Accepted Manuscript

Comparison between the 5-day cosynch and 7-day estradiol-based protocols for synchronization of ovulation and timed artificial insemination in suckled BOS taurus BEEF cows

Bilbao MG, Zapata LO, Romero Harry H, Perez Wallace S, M.F. Farcey, Gelid L, Palomares RA, M.S. Ferrer, Bartolome JA

PII: S0093-691X(18)30387-X

DOI: 10.1016/j.theriogenology.2019.01.027

Reference: THE 14857

To appear in: *Theriogenology*

Received Date: 22 June 2018

Accepted Date: 31 January 2019

COMPARISON BETWEEN THE 5-DAY COSYNCH AND 7-DAY ESTRADIOL-BASED PROTOCOLS FOR SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION IN SUCKLED BOS TAURUS BEEF COWS

Bilbao MG[ab], Zapata LO[b], Romero Harry H[c], Perez Wallace S[d], Farcey MF[b], Gelid L[c], Palomares RA[e], Ferrer MS[e], Bartolome JA [b*]

[a]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina;

[b]Facultad de Ciencias Veterinarias, Universidad Nacional de La Pampa, Argentina;

[c]EEA INTA Anguil, La Pampa, Argentina;

[d]Zoetis SRL, Buenos Aires, Argentina;

[e]Departments of Large Animal Medicine and Population Health, College of Veterinary Medicine, University of Georgia, USA

[*]Corresponding author. E-mail address: jbartolome@vet.unlpam.edu.ar

Abstract

The objective was to compare pregnancy per AI and follicular dynamic in suckled *Bos taurus* beef cows treated with either a 7-day progesterone + estradiol-based protocol or a 5-day progesterone CoSynch protocol for timed artificial insemination (TAI) during four breeding seasons. We hypothesized that estrous cycle status, days postpartum (DPP), fat depth and plasma progesterone concentration differentially modify the effect of treatments. Every year, 9 days before initiation of each breeding season, cows were randomly assigned to one of two groups.

Cows in the 7-d P+E group (n = 428) received a progesterone intravaginal device (DIB) and estradiol benzoate on Day -9. On Day -2 the device was removed, and cows received cloprostenol and estradiol cypionate. Forty-eight hours later (Day 0) cows received TAI. Cows in the 5-d P+CoS group (n = 428) received a DIB, and GnRH on Day -8. On Day -3, the device was removed, and cows received cloprostenol. A second dose of cloprostenol was given on Day -2. Cows received GnRH and TAI 72 h after device removal (Day 0). On Day -9, estrous cycle status was determined. In a subset of cows (n = 79) the size of the dominant follicle was determined between Days -2 and 0. In another subset of cows (n= 340), DPP, fat depth (mm) and plasma progesterone concentration (ng/mL) were evaluated on Day -9. Pregnancy per AI was determined 30 d after TAI. Pregnancy per AI was greater for cows in the 5-d P+CoS group than for cows in the 7-d P+E group (50.9 % vs. 41.3 %, $P = 0.01$) and was also greater in cyclic than in anestrus cows (54.3 % vs. 33.2 %, $P < 0.0001$). There was also a significant effect of breeding season ($P = 0.0002$) and sire ($P = 0.03$), and an interaction between treatment group and breeding season ($P = 0.03$). The dominant follicle was larger ($P < 0.0001$) in cows in the 5-d P+CoS group than the 7-d P+E group (10.7 ± 0.29 mm vs. 9.0 ± 0.28 mm). Pregnancy per AI was greater in cows with ≥ 55 DPP (47.0 % vs. 29.6 %, $P = 0.001$), fat depth ≥ 0.50 mm (44.7 % vs. 29.7 %), and with plasma progesterone concentration ≥ 1 ng/mL (47.2 % vs. 28.7 %, $P = 0.01$). In cows with plasma progesterone ≥ 1 ng/mL on Day -9, pregnancy per AI was greater in the 5-d P+CoS group (60.5 %) than in the 7-d P+E group (34.9 %), but there was no difference between treatment groups in cows with plasma progesterone < 1 ng/mL ($P = 0.07$). In conclusion, the 5-d P+CoS protocol resulted in greater size of the dominant follicle and pregnancy per AI in suckled *Bos taurus* beef cows subjected to TAI.

1. Introduction

Synchronization of ovulation and timed artificial insemination (TAI) allowed expanding the use of frozen semen in range beef cow-calf operations, which may result in genetic improvement and increased herd productivity [1,2]. This technology may also contribute to induce estrus in non-cyclic cows [3], minimize the potential impact of venereal diseases, and reduce the interval from calving to conception. Therefore, based on these multiple advantages, several synchronization strategies have been developed for beef cattle.

Administration of GnRH [4] alone or combined with progesterone [5], and estradiol combined with progesterone [6]induce follicular turnover, and after a luteolytic dose of $PGF_{2\alpha}$ and removal of the progesterone source 7 d later, synchronizes estrus between 48 and 72 h later. Then, either GnRH [7] or estradiol [8] can be used to synchronize ovulation and inseminate without the need for estrus detection. The Ovsynch protocol developed for dairy cows [9] was modified to reduce animal handling and be used in suckled beef cows [10]. Since GnRH is not very effective synchronizing emergence of a follicular wave in beef cows, and since proestrus is shortened by administration of GnRH 48 to 60 h later, the initial 7-day CoSynch protocol was modified into a 5-day CoSynch protocol [11]. This 5-day CoSynch protocol avoids persistence of the dominant follicle in cows that do not respond to the initial GnRH treatment and extends proestrus since GnRH and TAI are applied at 72 h [11].

Estradiol-17β administered at the beginning of a progestogen treatment for 7 days inhibit gonadotrophin secretion resulting in follicular regression and induction of a new follicular way 4 to 5 days later in *Bos taurus* beef heifers [12]. Estradiol benzoate administered 24-30 h after progesterone removal induced an LH surge and synchronize ovulation in *Bos taurus* beef cattle [13]. Estradiol and progesterone-based protocols, with or without eCG, are the most common treatments for synchronization of ovulation and timed insemination in suckled *Bos taurus* beef cows in South America [14]. The main reason for it is that the cost of estradiol is lesser than that of GnRH, estradiol is more effective inducing follicular turnover in cows and heifers and cow handling is reduced [15].

