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Comparison of equine chorionic gonadotropin (eCG) and oestradiol cypionate administered 24 h after CIDR removal during an oestrus synchronization protocol for artificial insemination in Mexican Criollo cattle

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ABSTRACT

Two experiments were conducted to evaluate the effect of administering equine chorionic gonadotropin (eCG) in substitution of estradiol, 24 h after the removal of a progesterone intravaginal device, on estrus and ovulatory response, and pregnancy rates of Rodeo Criollo cows (exp. 1, n = 21) and heifers (exp. 2, n = 39) subjected to a synchronization protocol (estradiol + CIDR + $PGF_{2\alpha}$) with estrus-detected artificial insemination. All females were inseminated 12 h after detected estrus. In cows, estrus and ovulation response, and maximum pre-ovulatory follicle diameter were similar ($P>0.05$) between equine chorionic gonadotropin and estradiol groups. However, the time to estrus was shorter ($P<0.05$) with estradiol, but equine chorionic gonadotropin increased ($P<0.05$) the estrus and ovulation grouping, as well as pregnancy rates. In heifers, estradiol increased ($P<0.05$) estrus response and reduced ($P<0.05$) time to estrus, without any difference ($P>0.05$) in ovulation rate. Both treatments resulted in low pregnancy rates, with a significantly lower ($P<0.05$) rate in heifers treated with equine chorionic gonadotropin. In conclusion, application of equine chorionic gonadotropin in substitution of estradiol, after the removal of a progesterone-releasing device in an estrus-detected AI protocol, increased the estrus and ovulation grouping and, consequently, pregnancy rate in cows, but not in heifers.

Key words: native cattle, estrus synchronization, equine chorionic gonadotropin

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Introduction

Indigenous cattle breeds are considered to be a valuable genetic resource due to their rusticity and high adaptability to harsh environmental conditions, so that various worldwide organizations promote their conservation. In fact, these cattle breeds have historical, social and cultural implications for some regions or countries. However, many of these breeds are currently threatened with extinction. The Food and Agriculture Organization of the United Nations (FAO, 2007) reported that 20 % of native cattle breeds are at risk and 9 % are already extinct. Therefore, the establishment of breeding programs using reproductive biotechnology is required for the rescue and conservation of those breeds.

Criollo breed cattle are found in the northern regions of Mexico and the south of the United States, and are also known as Corriente (QUEZADA-CASASOLA et al., 2014). This breed, a native of the State of Chihuahua, Mexico, has been exported on a large scale to the United States and Canada to be used in the sporting activities of Rodeos. As a result, its population in the place of origin is scarce, and currently it is in danger of disappearing (RIOS, 2010). In this regard, reproductive management techniques, such as estrus synchronization and artificial insemination (AI), are a powerful tool that may be used to improve the reproductive efficiency and the productivity of this cattle breed.

The estrus synchronization protocols, with fixed-time AI, that are usually applied to beef cattle have been tested on Criollo cattle in order to induce reproductive activity in heifers and to reduce the postpartum anestrus interval in cows (TORRES et al., 1997; RAMÍREZ et al., 1998; ZÁRATE-MARTÍNEZ et al., 2010). The protocol based on a progesterone-releasing intravaginal device (controlled internal drug release; CIDR) plus 1.0 mg estradiol, with applications of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and estradiol 24 h after CIDR removal, has produced high rates of estrus occurrence, but with a very low pregnancy rate. Using that protocol, ZÁRATE-MARTÍNEZ et al. (2010) reported 100 % of Criollo multiparous cows in estrus with a pregnancy rate of 9.1 % after applying fixed-time AI (56 h). Similarly, in Brangus cattle treated with the same hormonal protocol, MALIK et al. (2012) observed the same effect, with pregnancy rates of 28.6 % and 18.0 % after natural mating and AI, respectively. ZÁRATE-MARTÍNEZ et al. (2010) indicated that the low pregnancy rate with this protocol is due to the highly-variable ovulation time observed in Criollo cattle, therefore, the use of fixed-time artificial seems unsuitable. Possibly, by applying equine chorionic gonadotropin (eCG) instead of estradiol 24 h after CIDR removal with AI following the AM-PM rule (AI 12 h after the observed onset of estrus), an increase in the pregnancy rate of Criollos may be achieved, given the fact that ovulation time can become more predictable during the 12 h after onset of estrus (BARUSELLI et al., 2004). An injection of eCG after the removal of a CIDR increased the development rate of a dominant follicle, estradiol synthesis, ovulation rate and progesterone synthesis after AI (BARUSELLI et al., 2004; RAMÍREZ-GODÍNEZ et al.,

2000), as well as pregnancy rates, which were higher when compared with control groups (SOUZA et al., 2009; SÁ FILHO et al., 2010a; SÁ FILHO et al., 2010b) and cows treated with estradiol (FERNÁNDEZ-ABELLA and VILLEGAS, 2002).

