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Effect of the disinfection treatment of used intra-vaginal devices on the concentration of progesterone on cows under dry tropical conditions

Efecto del tratamiento de desinfección de los dispositivos intravaginales usados en la concentración de progesterona de vacas bajo condiciones de trópico seco

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ABSTRACT

The objective of the study was to compare the effect of disinfection/cleaning of CIDR devices on serum progesterone concentrations (P4) in Suizo x Cebú cows, which received during eight days a new intra-vaginal device (CIDR; T1), used device, sterilized by autoclave (T2) or a used device, disinfected with chlorine (T3). A complete randomized design with three replicates per treatment was applied. The used CIDR devices were used previously in cows. Blood samples were collected at 0, 15, 30 min, as well as at 1, 3 and 6 h and then each 12 h until tenth day , with respect to the moment of the insertion of the CIDR. The P4 concentration was determined by radioimmunoassay. Mean concentrations of P4 were similar among treatments (T1, 1.7 ± 0.9 ng/mL; T2, 1.31 ± 0.65 ng/mL; and T3, 1.33 ± 3.19 ng/mL; $P<0.05$). In conclusion, the process of disinfection/cleaning of the CIDR devices did not affect the availability of progestogen; therefore the CIDR could be used for estrous synchronization.

Keywords: CIDR, Autoclave, progesterone, cows.

RESUMEN

El objetivo de este estudio fue comparar el efecto de la desinfección/limpieza de los dispositivos CIDR sobre las concentraciones séricas de progesterona (P4) en vacas Suizo x Cebú, a las cuales se les colocó por ocho días un dispositivo intra-vaginal (CIDR) nuevo (T1), reutilizado esterilizado en autoclave (T2) o reutilizado desinfectado en cloro (T3). Se utilizó un diseño completamente al azar con tres vacas por tratamiento. Los dispositivos CIDR reutilizados habían sido usados en una ocasión en vacas. Se colectaron muestras sanguíneas a los 0, 15, 30 min, 1, 3 y 6 h y posteriormente cada 12 h hasta el día 10, a partir de la inserción del CIDR (hora 0). La concentración de P4 se determinó mediante radioinmunoensayo. Las medias de las concentraciones de P4 fueron similares entre tratamientos (T1, 1.7 ± 0.9 ng/mL; T2, 1.31 ± 0.65 ng/mL; y T3, 1.33 ± 3.19 ng/mL; $P>0.05$). En conclusión, el proceso de desinfección/limpieza de los dispositivos CIDR no afectó la disponibilidad del progestágeno, por lo que pueden ser reutilizados en el proceso de sincronización del estro.

Palabras clave: CIDR, Autoclave, progesterona, vacas.

INTRODUCTION

The intra-vaginal devices with progestin have been used for more than four decades with the objective of controlling the estrous cycles of cows and sheep (Lucy *et al.*, 2001, Martínez *et al*., 2002). The most widely used device in the United States is the controlled internal drug release device (CIDR), for use in livestock (Macmillan and Peterson 1993, Rathbone *et al.*, 1997). The CIDR is a vaginal insert in the form of T that contains 1.9 g (Canada, Mexico, Japan, Australia and New Zealand) or 1.38 g (United States and other countries) of progesterone (P4) in silicone molded on a nylon column (Rathbone *et al*., 2002; Mapletoft *et al*., 2003). The residual content of P4 after a 7-day insertion period of CIDR with 1.38 g, has been reported as 0.72 g (Rathbone *et al.*, 2002); therefore, it could be reused.

Although the manufacturer recommends use only once, the reuse of CIDR has been investigated (Martínez *et al*., 2003; Stevenson *et al*., 2003; Colazo *et al.*, 2004). Reused CIDR inserts suppress estrus for at least an additional 7 days, in both dairy and meat cattle (Richardson *et al.*, 2002); no differences were observed in pregnancy rates for cattle artificially inseminated at fixed time with new or used CIDR (Colazo *et al*., 2004).

Different approaches have been used to clean, disinfect or sterilize CIDR devices; however, there are apparently few reports comparing serum progesterone concentrations produced by second-use devices sterilized in an autoclave. Cerri *et al.* (2005) did not find differences in plasma concentrations of P4 in dairy cows that received a new device (1.38 g of P4), or an insert disinfected in an autoclave and used for 7 days. More scarce are the studies that compare the serum concentrations of P4 produced by CIDR washed and sterilized in an autoclave.