In a metanalysis study including a large number of cows, it was reported that days post-partum (DPP), body condition score (BCS) and cyclicity affected pregnancy per AI [16]. The impact of DPP, BCS and cyclicity on fertility could also be affected by the length of progesterone treatment, the type of protocol using different combinations of GnRH and estradiol, and length of proestrus. To the authors' knowledge, the 5-Day progesterone-based CoSynch protocol and the conventional progesterone + estradiol-based protocols have not been compared in multiparous suckled beef cows. Therefore, the objective was to compare pregnancy per AI and follicular dynamics between 5-day progesterone-based CoSynch and 7-day progesterone + estradiol-based protocol for synchronization of ovulation and TAI in multiparous beef cows during four breeding seasons. We hypothesized that estrous cycle status, DPP, fat depth and plasma progesterone concentration at initiation of the treatment will differentially affect the efficacy of treatments, and therefore, the interaction of these variables with treatments will be assessed.

2. Materials and methods

2.1. Study population

The study was conducted in the Aberdeen Angus cow-calf operation of the Experimental Station of the National Institute of Agricultural Technology EEA INTA Anguil, La Pampa, Argentina. A total of 856 multiparous suckled *Bos taurus* beef cows were included in the study. The day of the TAI was considered the first day of the breeding season. TAI was performed on 12/11/14 (breeding season 1), 11/9/15 (breeding season 2) 11/9/2016 (breeding season 3), and 11/21/17 (breeding season 4). Frozen semen from eight different sires was used (three in breeding season 1, one in breeding season 2 and two in breeding seasons 3 and 4), and each cow was inseminated only once per breeding season. Clean up bulls were introduced to the cow herd 15 d after TAI for a period of 75 d. The herd was free of brucellosis and vaccinated for reproductive diseases with a killed virus vaccine every 6 m (including BHV-1, BVDV, BRSV, PI3V, *Campylobacter fetus fetus*, *C. fetus veneralis*, *Leptospira interrogans pomona pomona*, *Haemophilus somnus*, 5 mL, sc, Bioabortogen® H, Biogenesis Bago, Argentina). Cows were grazing Weeping lovegrass (*Eragrostiscurvula*) during the entire breeding season. All procedures were performed with the approval of the Committee of Ethics in Biological Science Research (Facultad de Ciencias Veterinarias, Universidad Nacional de La Pampa, Argentina, Resolution 247/11) and according to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [17].

2.2. Experimental design

Every year, 9 d before the initiation of each breeding season, cows with more than 30 DPP were randomly assigned to one of two treatment groups. Group 7-d P+E. 7-day progesterone + estradiol-based group (n = 428), cows received a 0.5 g progesterone device

(DIB®, Zoetis Animal Health) and 2.5 mg of estradiol benzoate (2.5 mL, im, Gonadiol®, Zoetis, Argentina) on Day -9. On Day -2 the device was removed, and cows received 0.125 mg of cloprostenol (2 mL, im, Ciclase, Zoetis Animal Health) and 0.5 mg of estradiol cypionate (0.5 mL, im,Cipiosyn®, Zoetis Animal Health). Forty-eight hours later (Day 0) cows received TAI. Group 5-d P+CoS. 5-day Progesterone-based CoSynch group (n = 428), cows received a DIB, and 100 µg of GnRH analog (Gonadoreline acetate, 2 mL, im, Gonasyn GDR®, Zoetis) on Day -8. On Day -3, the device was removed, and cows received 0.125 mg of cloprostenol (2 mL, im, Ciclase®). A second dose of cloprostenol was given on Day -2. Finally, cows received 100 µg of gonadoreline acetate im and TAI 72 h after device removal (Day 0).

On Day -9, estrous cycle status (anestrus or cyclic) was determined in all cows based on clinical signs at palpation and ultrasonography of the genital tract per rectum. Cows with a CL or clinical signs of estrus (ovarian follicle ≥ 10 mm and uterine tone) were considered cyclic and cows without a CL and flaccid uterus were considered anestrus. Pregnancy per AI was determined by ultrasonography of the uterus per rectum (5 MHz transrectal linear transducer, HS-101V, Honda Electronics, Japan) 30d after TAI. Pregnancy rate was calculated as the number of cows pregnant at 30 d/number of cows inseminated x 100.

In a subset of 79 cows (breeding seasons 1 and 2, n = 40, Group 5-d P+CoS, n = 39, Group 7-d P+E) the diameter (mm) of the dominant follicle was determined daily between Days - 2 and 0 using transrectal ultrasonography of the ovaries [18]. In another subset of cows (n= 340, breeding seasons 3 and 4), DPP, plasma progesterone concentration and fat depth were evaluated on Day -9. Fat depth was measured between the 12th and 13th ribs,3/4 the length ventrally on the *longissimus dorsi* muscle [19,20], using a Pie Medical Falco Vet 100 diagnostic ultrasound machine with an 18 cm, 3.5 MHz linear array transducer, following the Iowa State University guidelines [21]. The coupler was vegetable oil, a no stand-off pad was used. Cattle were not clipped, and they stood in a normal, relaxed posture.

Blood samples were collected on Day -9 by venipuncture of the coccygeal vein into evacuated tubes containing EDTA (Vacutainer®; BD, Franklin Lakes, NJ, USA). The samples were immediately placed on ice. Samples were centrifuged at 1100 X g for 20 min, and plasma was stored at -20 ºC until assayed for progesterone. Plasma progesterone concentrations were determined at the Laboratory of Animal Reproduction at Facultad de Ciencias Veterinarias, Universidad Nacional de La Pampa, Argentina, using a direct, solid-phase RIA (RIA Progesterone, REF IM1188, IMMUNOTECH s.r.o.Hostivař, Czech Republic) according to previously described protocol [22], in a Multi Crystal Gamma Counter LB 2111 (Berthold Technologies, GmbH & Co., Bad Wildbad Germany). Measurements were completed in three assays. The sensitivity of each of them was 0.06, 0.038 and 0.047 ng/mL, respectively. The inter-assay CVs were 4.17, 1.62 and 10.45 %, and the intra-assay CVs were 2.57, 8.57 and 6.85 %, respectively. Cows were dichotomized as having progesterone concentration ≥1 ng/mL or < 1 ng/mL.