It must be noted that the use of eCG has not been previously evaluated in synchronization protocols applied to Criollo cows or heifers, so the aim of the present study was to evaluate the effect of the administration of eCG, in substitution for estradiol after CIDR removal, on estrus behavior, ovulation and pregnancy rates in heat-detected AI of Criollo cows and heifers, after a synchronization protocol based on estradiol, progesterone and $PGF_{2\alpha}$.

Materials and methods

The present study was carried out in the northern Mexican state of Chihuahua, and consisted of 2 experiments in which all procedures involving animals were conducted according to the approved local, official techniques of animal care and health in México (Federal law on animal health; Articles 19 to 22 and NOM-051-ZOO-1995: Humane care of animals during mobilization). Experiment 1 was conducted at the Teseachi ranch, which is owned and managed by the Autonomous University of Chihuahua (28º48´N, 107º25´W), using 21 open, non-lactating, approximately 5-year-old Criollo cows, with an average weight of 350 kg. Experiment 2 was conducted at the ranch of the Autonomous University of Ciudad Juarez (31º21 N, 105º59´W), on 39 Criollo heifers, with an average age of 15 months and an average weight of 212.5 kg. Both cows and heifers had a body condition score of 4 (scale 1 to 9; RICHARDS et al., 1986) and were considered as cyclic, as all of them had progesterone serum concentrations >1 ng/mL in one or two blood samples obtained 7 days apart, before the beginning of each experiment (JOHNSON et al., 2002). Progesterone serum concentrations were determined in the endocrinology laboratory at New Mexico State University, following the methodology described by SCHNEIDER and HALLFORD (1996). The assay included tubes which were coated with an antibody against P4 and 125I-progesterone as the tracer. The reported sensitivity of the assay was 0.1 ng/mL. The inter and intra-assay coefficients of variation were 2.6 % and 3.7 %, respectively. The low, medium and high detectable values were 2, 5 and 10 ng/mL, with coefficients of variation of 3.5 %, 2.6 % and 2.9 %, respectively.

In each experiment, all females were treated with a CIDR device (1.9 g progesterone, Pfizer, Hamilton, New Zealand) for 7 d and were injected with 2.76 mg of estradiol benzoate (Estrol, Loeffler, México, México) at CIDR insertion, and 25 mg of dinoprost trometamine (Lutalyse, Pfizer, Hamilton, New Zealand) at the moment of its removal (Fig. 1). After the removal of CIDR (24 h), the females were randomly divided in two groups to be treated with: 1) 1.0 mg of estradiol cypionate (ECP, Pfizer, Hamilton, New Zealand) or 2) 500 IU of eCG (Folligon, Intervet, Unterschleißheim, Germany). In

experiment 1, 11 cows were treated with estradiol and 10 with eCG, while in experiment 2, 19 and 20 heifers were treated with estradiol and eCG, respectively. Finally, all females were inseminated 12 h after onset of behavioral estrus, as detected by one out of four trained technicians. Estrus detection was performed continuously for 56 h, starting at the time of injection of eCG or estradiol. A female was considered to be in heat when allowed to be mounted by a herd mate for ≥ 3 s. After estrus detection, AI was performed by one of two trained technicians, who were randomly assigned to a similar number of females in each experiment. Frozen semen from one of two fertility-proven Criollo bulls was used randomly and equally in all females of both experiments.