The objective of this study was to evaluate the effect of the disinfection type (autoclave or in chlorine) received by the CIDR to be reused, on the serum progesterone concentration of Suizo x Cebu cows, in the dry tropics of Michoacán, Mexico.

MATERIAL AND METHODS

Location and climate

The research work was carried out in a ranch located in the community of San Lucas, Michoacán, Mexico (18 ° 35'' Latitude North and 100 ° 47'' West Longitude); which is at an altitude of 300 m a.s.l. The annual rainfall is 906 millimetres; with temperatures that oscillate between 20.2 and 37.6 °C (INEGI, 2005). The climate of the region is tropical dry steppe, where there is a critical dry season from February to June.

Animals and handling

Nine Suizo x Cebú cows were used, anestric and lactating, with 60 days postpartum, average weight of 400 + 10 kg and body condition of 4-5 units in the scale from 1 to 9;

where 1 corresponded to an emaciated animal and 9 to an obese one (Ayala *et al.,* 1995).

The cows were free grazing in sorghum stubble pastures (*Sorghum bicolor*), maize stubble (*Zea mays*) and native grasses (*Paspalum spp., Cynodon dactylon, Bouteloa sp.,* Etc.); as well as shrubs and leguminous plants. The cows were managed in a system with continuous suckling, where the calf remained with the mother until weaning.

The cyclicity of the cows was evaluated prior to the study by means of two palpations at 10-day intervals, considering that a cow was anestric, when on rectal palpation, on the surface of the ovary, the formation of follicles or the presence of a corpus luteum (Salas-Razo *et al.*, 2011); the latter was confirmed by progesterone concentrations lower than 1 ng/ml (Grajales *et al.,* 2006); which indicates the absence of an active corpus luteum.

Application of the CIDR

Once the physiological state of the cows (anestrics) was diagnosed, a CIDR was inserted intra-vaginally for 8 days; three treated groups were formed: 1) cows that received a new CIDR, which was impregnated with 1.9 g of progesterone (CN; n = 3) (Pfizer Laboratory); 2) cows with a previously used CIDR (reused), which after cleaning was sterilized in an autoclave at 121 ºC (CEA, n = 3); and 3) cows with reused CIDR, which was disinfected in chlorine, diluted in water at a rate of 3 ml of chlorine per liter of water (CDC, n = 3). When the CIDR was placed in all the cows, 1 mg of estradiol cypionate (Wittney Laboratory) was applied intramuscularly. Immediately after the withdrawal and 24 h later, 0.25 mg of estradiol cypionate was applied again.

Progesterone analysis

To determine the P4 concentrations, blood samples were taken at 0, 15, 30, 1, 3 and 6 hours and then every 12 hours, until day 10 from the time of insertion of the CIDR (two days after the withdrawal of the CIDR). The samples were obtained by puncturing the coccygeal vein in Vacutainer tubes, in which the blood was allowed to clot for one hour, and then centrifuged at 2500 rpm for 15 min. The serum was stored in aliquots that were stored at -20 ºC until analysis. The measurement of P4 was carried out using a solid phase radioimmunoassay kit. The sensitivity of the test was 0.04 ng/ml and the intra- and interassay variation coefficients 6 and 8.5%

Statistical analysis

A completely randomized design with three cows (repetitions) per treatment was used. All the data were subjected to one-way analysis of variance, for each measurement moment (0.15 30 min, 1, 3, 6 and 12 h, and later every 12 h), using the GLM procedure (SAS, 2009).

RESULTS AND DISCUSSION

The majority (75%) of the maximum peaks of P4 occurred between 6 and 12 h post-insertion of the intra-vaginal device (Figure 1). The mean concentrations (± SD) of serum P4 during the 8 days that the remained device, were similar (P> 0.05; for the CN groups (1.7 ± 0.9 ng/ml), CEA (1.31 ± 0.65 ng/ml) and CDC (1.33 ± 3.19 ng/ml). These results were due mainly to differences produced during the first 6 hours, after the insertion of the CIDR (Figures 1 and 2).