2.3. Statistical analysis

Baseline comparisons were established evaluating the distribution of cows in both groups using a Chi-square test (Proc Freq, SAS system®). The effect of treatment group (5-d P+CoS vs. 7-d P+E), breeding season (1, 2, 3 and 4), estrous cycle status (anestrus vs. cyclic), and sire (A, B, C, D, E, F, G and H) on pregnancy per AI was determined by univariate analysis with a Chi-square test and multivariable analysis using the backward elimination procedure (Proc Logistic, SAS system®) of multiple logistic regression [23]. The effect of treatment group, experimental day, and their interaction on follicular dynamics was evaluated using analysis of variance using the repeated measures method(Proc Mixed, SAS system®) using treatment group and the interaction treatment group and day as fixed variables, cow nested in treatment group as random variable and day as repeated variable. Since cloprostenol was first administered on Day -3 in cows in the 5-d P+CoS group and on Day -2 in cows in the 7-d P+E group, the effect of treatment on follicular dynamics was also evaluated considering the day from cloprostenol administration (first cloprostenol for 5-d P+CoS group). The interactions between treatment group and plasma progesterone concentrations on Day -9 (< 1ng/mL or ≥ 1 ng/mL), fat depth (≤ 0.5 mm or > 0.5 mm) and DPP (≤ 55 days or > 55 days) on pregnancy per AI adjusting for breeding season and bull was evaluated using multiple logistic regression (Proc Logistic, SAS system®). The effects of fat depth and DPP as continuous variables on the probability of pregnancy were also evaluated by logistic regression using STATA/IC 14.2 (StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845 USA). Significant effects were declared at $P \leq 0.05$ and tendencies declared at $0.05 < P \leq 0.10$.

3. Results

There was no difference in the distribution of cows by breeding season, estrous cycle status and sire in both groups (Table 1). In the univariate analysis, pregnancy per AI was greater in the 5-d P+CoS than the 7-d P+E group ($P = 0.004$; Table 2). Additionally, breeding season ($P < 0.0001$), estrous cycle status ($P < 0.0001$) and sire ($P < 0.0001$) affected pregnancy per AI (Table 2). In the multivariable analysis, pregnancy per AI was also greater in the 5-d P+CoS than the 7-

d P+E group (P = 0.01). There was also a significant effect of breeding season (P = 0.0002), estrous cycle status (P < 0.0001), and sire (P = 0.03), and an interaction between treatment group and breeding season (P = 0.03, Fig. 1) on pregnancy per AI.

In the subset of cows where ovarian ultrasonography was conducted, there was an effect of experimental day (P < 0.001) and treatment group (P < 0.0001), but not their interaction, on the size of the dominant follicle. The dominant follicle of cows in the 5-d P+CoS group (10.7 ± 0.29 mm) was larger than the dominant follicle of cows in the 7-d P+E group (9.0 ± 0.28 mm) on the day of TAI (Fig. 2 A). Considering the day from cloprostenol administration, there was an effect of day (P < 0.0001), treatment group (P = 0.01) and a tendency for interaction (P = 0.06) between treatment group and day from cloprostenol administration on the size of the dominant follicle. The dominant follicle of cows in the 5-d P+CoS group (10.1 ± 0.30 mm) was larger than the dominant follicle of cows in the 7-d P+E group (9.0 ± 0.30 mm) on the day of TAI (Fig. 2 B).

In the subset of cows were DPP, plasma progesterone concentration and fat depth were recorded on Day -9, there was no difference in the distribution of cows between groups (55.2 ± 1.4 d, 2.53 ± 0.39 ng/mL and 0.52 ± 0.02 mm for 5-d P+CoS group, and 56.8 ± 1.4 d, 2.95 ± 0.58 ng/mL, and 0.55 ± 0.02 mm for 7-d P+E group). In addition, there was no difference in the distribution of cows between groups according to category of DPP (P = 0.54), plasma progesterone concentration (P = 0.57) and fat depth (P = 0.19). There was an effect of treatment group (5-d P+CoS group, 44.0 %, 74/168, 7-d P+E group, 30.8 %, 53/172, P = 0.006), DPP (≤ 55 days, 29.6 %, 56/189, > 55 days, 47.0 %, 71/151, P = 0.001), fat depth (≤ 0.5 mm, 29.6 %, 50/168, > 0.5 mm, 44.7 %, 77/172, P = 0.004) plasma progesterone concentration (≥ 1 ng/mL, 47.2 %, 75/159, <1 ng/mL, 28.7 %, 52/181, P = 0.01) on pregnancy per AI. There was not interaction between treatment, DPP and fat depth. There was an interaction (P = 0.002) between breeding season and treatment group on pregnancy per AI. Moreover, there was a tendency for the interaction between treatment and plasma progesterone concentration (P = 0.07, Fig. 3) on pregnancy per AI. In cows with plasma progesterone concentration ≥1 ng/mL on Day -9, pregnancy per AI was greater for cows in the 5-d P+CoS group (60.5 %, 46/76) compared to cows in the 7-d P+E group (34.9 %, 29/83). On the other hand, there was no difference in pregnancy per AI between treatment groups in cows with plasma progesterone concentration < 1 ng/mL on Day -9 (30.4 %, 28/92 for cows in the 5-d P+CoS group and 26.9 %, 24/89 for cows in the 7-d P+E group). When fat depth and DPP were considered as continuous variables, there was a significant effect of those variables on the probability of pregnancy in both treatment groups (Fig. 4).

4. Discussion

This study was the first to compare two of the estrus synchronization protocols most widely used in North America (5-day progesterone + GnRH-based or 5-day CIDR CoSynch) and South America (7-day progesterone + estrogen-based) for *Bos taurus* beef cows. Pregnancy per AI was greater with the 5-day CoSynch (50.9%) than the estrogen-based protocol (41.4%). Protocols combining estrogen and progesterone are most commonly used in South America reporting pregnancy rates between 41 and 60% [24]. However, there were no studies comparing estrogen-based protocols with the 5-day CoSynch protocol that combines GnRH and progesterone. The increase in fertility seen here with the 5-dP+CoS protocol could be attributed to the larger size of the preovulatory follicle at the time of TAI and the longer duration of proestrus [25] in this group of cows compared with cows in the 7-d P+E group. Greater pregnancy per AI was reported using the 5-day CoSynch protocol when the second GnRH was administered 72 h after progesterone device removal compared to 7-day CoSynch with GnRH administered 60 h after progesterone removal [11]. In addition, the 5-day CoSynch protocol resulted in greater estradiol concentrations during proestrus and greater plasma progesterone concentration after induction of ovulation [26].