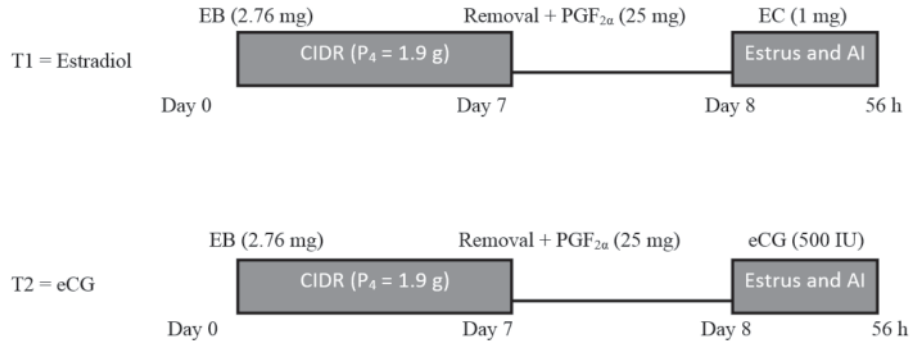

Fig. 1. Synchronization protocols used on Criollo cows and heifers that include estradiol benzoate (EB), progesterone (CIDR) and $PGF_{2\alpha}$. Experimental treatments are estradiol cypionate (EC) and equine chorionic gonadotropin (eCG) after CIDR removal. All females were inseminated 12 h after onset of estrus.

On the day of insertion of the CIDR, the largest follicle present was measured in each female of both experiments. Also, at 0, 24, 48, 72, 96 and 120 h after CIDR removal, transrectal ultrasound images of both ovaries of all females were obtained, to determine the growth pattern of the pre-ovulatory follicle and its time of ovulation. A female was considered as ovulated when a dominant follicle disappeared between two consecutive observations. Additionally, at D 40 after AI, each female was subjected to pregnancy diagnosis by transrectal ultrasound checking, using an Aloka 1200 device (Hitachi Aloka, Wallingford, CT) in experiment 1 (cows) and a Sonovet 600 device (Medison Inc., Cypress, CA) in experiment 2 (heifers), both with 7.5 MHz linear array transducers.

The study variables measured in each experiment were: estrus response (proportion of females that showed heat out of all treated females), hours to estrus (time interval from application of treatments to the onset of estrus), silent estrus (proportion of females that had not exhibited estrous behavior but had ovulated), estrus without ovulation (proportion of females that exhibited estrous behavior and did not ovulate), females that

ovulated (proportion of females that showed disappearance of pre-ovulatory follicle), maximum diameter of the pre-ovulatory follicle (diameter reached by the pre-ovulatory follicle before its disappearance), females with small and large follicles (proportion of females with pre-ovulatory follicles with a maximum diameter <10.7 and ≥ 10.7 mm, respectively; PERRY et al., 2007), pregnancy rate of treated females (proportion of females that were diagnosed as pregnant 40 d after AI of all treated) and pregnancy rate of females that showed estrus (proportion of females diagnosed as pregnant 40 d after AI of those that showed estrus)

Statistical analyses were performed separately for each experiment (cows and heifers). Those variables that were expressed as proportions were analyzed with chi-squared and Fisher's exact tests, using the PROC FREC. Hours to estrus and the maximum diameter reached by the pre-ovulatory follicle were analyzed under a completely randomized design using the PROC GLM. Comparisons of means were performed with Tukey tests and differences between means was determined when $P<0.05$. Additionally, time to onset of estrus was divided into two groups (12 to 24 and >24 to 36 h) to distribute the percentage of females that showed estrus. Likewise, the interval in which the pre-ovulatory follicle disappeared after the application of treatments was divided into three groups (48 to 72, 72 to 96 and 96 to 120 h) to distribute the percentage of ovulating females. For each interval, the percentage of females in estrus and ovulating were compared with chi-squared and Fisher's exact tests using the PROC FREC. All statistical analyses were performed with the statistical program SAS (version 9.0, 2004).

Results

Experiment 1. Cows. The mean diameters of the largest follicle detected by ultrasonography at insertion of the CIDR in cows treated with estradiol and eCG were 9.6 ± 1.2 and 9.2 ± 0.9 mm, respectively. A total of 19 (90 %) cows responded to the synchronization protocol with estrus signs in a 56-h time period after treatment ended. Eleven of those cows were treated with estradiol and the rest were treated with eCG (Fig. 2). Between 12 and 24 h after treatment application, the percentage of cows in estrus was higher ($P<0.05$) with estradiol than with eCG (36.4 vs. 12.5 %), but the opposite results were observed between >24 and 36 h post-treatment (63.5 and 87.5 % for estradiol and eCG, respectively). No cows showed estrus signs between hours 36 and 56 after the injection of estradiol or eCG.

Regarding the distribution of ovulating cows after the treatment application (Fig. 3), the percentage between 48 and 72 h was higher ($P<0.05$) in cows treated with eCG than in those treated with estradiol (70 vs. 44.4 %); but in the 72 to 96 h interval, more ($P<0.05$) cows treated with estradiol ovulated compared with cows treated with eCG (44.4 vs. 20 %). No differences in the percentage of ovulating cows were observed between treatment groups during the 96 to 120 h interval ($P>0.05$).

