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REDUCTION OF THE INTERVAL CALVING-CONCEPTION BY USE OF HORMONAL PROTOCOLS AND FIXED-TIME ARTIFICIAL INSEMINATION IN DAIRY COWS

Miriane Suplicy,[1] Haroldo Suplicy Filho[1], Luiz Ernandes Kozicki[2], José Carlos dos Santos Breda[2], Romildo Romualdo Weiss[3], Marcio Saporski Segui[2]

[1] Autônomo
[2] PUC-PR
[3] UFPR
Correspondência: Miriane Suplicy: misuplicy@hotmail.com

ABSTRACT: The aim of this study was to reduce the interval calving-conception in dairy cows by administration of two different hormonal protocols for estrus synchronization and fixed-time artificial insemination (FTAI). Holstein dairy cows (n=128) were divided in three groups (G): G1 (44 treated), G2 (34 treated) and G3 (50 as control). In the treated cows the following hormonal protocols were applied between 40 and 45 days post partum: G1- Day 0 (D0) - bovine intravaginal device containing progesterone (P_4) plus 1.0 mg of estradiol benzoate (EB), D8 - removal of the P_4, plus 0.5 mg of cloprostenol plus 400 IU of equine chorionic gonadotropin (eCG), D9 - 1.0 mg of EB and FTAI after 36 hours. G2 - D0 - P_4 intravaginal device plus 50 mcg of gonadotropin-releasing hormone (GnRH), D7 - removal of the P_4 plus cloprostenol plus 400 IU eCG; D9 - 50 mcg of GnRH and FTAI 16 hours after. G3 – Control group inseminated 12 hours after the visual estrus. Results: G1 and G2 were inseminated (FTAI) respectively in the D55.5±0.9 and D56.8±1.3 after calving, while the G3 in the D84.5±5.7 ($p<0.05$). The pregnancy rate in the G1, G2 and G3 was respectively 47.0, 46.1 and 35.8% ($p>0.09$). In conclusion: there was significant reduction of the interval calving/conception in treated cows (G1,G2)($p<0.05$) and better pregnancy rate regarding to G3
Key Words: interval post partum period; pregnancy rate; dairy cows; progesterone; time artificial insemination

REDUÇÃO DO INTERVALO PARTO-CONCEPÇÃO MEDIANTE O EMPREGO DE PROTOCOLOS HORMONAIS VISANDO A INSEMINAÇÃO ARTIFICIAL EM TEMPO FIXO (IATF) EM VACAS LEITEIRAS

RESUMO: O objetivo deste estudo foi reduzir o intervalo parto - concepção em vacas leiteiras, mediante a administração de dois diferentes protocolos hormonais para a sincronização do estro e inseminação artificial em tempo fixo (IATF). Vacas leiteiras da raça Holandesa (n=128) foram divididas em três grupos (G): G1 (n=44); G2 (n=34) e G3 (n=50). Nos grupos tratados G1 e G2 administrou-se entre o 40º e o 45º dia pós parto: G1- Dia 0 (D0) - dispositivo intravaginal bovino contendo progesterona (P_4) mais 1,0 mg de benzoato de estradiol (BE- IM), D8 - remoção da P_4 mais 0,5 mg de prostaglandina F2 alfa (PGF2α) mais 400 UI de gonadotrofina coriônica equina (eCG); D9 - 1,0 mg de BE e IATF após 36 horas; G2**:** D0 - P_4 intravaginal, mais 50 microgramas de hormônio liberador de gonadotrofina (GnRH); D7 - remoção da P_4 mais PGF2α mais 400 UI de eCG; D9 50 microgramas de GnRH e IATF 16 horas após; G3: Grupo controle inseminado 12 horas após a observação visual do estro. Resultados: No G1 e no G2 a IATF foi feita no D55,5±0.9 a no D56,8±1.3 após o parto, enquanto que no G3 a inseminação artificial foi executada no D84,5±5.7 ($p<0,05$). A taxa de prenhez no G1, G2 e G3 foi respectivamente de 47,0, 46,2 e 35,8 % ($p=0,09$). Conclusão: observou-se significativa redução do intervalo parto-concepção nas vacas tratadas assim como melhor taxa de prenhez ($p=0.09$) em relação ao grupo controle.
Palavras-chave: período pós parto; taxa de prenhez; vacas leiteiras; progesterona; inseminação artificial em tempo fixo

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INTRODUCTION

The biotechnology of reproduction should be used to increase the efficiency of dairy cows on the interval calving-conception., in order to achieve the aim l of one parturition per cow per year. The use of progestins for FTAI has been heavily investigated in recent years (Baruselli *et al.*2008; Bó *et al.*, 2008; Mapletoft *et al.*, 2008; Rodrigues *et al.*, 2008; Santos, 2008; Colazo *et al.*, 2009).