The benefit of a GnRH-based protocol on pregnancy per AI may not be applicable to *Bos indicus* cattle since the ability of GnRH to induce an LH surge seems to be compromised specially when plasma progesterone levels are high [27]. In addition, elevated progesterone concentrations during follicular growth may reduce the size of the dominant follicle at the time of ovulation in *Bos indicus* [28] and it may be a concern for CoSynch protocols that induce accessory CL in cows that ovulate in response to GnRH. Protocols including estradiol and progesterone generated better pregnancy per AI than protocols combining GnRH and progesterone in *Bos indicus* cows and increasing the duration of progesterone treatment to 8 or 9 d and the inclusion of eCG at progesterone removal further enhance fertility in herds with high incidence of anestrus [29]. In addition, the presence of CL at the beginning of the protocol were associated with reduced pregnancy per AI in *Bos indicus*. [30]. Since the cows of the present study were *Bos taurus* with good BCS at the first breeding season, we decided to use a 7-d duration progesterone treatment for the estradiol-based protocol without the inclusion of eCG at progesterone removal.

Optimization of the dominant follicle size has been an important target in synchronization of ovulation and TAI protocols [31,32]. In beef cattle, increasing the size of the dominant follicle resulted in increased estradiol concentration [33,34], improved ovulatory response [10, 35] and

CL function [34,35,36,37], which may result in a greater pregnancy rate [38,39]. In the current study, the larger diameter of the ovulatory follicle in the 5-d P+CoS group could have been responsible for the observed greater pregnancy per AI. However, comparing the size of the dominant follicle and its effect on pregnancy outcomes between these two treatments is difficult since in the 5-day P+CoS protocol follicular recruitment has been documented to start 28 to 32 h after the first GnRH treatment [40], progesterone treatment lasts 5 d, and proestrus lasts 72 h [11].In contrast, in the 7-day estradiol-based protocol, follicular recruitment was reported to be initiated 4 d after the first administration of estradiol benzoate, progesterone treatment lasts 7 d, and proestrus lasts 48 h [15]. Therefore, cows in the 5-day group are expected to initiate follicular recruitment earlier and have a longer proestrus, allowing for further follicular growth until the time of AI. This was confirmed in this study, where the dominant preovulatory follicle was larger in cows in the 5-day group not only when experimental day was considered but also, considering the day of $PGF_{2\alpha}$ administration as Day 0.

Another factor influencing fertility in protocols for synchronization of ovulation and TAI is the ability of $PGF_{2\alpha}$ to induce luteal regression. The 5-d CoSynch protocol included GnRH on Day 0 which induces accessory CL in approximately 60 % of the cows and therefore two doses of $PGF_{2\alpha}$ 8 to 24 h apart are recommended [11]. It has also been reported than two doses of $PGF_{2\alpha}$ administered simultaneously at progesterone intravaginal device removal were also effective when compared with two doses 8 h apart [41]. However, in beef heifers, administration of $PGF_{2\alpha}$ 6 h apart in a 5-d CoSynch protocol including GnRH on Day 0, improved pregnancy per AI compared with double dose of $PGF_{2\alpha}$ at progesterone device removal [42]. Therefore, in the present study, we decided to administer two doses of $PGF_{2\alpha}$ 24 h apart in an attempt to induce complete luteolysis.

Breeding season, estrous cycle status, DPP, fat depth and plasma progesterone concentrations at synchronization also affected pregnancy per AI in this study. Estrous cyclicity, DPP and BCS are the three most common factors affecting fertility in suckled beef cows [16]. The proportion of cows in anestrus considering clinical findings at transrectal palpation and ultrasonography was 38.7 % in the present study, in agreement with previous reports [43]. Pregnancy per AI was 20 % greater in cyclic than in anestrus cows, and there was no interaction between protocol and estrous cycle status. Pregnancy per AI was also greater in cows with plasma progesterone concentration ≥ 1 ng/mL, but the improvement was more pronounced in cows synchronized using the 5-d P+CoS protocol. Estradiol-based protocols in anestrus cows could result in failure to induce follicular turnover and induction of estrus without ovulation [25], explaining the lesser pregnancy per AI in cows in this group. For cows synchronized with the a 5-day CoSynch protocol, the ovulatory response to initial GnRH under reduced plasma progesterone concentration is high in cyclic cows [34] but low in anestrus cows [44]. However, there was no difference in pregnancy per AI in anestrous beef cows that ovulated or not after administration of GnRH at the time of a progesterone device insertion [45].

Breeding season affected pregnancy per AI and also influenced the effect of treatment since there was a significant interaction between these two variables. The interaction between treatment and breeding season could be explained by differences in DPP, BCS and estrous cycle status among years. The breeding season during the first year of the study started on December 11th but was initiated approximately 20 d earlier in the following season due to operative circumstances (median DPP was 69 d, 54 d, 51 d and 52 d for breeding seasons 1, 2, 3 and 4, respectively). Pregnancy rate was lesser in cows < 55 DPP. Advancing the breeding season reduced the DPP at synchronization resulting in a dramatic reduction in pregnancy per AI in the second year. Pregnancy per AI subsequently slightly recovered during the 3rd and 4th year. The reduction in fertility during early postpartum seem to be caused by delayed resumption of cyclicity rather than by lack of uterine involution [46]. Induction of ovulation of the first dominant follicle in early postpartum resulted in high incidence of short luteal phases [47].

The impact of BCS in pregnancy per AI in cows subjected to protocols of synchronization of ovulation and TAI has been reported [2,16,24]. In the present study, pregnancy per AI was greater in cows with fat depth > 5 mm. Fat depth was measured instead of BCS to reduce the variability of the data since the study was conducted during four different breeding seasons. Cows that maintained BCS during the postpartum period had a shorter interval to estrus, greater levels of basal LH and enhanced response to GnRH induced LH release [48].A study including 3,269 cows in seven different studies reported a linear increase of 18 % in the number of cyclic cows for each unit of BCS increase from ≤ 3.5 to ≥ 6.0 of a 1 (thin) to 9 (fat) scale [45].Rump fat and BCS were greater and serum concentration of BHB and NEFA reduced during the six weeks after parturition in cows that ovulated before first AI and become pregnant [49].