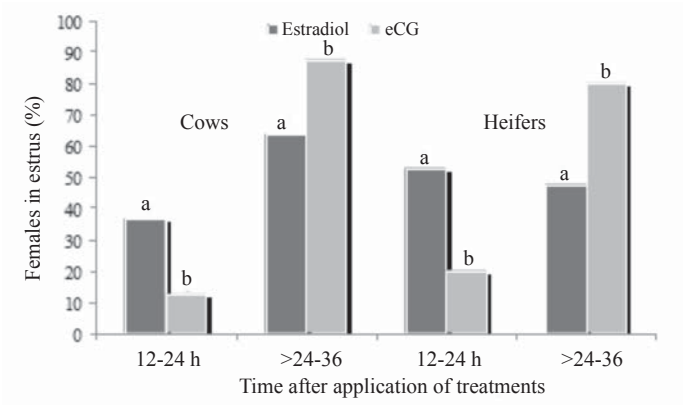

Fig. 2. Distribution of Criollo cows and heifers in estrus in similar time intervals after application of estradiol or eCG 24 h after withdrawal of the vaginal CIDR device ([a,b] different letters within a time interval indicate differences at P<0.05).

Table 1. Estrous behavior, ovulatory response and pregnancy rate of Criollo cows treated with eCG or estradiol 24 h after withdrawal of the vaginal CIDR device.

	Treatments	
Parameter	Estradiol	eCG[1]
Estrus response (%)	100.0 (11/11)[a]	80.0 (8/10)[a]
Time to estrus (h)[2]	24.9 ± 2.8[a]	31.5 ± 2.8[b]
Silent estrus (%)	0.0 (0/11)[a]	20.0 (2/10)[a]
Estrus without ovulation (%)	0.0 (0/11)[a]	0.0 (0/10)[a]
Cows that ovulated (%)	100.0 (11/11)[a]	100.0 (10/10)[a]
Pre-ovulatory follicles		
Maximum diameter (mm)	10.4 ± 1.6[a]	10.2 ± 1.6[a]
Small size (%)	45.5 (5/11)[a]	50.0 (5/10)[a]
Large size (%)	54.5 (6/11)[a]	50.0 (5/10)[a]
Pregnancy rate (%)		
of treated cows	27.3 (3/11)[a]	60.0 (6/10)[b]
of cows in estrus	27.3 (3/11)[a]	75.0 (6/8)[b]

[a,b] Different letters within a row indicate significant difference (P<0.05); [1] Equine chorionic gonadotropin; [2] Time to estrus after application of estradiol or eCG

Table 1 shows the results of estrus behavior, ovulation response and pregnancy rate after AI of treated cows. The percentages of cows in estrus were similar (P>0.05) between cows treated with estradiol or eCG, although estradiol produced 20 % more cows in estrus than eCG. Cows treated with estradiol had a shorter interval of time to estrus (P<0.05) than those treated with eCG (24.9 vs. 31.5 ± 2.8 h). Percentages of cows that showed ovulation, silent estrus and estrus without ovulation were similar (P>0.05) between treatments. The maximum diameter of the pre-ovulatory follicle did not vary in terms of the effect of treatments (P>0.05), nor did the percentage of cows with small or large pre-ovulatory follicles (P>0.05). The overall pregnancy rate was higher (P<0.05) in cows treated with eCG than in cows treated with estradiol.

Experiment 2. Heifers. The mean diameters of the largest follicle detected by ultrasonography at insertion of the CIDR in heifers treated with estradiol and eCG were 9.1 ± 0.8 and 8.6 ± 1.1 mm, respectively. A total of 22 (56.4 %) heifers responded to the synchronization protocol with estrus signs in a 56-h time period after treatment ended. Seventeen of those heifers were treated with estradiol and 5 were treated with eCG. The percentage of heifers in estrus between 12 and 24 h was higher (P<0.05) in the estradiol group than in the eCG group (53 vs. 20 %), and in the interval of >24 to 36 h, the percentage of heifers in estrus was higher (P<0.05) with eCG than with estradiol (80 vs. 47 %; Fig. 2). No heifers showed estrus signs between hours 36 and 56 after the injection of eCG or estradiol. In the distribution of heifers that ovulated (Fig. 3), similar proportions (P>0.05) of ovulating heifers were observed at all intervals of time (48 to 72, 72 to 96 and 96 to 120 h) between treated groups.