Pfeifer *et al.* (2005) confirmed that a major cause of the low rate of artificial insemination (AI) in dairy cattle is the failure to detect estrus, supported by Sartori (2007). The dairy cattle under grazing conditions, often are affected by anestrus post-partum, delaying the interval from calving to conception, affecting negatively the reproductive performance (Lucy *et al.*, 2004; Cavalieri *et al.*, 2006).

To reduce the period of postpartum anestrus, hormonal treatments may be used, such as the administration of progesterone (P_4) or gonadotropin-releasing hormone (GnRH) combined with prostaglandin F2 alpha (PGF2 alpha), estradiol benzoate (EB) or equine chorionic gonadotropin (Bo *et al.*, 2008). The duration of postpartum anestrus involves several physiological mechanisms, acting individually or together, preventing ovulation, which can be reduced by hormone treatment (Rabassa *et al.*, 2007). Bartolome *et al.* (2004) investigated cows using the Ovsynch protocol (D0 GnRH - D7 $PGF_{2\alpha}$ - D9 GnRH) in postpartum period, resulting in a pregnancy rate of 25.2%. According Bo *et al.* (2008), several research groups have combined the use of a controlled release of P_4 with the Ovsynch protocol in dairy cows. An experiment showed significant improvement on pregnancy rates (55.2 *vs.* 34.7%;) for cows treated and no treated with P_4 at the time of first application of GnRH in the Ovsynch protocol (Bó *et al.*, 2008). A review of Stevenson *et al.* (2006) has shown variations on the results.

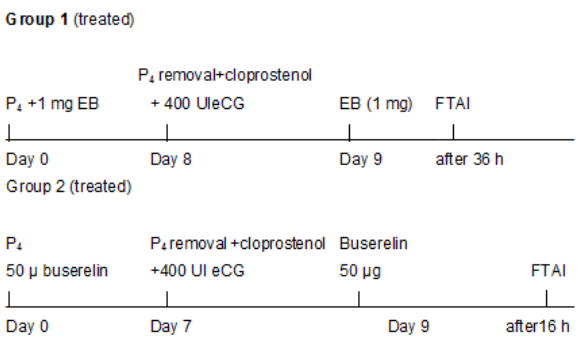
According Souza *et al.* (2006) and Bo *et al.* (2008), treatment with eCG increased the pregnancy rates in the FTAI. Veneranda *et al.* (2006) studied cows in 61.7th day post partum, verifying a pregnancy rate of 44.9% by use of protocol involving P_4, EB and eCG. These results suggest that the incorporation of eCG plus progestins improves the pregnancy rate. Bo *et al.* (2008) suggested that further studies should be undertaken to determine the best interval between GnRH and treatment with eCG in dairy cows. Souza *et al.* (2007) verified the effects of eCG plus estradiol cypionate (group 1) and eCG plus GnRH (group 2) on the pregnancy rate. They found that the pregnancy rates were 29.1% and 33.8% ($p>0.05$), respectively. Colazo *et al.* (2009) compared the effects of GnRH versus luteinizing hormone (LH) on the ovulatory response and pregnancy rate after FTAI. In this study GnRH, LH and PGF2 alpha were administered, resulting in 42.0% pregnancy rate, while in the group that was used the protocol GnRH, PGF2 alpha and GnRH, achieved 28% pregnancy rate.

The aim of this study was to reduce the post-partum anestrus period of dairy cows with high milk production by administration of two different hormonal protocols for estrus synchronization.

MATERIAL AND METHODS

Black and White Holstein pluriparous cows (n=128), average age 35.5±38.0 (months) (range 25.5-75.0) were used. The milk production was 12500 liters (305 days). The body

condition score ranged from 3.0 to 3.5 in a scale of 0 to 5. The animal's nutrition consisted of pasture oats, ryegrass, forage sorghum, corn silage and concentrate. Cows were divided into three groups (G): G1 and G2 (treated groups), formed by 44 and 34 cows respectively, submitted to FTAI. The G3 (50 animals) was the control. The study was conducted at Lapa (PR, south Brazil) (25'46'02 latitude, longitude 49'10 W, Altitude 908 meters), from July to November. Used hormones: P_4 = Progesterone intravaginal device (DIB - Shering Plough); Buserelin acetate (Intervet); EB=estradiol benzoate (Shering Plough); eCG=equine chorionic gonadotropin (Intervet); Cloprostenol (Shering Plough)
The applied protocols for the cows



The G3 (control group) was not submitted to any protocol. Animals of this group were inseminated 12 hours later after the spontaneous estrus demonstration. The treated groups (G1 and G2) were submitted respectively to the experiment on D40 and D45 after parturition and inseminated (FTAI) once around the D56. Fifty days after the AI ultrasound pregnancy diagnosis was carried out.