5. Conclusion

The 5-d P+CoS protocol resulted in greater pregnancy per AI compared to the 7-d P+E protocol in *Bos taurus* suckled beef cows subjected to TAI. Cows treated with the 5-d P+CoS protocol had larger dominant follicles at TAI than those receiving the 7-d P+E protocol. Estrous cycle status, DPP, fat depth and breeding season also affected pregnancy per AI. The increase in pregnancy per AI with the 5-d P+CoS protocol was greater when plasma progesterone concentration at initiation of the treatment was greater than 1 ng/mL.

Acknowledgments

The authors thank the personnel of the EEA INTA Anguil for assistance during the study and Project FITR 2013, Agencia Nacional de Promoción Científica y Tecnológica (Resolución 269/15) and Zoetis SRL for financial support. Thanks to Dr. Roy Berghaus, College of Veterinary Medicine, University of Georgia, for his contribution with regression analysis.

References

[1] Rodgers JC, Bird SL, Larson JE. An economic evaluation of estrus synchronization and timed artificial insemination in suckled beef cows. J Anim Sci 2012;10:1297-308.

[2] Lamb GC, Mercadante VRG. Synchronization and artificial insemination strategies in beef cattle. Vet Clin Food Anim2016;32:335-47.

[3] Stevenson JS, Hoffman DP, Nichols DA, McKee RM, Krehbiel CL. Fertility in estrus-cycling and noncycling virgin heifers and suckled beef cows after induced ovulation. J Anim Sci 1997;75:1343-50.

[4] Thatcher WW, Macmillan KL, Hansen PJ, Drost M. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology 1989;31:149-64.

[5] Geary TW, Whittier JC, Hallford DM, MacNeil MD.Calf removal improves conception rates to the ovsynch and CO-Synch protocols. J Anim Sci 2001;79:1-4.

[6] Adams GP, Matteri RL, Kastelic JP, Ko JC, Ginther OJ. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J ReprodFertil1992;94:177-88.

[7] Troxel TR, Kesler DJ. The effect of progestin and GnRH treatments on ovarian function and reproductive hormone secretions of anestrus postpartum suckled beef cows. Theriogenology 1984;21:699-711.

[8] Roche JF. Attempts to determine the optimal time of artificial insemination in heifers. J Reprod Fert 1974;41:223-5.

[9] Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 1995;44:915-23.

[10] Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. J Anim Sci 2001;79:2253-9.

[11] Bridges GA, Hesler LA, Grum DE, Mussard ML, Gasser CL, Day ML. Decreasing the interval between GnRH and PGF2alpha from 7 to 5 days lengthening proestrus increased timed-AI pregnancy rates in beef cows. Theriogenology 2008;69:843-51.

[12] Bo GA, Adams GP, Pierson RA, Caccia M, Tribulo H, Mapletoft RJ. Follicular wave dynamics after estradiol-17β treatment of heifers with or without a progestogen implant Theriogenology 1994;41:1555-69.

[13] Lammoglia MA, Short RE, Bellows SE, Bellows RA, MacNeil MD, Hafs HD. Induced and synchronized estrus in cattle: dose titration of estradiol benzoate in peripubertal heifers and postpartum cows after treatment with an intravaginal progesterone-releasing insert and prostaglandin F2a. J Anim Sci 1998;76:1662-70.

[14] Bo GA, Baruselli PS, Mapletoft RJ. Synchronization techniques to increase the utilization of artificial insemination in beef and dairy cattle. AnimReprod2013;10:137-42.

[15] Bó GA, de la Mata JJ, Baruselli PS, Menchaca A. Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. Theriogenology 2016;86:388-96.

[16] Stevenson JS, Hill SL, Bridges GA, Larson JE, Lamb GC.Progesterone status, parity, body condition, and days postpartum before estrus or ovulation synchronization in suckled beef cattle influence artificial insemination pregnancy outcomes. J Anim Sci 2015;93:2111-23.

[17] Mcglone J, Ford SS, Mitloehner F, Grandin T, Ruegg P, Swanson J, et al. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 2010.

[18] Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. Theriogenology 1984;21:495-504.

[19] Bullock KD, Bertrand JK, Benyshek LL, Williams SE, Lust DG. Comparison of real-time ultrasound and other live measures to carcass measures as predictors of beef cow energy stores. J Anim Sci 1991;69:3908-16.

[20] Brethour JR. The repeatability and accuracy of ultrasound in measuring backfat of cattle. J AnimSci1992;70:1039-44.

[21] Guitou HR, Monti A, Sutz G, Baluk I. Interpretación y uso correcto de las diferencias esperadas entre progenie (DEP´s) como herramienta de selección para la calidad de carne. Segunda parte. Revista Col de CiencPec2007;20:363-76.

[22] Meikle, A, L Sahlin, A Ferraris, B Masironi, J E Blanc, M Rodríguez-Irazoqui, M Rodríguez-Piñón, H Kindahl, and M Forsberg. Endometrial MRNA Expression of Oestrogen

Receptor Alpha, Progesterone Receptor and Insulin-like Growth Factor-I (IGF-I) throughout the Bovine Oestrous Cycle. AnimReprod Sci 2001; 68(1–2):45-56.

[23] Agresti, A. An introduction to categorical data analysis, 1st ed. New York: John Wiley & Sons, Inc.; 1996.

[24] Bo GA, Baruselli PS. Synchronization of ovulation and fixed-time artificial insemination in beef cattle. Animal 2014;8:144-50.

[25] Day ML. Hormonal induction of estrous cycles in anestrous *Bos taurus* beef cows. AnimReprod Sci 2004;82-83:487-94.

[26] Bridges GA, Mussard ML, Helser LA, Day ML. Comparison of follicular dynamics and hormone concentrations between the 7-day and 5-day CO-Synch + CIDR program in primiparous beef cows. Theriogenology 2014;81:632-8.

[27] Batista EOS, Del Valle TA, Ortolan MDDV, Rennó FP, Nogueira GP, Souza AH, Baruselli PS. The effect of circulating progesterone on magnitude of the GnRH-induced LH surge: Are there any differences between *Bos indicus* and *Bos taurus* heifers? Theriogenology 2017;104:43-48.