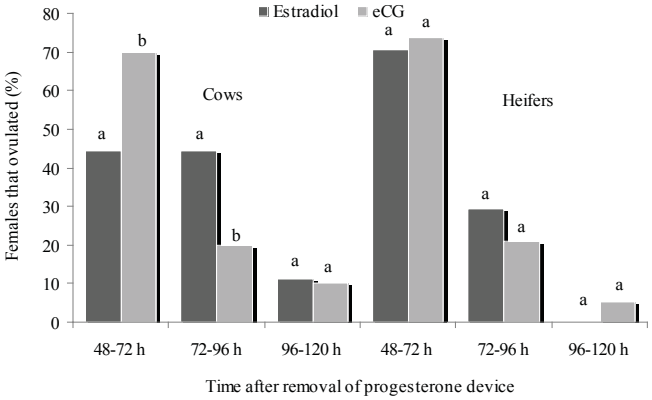

Fig. 3. Distribution of Criollo cows and heifers that ovulated in similar time intervals after application of estradiol or eCG 24 h after withdrawal of the vaginal CIDR device ([a,b] different letters within a time interval indicate differences at P<0.05)

Table 2 shows the results of estrous behavior, ovulatory response and pregnancy rate after AI. Estrus response was higher ($P<0.05$) and time to estrus was lower ($P<0.05$) in heifers treated with estradiol than in heifers treated with eCG. The percentage of silent estrus was higher ($P<0.05$) after application of eCG, while the percentage of estrus without ovulation was not affected ($P>0.05$) by the treatment. Likewise, the percentage of ovulating heifers was similar ($P>0.05$) between treatments.

Table 2. Estrous behavior, ovulatory response and pregnancy rate of Criollo heifers treated with eCG or estradiol 24 h after withdrawal of the vaginal CIDR device.

Parameter	Treatments	
	Estradiol	eCG[1]
Estrus response (%)	89.5 (17/19)[a]	25.0 (5/20)[b]
Time to estrus (h)[2]	25.8 ± 2.9[a]	30.6 ± 2.9[b]
Silent estrus (%)	10.5 (2/19)[a]	75.0 (15/20)[b]
Estrus without ovulation (%)	10.5 (2/19)[a]	0.0 (0/20)[a]
Heifers that ovulated (%)	89.5 (17/19)[a]	100.0 (20/20)[a]
Pre-ovulatory follicles		
Maximum diameter (mm)	11.3 ± 1.4[a]	10.1 ± 1.4[a]
Small size (%)	41.2 (7/17)[a]	60.0 (12/20)[b]
Large size (%)	58.8 (10/17)[a]	40.0 (8/20)[b]
Pregnancy rate (%)		
of treated heifers	31.6 (6/19)[a]	10.0 (2/20)[b]
of heifers in estrus	35.3 (6/17)[a]	40.0 (2/5)[a]

[a,b] Different letters within a row indicate significant difference ($P<0.05$). [1] Equine chorionic gonadotropin, [2] Time to estrus after application of estradiol or eCG

The average maximum diameter of the pre-ovulatory follicles was similar ($P>0.05$) in heifers treated with estradiol or eCG, but the rate of heifers with small or large pre-ovulatory follicles differed ($P<0.05$) between treatments, with a higher ($P<0.05$) incidence of small follicles when heifers were treated with eCG. The rate of large follicles was higher ($P<0.05$) in heifers treated with estradiol than in heifers treated with eCG. The pregnancy percentage in treated heifers was higher ($P<0.05$) with estradiol treatment compared with eCG treatment, however, a similar percentage of pregnant heifers was obtained ($P>0.05$) between treatments when this study variable was calculated from heifers that showed estrus.

Discussion

The results of the present study indicate that estrus and ovulation, as a response to the application of estradiol or eCG, after removal of the CIDR, were different in cows

and heifers, which may have occurred because the heifers involved in the study, although detected as cyclical according to their progesterone serum concentrations, may have not reached reproductive maturity at the time of insemination, in terms of the optimal functioning of the hypothalamic-hypophyseal-gonadal axis (MEZA-HERRERA et al., 2009; STEVENSON et al., 2006).