Statistical analysis

The quantitative variable interval calving-conception was compared by Student t test and the pregnancy rate by Chi Square test at the level of ($p<0.05$).

RESULTS AND DISCUSSION

The treated groups (G1 and G2), aiming reduce the voluntary waiting period after parturition, were inseminated (on average) in day 55.5 and 56.8 respectively, resulting in differences ($p<0.05$ versus G3 (84.5 days after parturition IA). Pregnancy rates in both treated groups were better than in the control one (Table 1).

Table 1 – Reduction of voluntary waiting period and pregnancy rate in Holstein dairy cows (n=128) submitted to hormonal protocols and fixed time artificial insemination, Lapa (PR).

Groups	Animals (n)	Interval calving/FTAI (days) (mean±sem)	Pregnancy rate (%)
G1	44	55,5±0.9[a]	47.0[a]
G2	34	56,8±1.3[a]	46.1[a]
G3(AI at estrus)	50	84,5±5.7[b]	35.8[a]

Different letters in the same column means significance ($p<0.05$).

The calving interval in dairy cattle has increased over the past decades due to several factors such body condition score, infections, uterine involution, thermal stress, deficiency in estrus observation (Sartori, 2007), ovarian cysts (Fantini Filho *et al.*,2003) among others. There is high incidence of postpartum anestrus, affecting negatively the reproductive performance (Lucy, 2001, Lucy *et al.*, 2004, Cavalieri *et al.*, 2006). Alternatives to solve the problem of low service rate in the postpartum period like the adoption of FTAI had been proposed (Rodrigues *et al.*, 2008; Sartori, 2007). Cavalieri *et al.* (2006) and Rodrigues *et al.* (2008) reported the advantages of protocols by use of progesterone intravaginal devices associated to estradiol benzoate or GnRH in dairy cows. Table 1 results show a significant reduction in the interval calving-conception ($p<0.05$) between the treated groups (G1 and G2) versus control (G3).

Animals of the G1 and G2 were submitted to FTAI usually in the 8th week post partum, versus G3 inseminated by spontaneous visible estrus only at the 12th week post

partum. There were advantages in animals of groups submitted to hormonal protocols to become pregnant one month before the control group. There was better pregnancy rate in treated groups (p>0.09) corroborating reports of Rodrigues *et al.* (2008).

Cows of G1 and G2 showed estrus in the 55.5th and 56.8th day, respectively, versus in the 84,5th (G3). These results are confirmed by Nasser *et al.* (2004), Bo *et al.* (2007), Bryan *et al.* (2008) and Souza (2008), who registered the advantages of using similar protocols in FTAI in the intgerval calving-conception.

Analyzing the data on pregnancy rates in this study, there was no statistical difference among the groups. However, cows from both treated groups (G1, G2) showed better pregnancy rates than the control (45.8, 43.7 and 37.9) respectively. For the G1 (protocol P_4+EB+eCG+EB+FTAI 36 h after), in a similar protocol, Veneranda *et al.* (2006) and Bó *et al.* (2008), obtained pregnancy rate of 44.9%, a result very close to this one. Our results are corroborated likewise by Souza *et al.* (2007), Bo *et al.* (2008) and Mian and Brogliatti (2007). They report that administration of eCG plus GnRH, after previous progesterone treatment increases the pregnancy rate in cows. In the present study we carried out the modified Ovsynch protocol in G2 to check for possible differences to the protocol of G1 (EB as an inducer of ovulation). The EB administration should be performed within 24 hours after removal of the P_4 to obtain better ovulation rates (Marques *et al.*, 2003), since the pre-ovulatory peak of LH occurs approximately 21.5 hours after application (Lammoglia *et al.*, 1998) as well as the GnRH applied a new wave of follicular growth begins at the 2 or 3 days (Bodensteiner *et al.*, 1996). There was no improvement in pregnancy rates using Ovsynch protocol to another program corroborating reports Rabiee *et al.* (2005).

CONCLUSION

It was concluded that it is possible to reduce the interval calving-conception in dairy cows by use of hormonal protocols with EB or GnRH and FTAI with significant advantages, mainly in regarding to pregnancy rate.

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