[28] Bo GA, Baruselli PS, Martinez MF. Pattern and manipulation of follicular development in *Bos indicu*s cattle AnimReprod Sci 2003;78:307–26.

[29] Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. AnimReprod Sci 2004;82-83:479-86.

[30] Nishimura TK, Martins T, da Silva MI, Lafuente BS, de GarlaMaio JR, Binelli M, Pugliesi G, Saran Netto A. Importance of body condition score and ovarian activity on

determining the fertility in beef cows supplemented with long-acting progesterone after timed-AI. AnimReprod Sci 2018;198:27-36.

[31] Wiltbank MC, Sartori R, Herlihy MM, Vasconcelos JL, Nascimento AB, Souza AH, Ayres H, Cunha AP, Keskin A, Guenther JN, Gumen A. Managing the dominant follicle in lactating dairy cows. Theriogenology 2011,76:1568-82.

[32] Baruselli PS, Sá Filho MF, Ferreira RM, Sales JN, Gimenes LU, Vieira LM, et al. Manipulation of follicle development to ensure optimal oocyte quality and conception rates in cattle. ReprodDomestAnim 2012,47(4):134-41.

[33] Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, Roberts AJ, Geary TW. Relationship between follicle size at insemination and pregnancy success. Proc Natl Acad Sci USA 2005;102:5268-73.

[34] Atkins JA, Smith MF, Wells KJ, Geary TW. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: Cycling cows. J Anim Sci 2010a;88:2300-10.

[35] Pugliesi G, Santos FB, Lopes E, Nogueira É, Maio JR, Binelli M. Improved fertility in suckled beef cows ovulating large follicles or supplemented with long-acting progesterone after timed-AI. Theriogenology 2016;85:1239-48.

[36] Echternkamp SE, Cushman RA, Allan MF. Size of ovulatory follicles in cattle expressing multiple ovulations naturally and its influence on corpus luteum development and fertility. J Anim Sci 2009;87(11):3556–68.

[37] Dadarwal D, Mapletoft RJ, Adams GP, Pfeifer LFM, Creelman C, Singh J. Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination. Theriogenology 2013;79:859-66.

[38] Perry GA, Smith MF, Roberts AJ, Macneil, MD, Geary TW. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J Anim Sci 2007;85(3):684-89.

[39] SáFilho MF, Crespilho AM, Santos JE, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. AnimReprod Sci 2010;120(1–4):23-30.

[40] Wolfenson, D., Thatcher, W.W., Savio, J.D., Badinga, L., Lucy, M.C. The effect of a GnRH analogue on the dynamics of follicular development and synchronization of estrus in lactating cyclic dairy cows. Theriogenology 1994;42:633-44.

[41] Bridges GA, Ahola JK, Brauner C, Crupper LH, Currin JC., et al., Determination of the appropriate delivery of PGF2α in the 5-day CO-Synch + CIDR protocol in suckled beef cows. J Anim Sci 2012;90:4814-22.

[42] White SS, Kasimanickam RK, Kasimanickam VR. Fertility after two doses of PGF2α concurrently or at 6-hour interval on the day of CIDR removal in 5-day CO-Synch progesterone-based synchronization protocols in beef heifers. Theriogenology 2016;86:785-90.

[43] Lucy MC, Billings HJ, Butler WR, Ehnis LR, Fields MJ, Kesler DJ, et al. Efficacy of an intravaginal progesterone insert and an injection of PGF2α for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers and dairy heifers. J Anim Sci 2001;79:982-95.

[44] Atkins JA, Smith MF, Wells KJ, Geary TW. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part II: Anestrous cows. J Anim Sci 2010b;88:2311-20.

[45] Stevenson JS, Johnson SK, Milliken GA. Incidence of Postpartum Anestrus in Suckled Beef Cattle: Treatments to Induce Estrus, Ovulation, and Conception. Prof Anim Scientist 2003;19:124-34.

[46] Short RE, Bellows RA, Staigmiller RB, J. G. Berardinelli JG, Custep EE. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J Anim Sci 1990;68:799-816.

[47] Crowe MA, Goulding D, Baguisi A, Boland MP, Roche JF. Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. J ReprodFertil 1993;99:551-55.

[48] Rutter LM, Randel RD. Postpartum nutrient intake and body condition: effect on pituitary function and onset of estrus in beef cattle. J Anim Sci 1984;58:265-74.

[49] Hill SL, Olson KC, Jaeger JR, Stevenson JS. Serum and plasma metabolites associated with postpartum ovulation and pregnancy risks in suckled beef cows subjected to artificial insemination. J Anim Sci 2018,96:258-272.

Table 1. Distribution of cows and baseline comparisons for breeding season, estrus cycle status and sire for both groups. P > 0.05.

	Treatment	5-d P+CoS (n = 428)		7-d P+E (n = 428)	
Variable	Group	%	n	%	N
Breeding season	1	33.4	143	31.5	135
	2	21.5	92	23.8	102
	3	25.0	107	24.3	104
	4	20.1	86	20.3	87
Cyclicity	Anestrus	37.6	161	39.7	170
	Cyclic	62.4	267	60.3	258
Sire	A	11.0	47	11.0	47
	B	11.0	47	13.1	56
	C	11.4	49	9.3	40
	D	14.0	60	11.2	48
	E	11.0	47	11.2	48
	F	21.5	92	23.8	102
	G	10.3	44	10.0	43
	H	9.8	42	10.3	44

Table 2.The effect of treatment group, breeding season, estrus cycle status and sire on pregnancy per AI (univariate analysis).[a]P=0.004, [b]P<0.0001

	Variable	Pregnancy per AI	
		%	n
Treatment[a]	5-d P+CoS	50.9	218/428
	7-d P+E	41.4	177/428
Breeding season[b]	1	59.4	165/278
	2	32.9	64/194
	3	42.2	89/211
	4	44.5	77/173
Cyclicity[b]	Anestrus	33.2	110/331
	Cyclic	54.3	285/525
Sire[b]	A	58.5	55/94
	B	47.6	49/103
	C	68.5	61/89
	D	37.0	40/108
	E	51.6	49/95
	F	32.9	64/194
	G	47.1	41/87
	H	41.9	36/86

Fig. 1. Pregnancy rate by breeding season and treatment group in all cows (n = 856), adjusted by estrus cycle status and sire (treatment group by breeding season, P = 0.03).