The serum concentrations of P4 obtained from the new CIDR device during 8 days are comparable to the concentrations reported in another study, using the CIDR with 1.38 g (Rathbone *et al*., 2002). The P4 concentrations obtained with the sterilized CIDR in an autoclave were similar to those obtained by Martínez *et al*. (2007), who used a reused device that originally contained 1.9 g of P4 in ovariectomized cows. The concentrations of P4 reached their maximum point in the first 6 h after the insertion, maintaining it until approximately 12 hours; however, the same did not happen for the CIDR sterilized with chlorine, which suffered a considerable decrease in the concentration of P4. This decrease was followed by a constant decrease until the elimination of CIDR on day 8. Macmillan *et al.* (1991) reported that after insertion of a new CIDR (1.9 g of progesterone) into ovariectomized heifers, plasma progesterone concentrations increased to approximately 8.7 ng/ml for 6 h, and then reduced to 6.8 and 2.5 ng/ml on days 1 and 12 post-insertion, respectively. Cerri *et al*. (2005) comparing plasma concentrations of P4 after the insertion of a new CIDR or one used for 7 days and sterilized in an autoclave, observed that P4 concentrations increased immediately after insertion, reaching its maximum concentration at 90 minutes; following the same pattern during the rest of the insertion period.

Although there was no significant difference between treatments, it is possible that the low concentrations of P4 observed with the disinfected devices in chlorine compared to the devices sterilized in autoclave, were caused by a prolonged exposure to the disinfectant solution. Therefore, it is very likely that steam sterilization of the device in T3 increased the elution rate during the first hours, after insertion compared to T2. Such an effect indicates that the autoclaving process modifies in some way the structure of the implant or the arrangement of the P4 within the insert.

According to McPhee *et al.* (1983), the plasma progesterone profiles of the intra-vaginal progesterone devices (PRID) reused and sterilized in gas were lower compared to the PRID treated in autoclave; where post-reintegration steady-state plasma concentrations similar to a new insert were observed. The results were attributed to the formation of a large amount of crystalline P4 on the surface of the PRID in an autoclave.

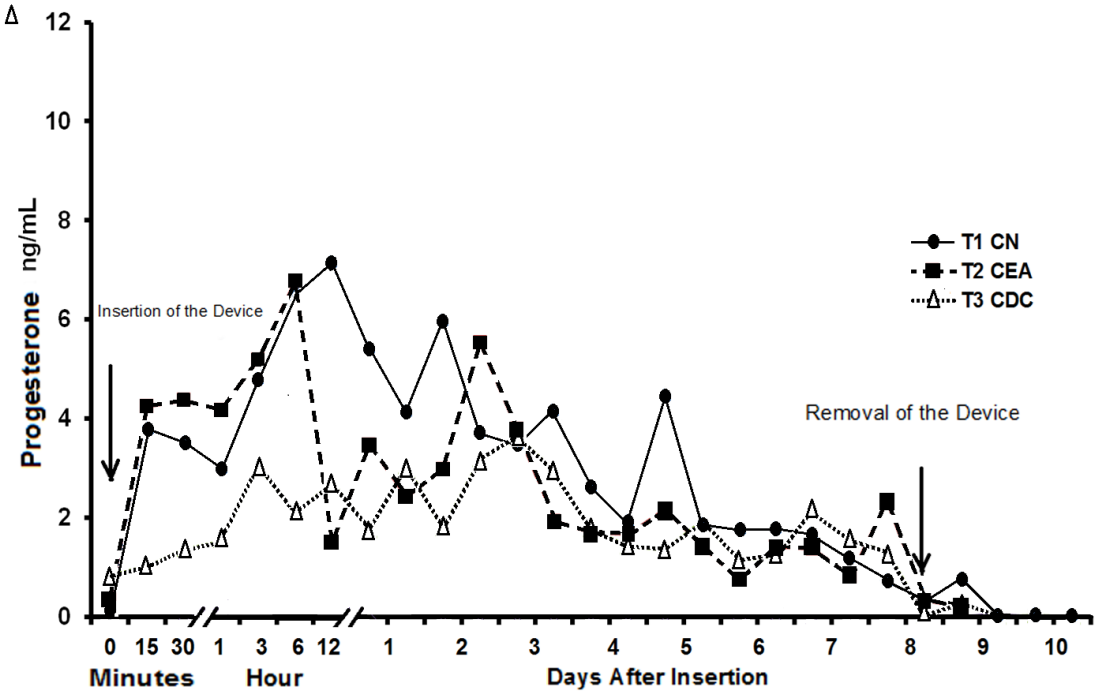

Figure 1. Average concentrations of serum progesterone (P4, ng/mL) in Suizo x Cebú cows produced by new CIDR devices (T1 CN), sterilized in autoclave (T2 CEA) and disinfected in chlorine (T3 CDC).

