the same TAI protocol (Figure 1), with the only difference between groups being the number of times the CIDR had been used (CIDR1, device of first use; CIDR2, second use; and CIDR3, third use). On day nine (d9), the calves were removed from the dams for 48 h, and were returned on d11. Before commencement of the study, the ovaries were assessed by ultrasonography (Tringa model, 5-MHz transducer; Pie Medical) to determine the cyclicity or anestrus status using the following criteria: the presence of a corpus luteum (CL) or follicles of >8.0 mm (modified Baruselli *et al.*, 2004) were considered cycling cows, and the absence of a CL or follicles of <8 mm were considered animals in anestrus. The pregnancy check was performed by ultrasonography, eigth weeks after the TAI.

The same protocol was used for all groups, except on the number of times the CIDR had been used (first, second, and third use).

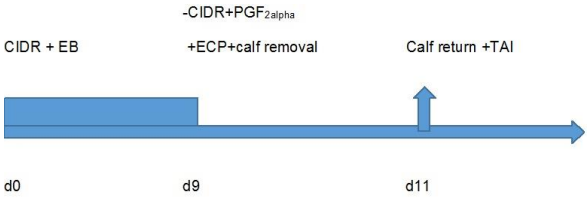

Figure 1-.CIDR (1.9 g Progesterone – Pfizer Saude Animal, São Paulo, Brasil); EB (2mg estradiol benzoate - Estrogin, Biofarm – SP); US (ultrasonography of the ovaries); PGF2 alfa (12.5 mg de dinoprost trometamin - Pfizer Saude Animal, São Paulo, Brasil); ECP (0.6 mg estradiol cipionate – Zoetis –Pfizer Saude Animal, São Paulo, Brasil); CR (calf removal) ; -CR (=return of the calf to dam); TAI (timed artificial insemination).

STATISTICAL ANALYSIS

The data were analyzed using the PROC GLM and PROC CORR procedures of SAS (SAS, Carey, NC, 1996) to determine the effects of treatment, PR, open days after delivery, and BCS among the groups. The differences between treatments were compared with Fisher's exact test and Student's t-test, and were considered significant at the level of P = 0.05. The variables open days, BCS and pregnancy rate were correlated (Pearson correlation) in order to measure the degree of association between the variables.

RESULTS AND DISCUSSION

Since the first protocols for TAI in cattle were developed (Patterson *et al.*, 1989, Savio *et al.*, 1993), the CIDR by slow release of P4 have been intensively studied (Sales *et al.*, 2012). The intravaginal devices initially developed in the industry were for one-time use only. To reduce the costs associated with the protocol, studies have investigated the re-use of the devices in cows without reducing the PR (Schneider *et al.*, 2009; Melo *et al.*, 2012). In the present study, a commercial herd was subjected to CIDR devices of first, second, and third use to determine the effects of re-use of the devices on the PR. In the present study, the PR was higher in the CIDR1 group (63.7%) than in the CIDR2 and CIDR3 groups (53.7% and 26.9%, respectively) (P<0.05) (Table 1).

Table 1 – Pregnancy rate (PR), days open and body condition score (BCS) in *Bos indicus* cows subjected to CIDR 1, 2 and 3 use in timed-artificial insemination protocol (n = 547).

Groups	PR (n)	(%)	Open days (days)	BCS (x ± s)
CIDR 1	125/196	(63.7)[a]	55.7 ± 8.8[a]	3.1 ± 0.3[a]
CIDR 2	101/188	(53.7)[b]	47.0 ± 8.9[b]	3.5 ± 0.5[b]
CIDR 3	44/163	(26.9)[C]	45.6 ± 1.2[b]	3.7 ± 0.5[b]

Different letters in the column are significant at the level of P<0.05)

The PR obtained in the CIDR1 group, compared with that in the CIDR2 and CIDR3 groups differs from that reported by other studies, where no differences were observed among animals, regardless of the number of times the CIDR device had been reused (Macmillan, 2002; Nascimento *et al.*, 2006; Schneider *et al.*, 2009; Melo *et al.*, 2012). The PR observed in the CIDR1 group was higher than that reported by the previously cited authors, who found