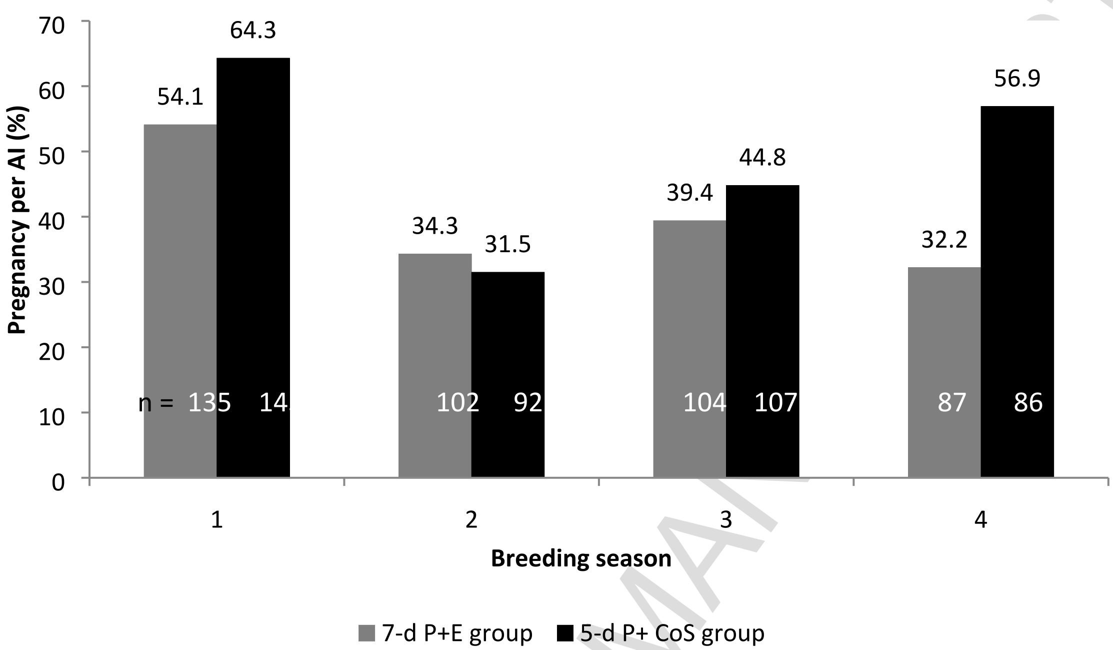
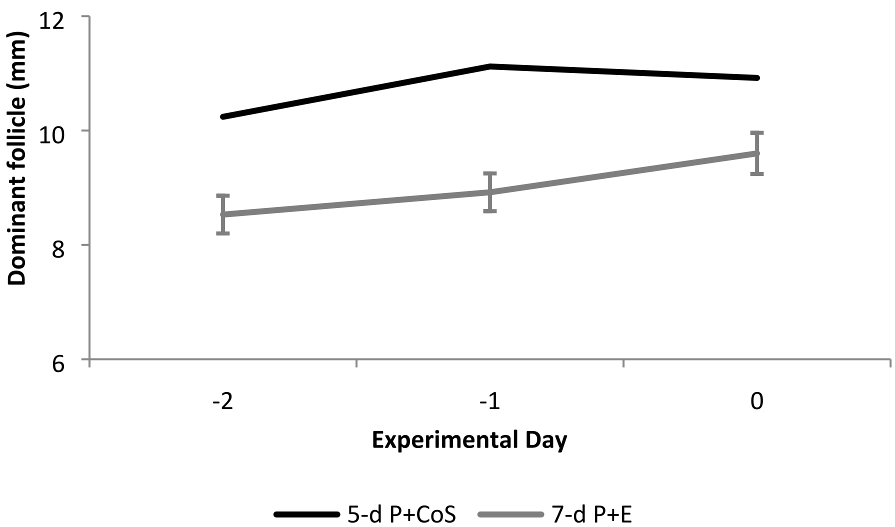


Fig.2. Size of the dominant follicle (mean ± SEM) for cows in both groups (5-d P+CoS group: n = 40; 7-d P+E group: n = 39). Effect of day, $P < 0.0001$, treatment group, $P < 0.0001$, day by treatment group interaction, $P = 0.22$ (A), and size of the dominant follicle (mean ± SEM) related to the day of cloprostenol administration (Day 0) for cows in both groups. Effect of day, $P < 0.0001$, treatment group, $P < 0.01$, day by treatment group interaction, $P = 0.06$, ** $P = 0.006$ (B).

A



B

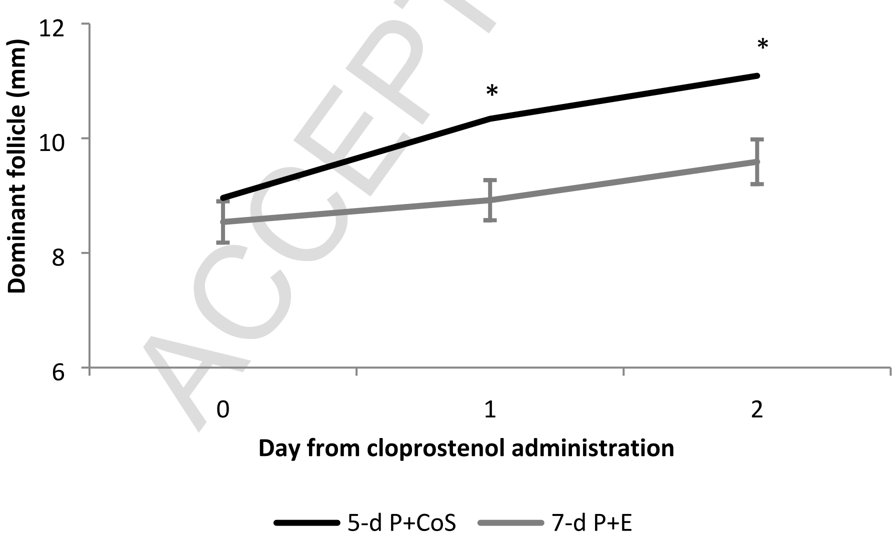


Fig. 3. Pregnancy rate by treatment group and plasma progesterone concentration on Day -9 (n =340), adjusted by breeding season, days postpartum, sire, fat depth and interactions on pregnancy per AI (treatment group by plasma progesterone concentration, P = 0.07).