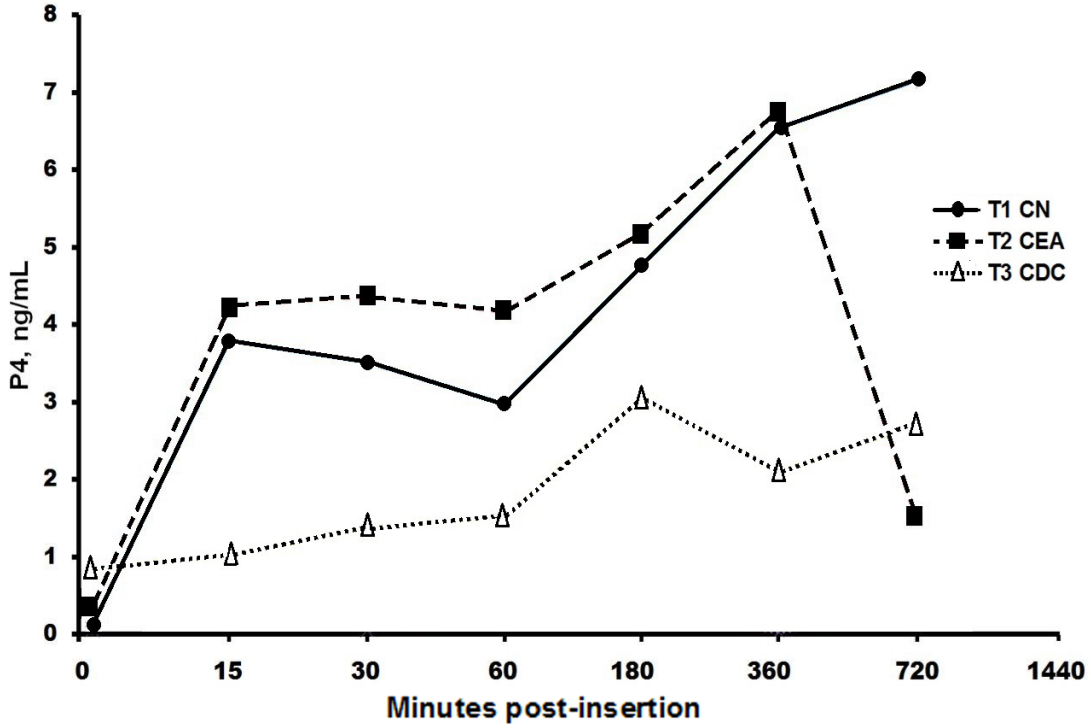

Figure 2. Average serum progesterone concentrations (P4, ng / ml) in Suizo x Cebú cows produced by new devices (T1 CN), sterilized in autoclave (T2 CEA) and disinfected in chlorine (T3 CDC) during the first 12 h post -insertion.

There are structural and functional similarities between the PRID and CIDR devices, since both devices are constructed using micronized progesterone in silicone rubber skin that is molded in a nylon (CIDR), or stainless steel structure (PRID; Rathbone *et al*., 1998). Due to this similarity, it is possible that the thermal sterilization process used in the present study may have resulted in the same effect observed in autoclaved PRID devices.

Some researchers (Colazo *et al.,* 2004) have used CIDR immersed in a detergent solution based on povidone iodine for 2 hours; followed by washing, rinsing with water, drying in the air and sterilizing with steam in an autoclave equipment; while others have used restricted schemes, only for chemical disinfection (Van Cleeff *et al* 1992; Martínez *et al.,* 2007) and gas sterilization (Schmitt *et al.*, 1996b). Padula and Macmillan (2006) demonstrated that changes in the vaginal and uterine microflora in the early postpartum of beef cows occurred during 14 days post-insertion of a CIDR. These authors indicate that the microbial cultures in swabs after the withdrawal of the CIDR, produced intense bacterial growth, being the isolated species *Pseudomonas aeruginosa* and *Actinomyces pyogenes.*

CONCLUSION

The disinfection/cleaning process of the CIDR devices did not affect the content and availability of their progestogen, so they can be used again in the process of estrus synchronization in cows under dry tropic conditions.