a significant variation in the PR of the cows that had received a CIDR of first use (38.5%, Nascimento *et al.*, 2006; 58.2%, Melo *et al.*, 2012). In our study, the PR between the groups decreased with re-use of the CIDR, contrary to the reports of Nascimento *et al.* (2006), where the animals that received CIDR3 devices showed a higher PR than those that received CIDR1 devices, based on animals of the same breed and with the same study aim as the present study. The differences between our findings and those reported in the literature become more evident in the studies of Melo *et al.* (2012), in which a higher PR was achieved in the cows that received CIDR4 devices (56.3%) than those that received CIDR2 (54.5%) or CIDR3 (55.9%) devices. The differences become more important when comparing data from the studies by Nascimento *et al.* (2006) and Rocha *et al.* (2007), reporting that the animals that received CIDR3 devices had a higher PR than those that received CIDR1 devices. The differences in PR could be attributed to the BCS being different in the cows used by Schneider *et al.* (2009), as well as to the parity (Colazo *et al.*, 2004) or nursing (Baruselli *et al.* 2004). Colazo *et al.* (2004) found similar results to the present study, after administering CIDR2 and CIDR3 devices to Bos taurus beef cows and obtaining a PR reduction according to the number of times the CIDR was used.

In the present study, we correlated open days postpartum and PR among the groups. The CIDR1 group was subjected to TAI at 55.7 days (median) after delivery, which is significantly later than that of the CIDR2 (47.0) and CIDR3 (45.6 days) groups, resulting in PR differences ($P<0.05$) (Table 1) in favor of the CIDR1 group. Bostedt *et al.* (1985) monitored cows from delivery to 72 days postpartum, noting that the estrus cycles, ovarian activity, and P4 profile became more regular as the period of service increased. Silveira *et al.* (2010) investigated the TAI in two groups of beef cows ([Group 1: 28–44 days; mean, 39.6 days postpartum] vs. [Group 2, with a delayed postpartum period: 45–90 days; mean, 57 days]) and found a higher PR in the group with the largest number of open days after delivery. Furthermore, the authors have reported an improvement in PR in cows subjected to the TAI protocol with < 45 open days as well as in no-cycling animals (anestrus) using equine chorionic gonadotropin or temporary weaning of calves. Sá Filho *et al.* (2009) applied the TAI protocol in cows with 30–60 and 61–90 open days, resulting in an improvement in the PR in cows with a higher postpartum period. Pinheiro *et al.* (2009) investigated beef cows with an interval of < 45, 45–70 and > 70 open days; however, they did not verify the difference in the PR.

Another aspect related to this study concerns the BCS. The BCS of the CIDR1, CIDR2, and CIDR3 groups was 3.1, 3.5, and 3.7, respectively (Table 1). Based on the data analysis, the PR was higher in the CIDR1 group than in the other groups ($P<0.05$). Schneider at al. (2009) investigated the use of CIDR devices in cows that were assigned to two groups based on a BCS of > 3.0 or < 3.0 and found a higher PR in the group with BCS < 3.0 ($P > 0.05$). Baruselli *et al.* (2004) stated that the minimum BCS for beef cattle would be in the 2.5 range (1–5). Melo *et al.* (2012) reported that a BCS that is considerably low or high is not optimal for breeding cattle and showed that the PR was higher in Nelore cows with a BCS of 3.0–3.2 than in those with a BCS of 3.7–4.2.

Schillo (1992) stated that a higher BCS can suppress the release of gonadotropin releasing hormone, causing a reduction in the frequency of luteinizing hormone (LH) pulses and a

failure in the development of DF and ovulation. Thus, high cortisol levels in cows with a higher BCS could generate more endogenous opioids responsible for the suppression of LH pulses (Stevenson *et al.*, 1994). However, there are few reports on a BCS > 3.1 being related to the PR. Animals with a higher BCS could carry a greater accumulation of periovarian fat, which impairs the oocyte pickup by the oviduct infundibulum after ovulation (Grunert and Berchtold, 1982).

Besides these related aspects, the correlation test was performed, to check the degree of association between open days, the BCS and PR in groups. There was a correlation between open days from CIDR1 and 2 groups to BCS CIDR1 and 2 ($P < 0.05$); there was also correlation between BCS of CIDR1 and 3 with PR CIDR1 and CIDR3 ($P < 0.05$), but no correlation between open days versus PR was performed.

CONCLUSION

The protocol based on CIDR devices of first use showed a higher PR than that based on a second or third use; BCS of 3.1 resulted in a higher PR than that of 3.5 and 3.7. The cycling animals at the beginning of the protocol application showed a higher PR than those in anestrus.

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