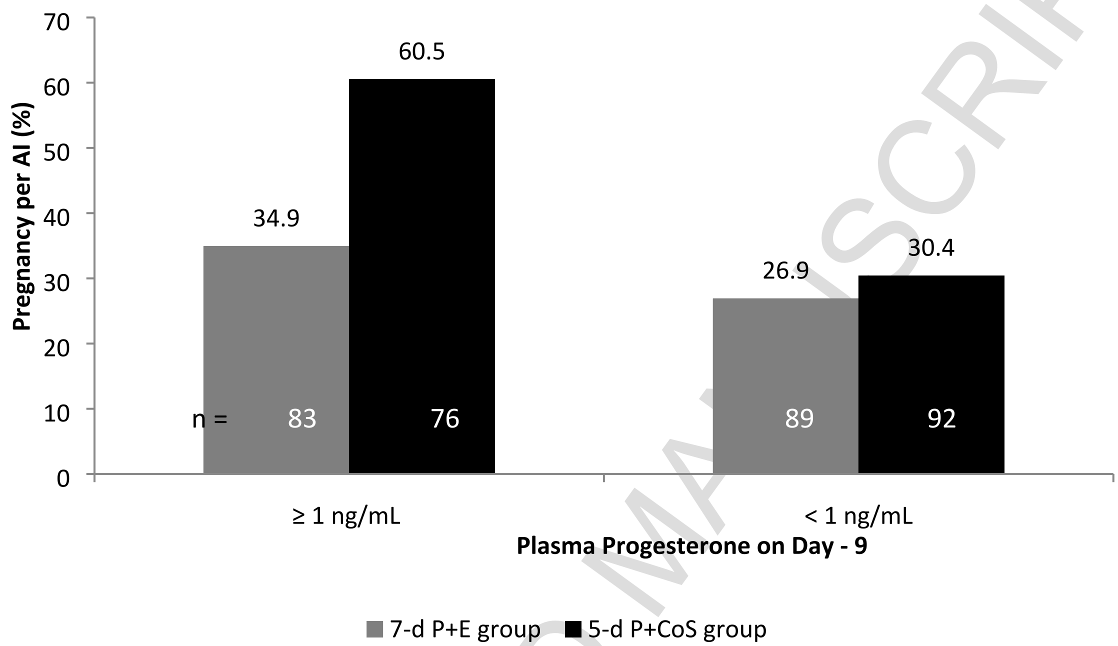
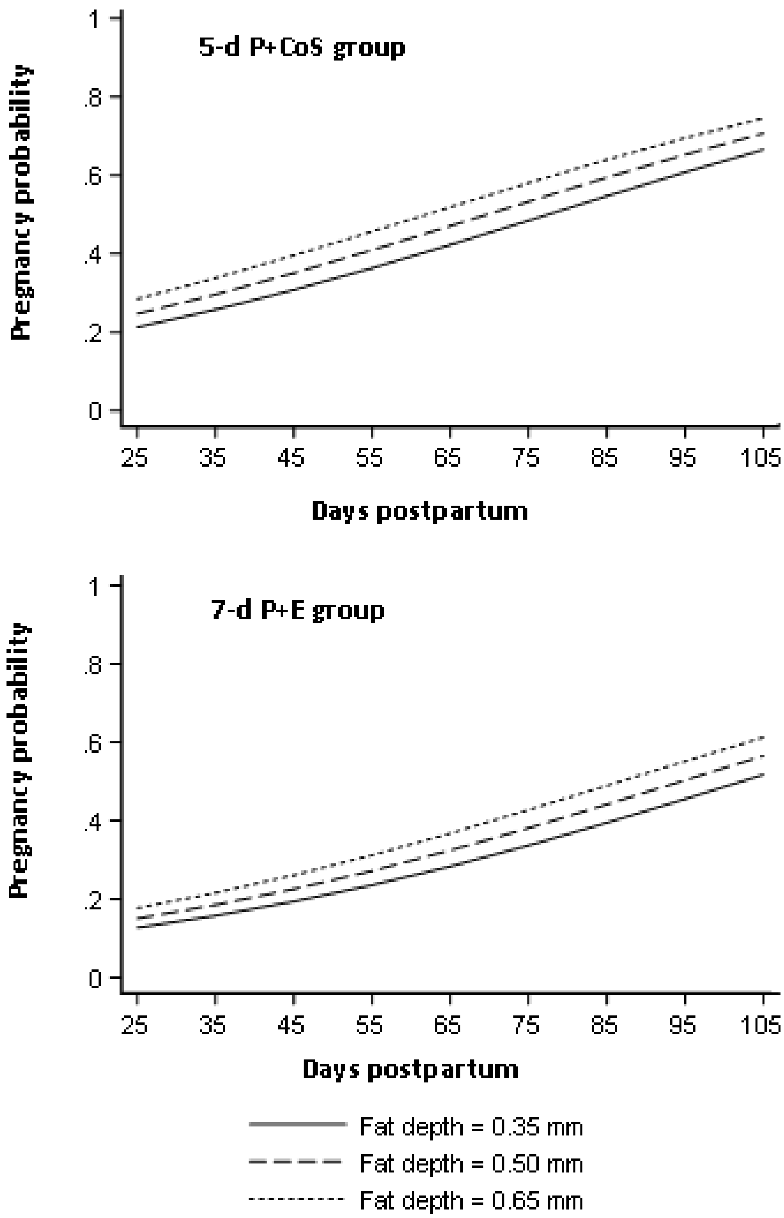


Fig. 4.Predicted probabilities of pregnancy by treatment group (5-d P+CoS group: n = 168; 7-d P+E group: n = 172), using days postpartum, and fat depth as continuous predictors (P < 0.01).



COMPARISON BETWEEN THE 5-DAY COSYNCH AND 7-DAY ESTRADIOL-BASED PROTOCOLS FOR SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION IN SUCKLED BOS TAURUS BEEF COWS

Highlights

The 5-Day Cosynch resulted in higher pregnancy per AI than a 7-Day estradiolprotocol

The dominant follicle at TAI was larger for the 5-Day Cosynch protocol

Pregnancy per TAI was increased in cows with high progesterone

Days postpartum, body condition and breeding season affected pregnancy per TAI