BIBLIOGRPHY

AYALA A, Honhold R, Delgado R, Magaña J. 1995. A visual condition scoring scheme for Bos indicus and crossbred cattle. In: Anderson, S. and Wadsworth, J (editors) Proceeding of an International Workshop on Dual Purpose Cattle Research. IFS/FMVZ-UADY, Mérida, Yucatán, México.

CERRI RLA, Rutgliano HM, Bruno RGS, Santos JEP. 2005. Progesterone (P4) concentrations and ovarian response after insertion of a new or a 7-day used intra-vaginal P4 insert (IPI) in proestrus lactating cows. *Journal of Dairy Science*. 83 (Suppl. 1): 37. ISSN: 1525-3163. ISSN: 0022-0302

COLAZO MG, Kastelic JP, Whittaker PR, Gavaga QA, Wilde R, Mapletoft RJ. 2004. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. *Animal Reproduction Science*. 81: 25–34. ISSN: 0378-4320 https://doi.org/10.1016/j.anireprosci.2003.09.003

GRAJALES H, Hernánez A, Prieto E. 2006. Determinación de parámetros reproductivos basados en los niveles de progesterona en novillas doble propósito en el trópico

colombiano. Livestock Research for Rural Development. 18. Article #144. Disponible en http:/www.lrrd.org/lrrd18/10/graj18144.htm

INEGI. 2005. Instituto Nacional de Estadística Geografía e Informática: X Censo de población y vivienda (Cartografía Geoestadística del Estado de Michoacán). México. Volumen I, Tomo 16, pp. 171.

LUCY MC, Billings HJ, Butler WR, Ehnis LR, Fields MJ, Kesler DJ, Kinder JE, Mattos RC, Short RE, Thatcher WW, Wettemann RP, Yelich JV, Hafs HD. 2001. Efficacy of an intra-vaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. *Journal of Animal Science*. 79:982-995. ISSN: 1525-3163. doi:10.2527/2001.794982x Disponible: file:///C:/Users/scorrea/Downloads/jas-79-4-982.pdf

MACMILLAN KL, Peterson AJ. 1993. A new intra-vaginal progesterone releasing device (CIDR-B) for oestrous synchronization, increasing pregnancy rates and the treatment of postpartum anoestrus. *Animal Reproduction Science*. 33: 1–25. ISSN: 0378-4320. https://doi.org/10.1016/0378-4320(93)90104-Y

MACMILLAN KL, Taufa VK, Barnes DR, Day AM. 1991. Plasma progesterone concentrations in heifers and cows treated with a new intra-vaginal device. *Animal Reproduction Science.* 26: 25–40. ISSN: 0378-4320. https://doi.org/10.1016/0378-4320(91)90063-6

MAPLETOFT RJ, Martinez MF, Colazo MG, Kastelic JP. 2003. The use of controlled internal drug release devices for the regulation of bovine reproduction. *Journal of Animal Science.* 81 (E. Supplement 2): E28–E36. ISSN: 1525-3163. doi:10.2527/2003.8114_suppl_2E37x Disponible: file:///C:/Users/scorrea/Downloads/jas-81-14_suppl_2-E28.pdf

MARTINEZ MF, Kastelic JP, Adams GP, Mapletoft RJ. 2002. The use of a progesterone-releasing device (CIDR-B) or melengesterol acetate with GnRH, LH, or estradiol benzoate for fixed-time AI in beef heifers. *Journal of Animal Science*. 80:1746-1751. ISSN: 1525-3163. doi:10.2527/2002.8071746x Disponible file:///C:/Users/scorrea/Downloads/jas-80-7-1746%20(1).pdf

MARTINEZ MF, Colazo MG, Kastelic JP, Mapletoft RJ. 2003. Effects of estradiol and progesterone on plasma steroid and gonadotropin concentrations in CIDR-treated ovariectomized cows. *Theriogenology*. 59: 224-228. ISSN: 0093-691X. DOI: 10.1016/j.domaniend.2006.04.009

MARTINEZ MF, Kastelic JP, Colazo MG, Mapletoft RJ. 2007. Effects of estradiol on gonadotrophin release, estrus and ovulation in CIDR-treated beef cattle. *Domestic*

Animal and Endocrinology. 33: 77–90. ISSN: 0739-7240 https://doi.org/10.1016/j.domaniend.2006.04.009

MCPHEE SR, Doyle MW, Davis IF, Chamley WA. 1983. Multiple use of progesterone releasing intra-vaginal devices for synchronization of oestrus and ovulation in cattle. *Australian Veterinary Journal*. 60: 40–43. ISSN: 1751-0813 DOI: 10.1111/j.1751-0813.1983.tb05859.x. Disponible: http://onlinelibrary.wiley.com/doi/10.1111/j.1751-0813.1983.tb05859.x/epdf?r3_referer=wol&tracking_action=preview_click&show_checkout=1&purchase_referrer=onlinelibrary.wiley.com&purchase_site_license=LICENSE_DENIED

PADULA AM, Macmillan KL. 2006. Effect of treatment with two intra-vaginal inserts on the uterine and vaginal microflora of early postpartum beef cows. *Australian Veterinary Journal.* 84: 204–208. ISSN: 1751-0813. DOI: 10.1111/j.1751-0813.2006.tb12800.x Disponible: http://onlinelibrary.wiley.com/doi/10.1111/j.1751-0813.2006.tb12800.x/epdf?r3_referer=wol&tracking_action=preview_click&show_checkout=1&purchase_referrer=onlinelibrary.wiley.com&purchase_site_license=LICENSE_DENIED

RATHBONE MJ, Macmillan KL, Bunt CR, Burggraaf S, Burke C. 1997. Conceptual and commercially available intra-vaginal veterinary drug delivery systems. *Advanced Drug Delivery Reviews.* 28: 363–392. ISSN: 0169-409X https://doi.org/10.1016/S0169-409X(97)00089-6

RATHBONE MJ, Macmillan KL, Inskeep K, Burggraaf S, Burke CR. 1998. Fertility regulation in cattle. *Journal of Controlled Release*. 54: 117–148. ISSN: 0168-3659. https://doi.org/10.1016/S0168-3659(98)00003-0

RATHBONE MJ, Bunt CR, Ogle CR, Burggraaf S, Macmillan KL, Burke R, Pickering KL. 2002. Reengineering of a commercially available bovine intra-vaginal insert (CIDR insert) containing progesterone. *Journal of Controlled Release*. 85:105–115. ISSN: 0168-3659 https://doi.org/10.1016/S0168-3659(02)00288-2

RICHARDSON AM, Hensley BA, Marple TJ, Johnson SK, Stevenson JS. 2002. Characteristics of estrus before and after first insemination and fertility of heifers after synchronized estrus using GnRH, PGF2, and progesterone. *Journal of Animal Science*. 80: 2792–2800. ISSN: 1525-3163 doi:10.2527/2002.80112792x. Disponible: file:///C:/Users/scorrea/Downloads/jas-80-11-2792.pdf

SALAS-RAZO G, Gutiérrez-Vásquez E, Ku-Vera JC, Aké-López R. (2011). Reinicio de la actividad ovárica posparto y concentración plasmática de metabolitos lípidos y progesterona en vacas suplementadas con grasa de sobrepaso. *Tropical and Subtropical Agroecosystems,* 14:385-392. (ISSN: 1870-0462)

SAS. 2009. User's Guide: Statistics, Version 9.2th Edition. SAS Institute Inc., Cary, North Carolina. USA.

SCHMITT EJP, Drost M, Diaz T, Roomes C, Thatcher WW. 1996. Effect of a gonadotropin-releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. *Journal of Animal Science*. 74: 154–161. ISSN: 1525-3163. doi:10.2527/1996.741154x Disponible: file:///C:/Users/scorrea/Downloads/jas-74-1-154.pdf

STEVENSON JS, Lamb GC, Johnson SK, Medina-Britos M A, Grieger DM, Harmoney KR., Cartmill JA, El- Zarkouny SZ, Dahlen CR, Marple TJ. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. *Journal of Animal Science*. 81: 571–586. ISSN: 1525-3163. doi:10.2527/2003.813571x Disponible: file:///C:/Users/scorrea/Downloads/jas-81-3-0810571.pdf

VAN CLEEFF JK, Lucy MC, Wilcox CJ, Thatcher WW. 1992. Plasma and milk progesterone and plasma LH in ovariectomized lactating cows treated with new or used controlled internal drug release devices. *Animal Reproduction Science*. 27: 91–106. ISSN: 0378-4320 https://doi.org/10.1016/0378-4320(92)90049-J