The results of estrus response suggest that both estradiol and eCG may be used to stimulate the ovaries of Criollo cows synchronized with CIDR-based synchronization protocols. However, estradiol yielded an estrus response in a shorter interval of time but it was more dispersed, which is due to differences in the mechanisms of action of the treatment hormones. Estradiol is the hormone responsible for generating the signs of estrus, so exogenous application of this hormone increases its blood concentration rapidly and estrus appears in a shorter period of time (MENEGHETTI et al., 2009). Meanwhile, eCG has effects similar to FSH, inducing the growth of the dominant follicle, and the subsequent and gradual secretion of estradiol, depending directly on the maximum follicular size reached (RAMÍREZ-GODÍNEZ et al., 2000). Similar results of estrus behavior were observed by DUFFY et al. (2004) in beef cows treated with estradiol or eCG. In heifers, the lower occurrence of estrus in those treated with eCG was probably caused by a failure of the hormone to stimulate the follicular growth and functional development in an efficient manner, which might have led to the synthesis of low amounts of estradiol, with the corresponding absence of signs of estrus in most females. This may be verified by the percentage of heifers treated with estradiol or eCG that showed small pre-ovulatory follicles (41.2 and 60 %, respectively). Although the most effective dose of eCG for synchronizing time of estrus is for the most part unknown and its advantages are still unclear (FERREIRA et al., 2013), the results showing a low occurrence of estrus indicate that Criollo heifers may require a different dose of eCG in order to obtain a higher rate of estrus response. Perhaps a progesterone-based protocol that includes an application of PGF2α prior to CIDR (LAMB et al., 2010) removal may be more suitable for Criollo heifers.

The percentage of cows (100 %) and heifers (95 %) that ovulated was considerably high, with no differences between treatment groups in either experiment. This indicates that both estradiol and eCG were effective to induce ovulation when applied as part of progestin-based synchronization protocols. Nevertheless, ovulation in cows treated with eCG occurred in a more concentrated and predictable period of time after treatments, compared to cows treated with estradiol (70 *vs*. 44 % between 48 and 72 h). It is known that eCG synchronizes and groups estrus, promoting the stimulation and development of follicles in a synchronized manner as well, meaning that events such as pre-ovulatory LH peak and ovulation appear grouped (SOUZA et al., 2009; SÁ FILHO et al., 2010a). In contrast, estradiol does not stimulate follicular development, causing ovulation only in those cows

that have a pre-ovulatory follicle, and the rest may show estrus signs but ovulation will occur only when a pre-ovulatory follicle with LH receptors is present (COLAZO et al., 2003). Previous studies indicate that, when administered, estradiol is capable of reducing the variation in the occurrence of the LH pre-ovulatory peak, but that may depend on the calculated dose and its time of application after the progestin regime (COLAZO et al., 2003; COLAZO et al., 2005). In heifers, it was observed that application of estradiol or eCG 24 h after CIDR withdrawal was equally effective to induce ovulation in a grouped manner over a short period of time (estradiol = 70.6 and eCG = 73.6 % between 48 and 72 h after progestin). These results differ partially from those obtained in cows in experiment 1, in which cows treated with estradiol ovulated in a less synchronized manner. COLAZO et al. (2005) and SOUZA et al. (2009) pointed out that different environmental and genetic factors may alter the response of cattle to estrus synchronization protocols.

Unlike the cows, the heifers showed abnormalities related to their estrous and ovulatory behavior, being most noticeable after eCG treatment (75 vs. 10.5 %). The high percentage of ovulating heifers without estrous signs by eCG effect was possibly caused by the small size of their pre-ovulatory follicles (10.1 ± 1.4 mm), thus, the amount of estradiol that is secreted by such follicles was not sufficient to trigger the onset of behavioral heat. Small follicles were also observed in heifers treated with estradiol, however, exogenous application of this hormone favored estrus occurrence. To our knowledge, previous studies have not reported the occurrence of silent estrus or estrus without ovulation in Criollo or beef cattle breeds, when females are treated with estrus synchronization protocols that include CIDR, estradiol and $PGF_{2\alpha}$. Nevertheless, ZARATE-MARTÍNEZ et al. (2010) reported that Criollo cows with low body condition scoring during the postpartum period did not show any estrous signs, nor ovulation; however, as body condition increased, a large number of cows that ovulated appeared, but still without visual estrous signs. This may suggest that estrous behavior abnormalities are common in these cattle, and that in heifers, a higher dose of eCG (>500 IU) is required to increase estrus occurrence after this synchronization protocol.

Aside from attempting to obtain a higher and more grouped occurrence of estrus and ovulation, the main reason for replacing estradiol with eCG 24 h after removal of a CIDR in Criollo females was to increase follicular development and the maximum size of the pre-ovulatory follicle, thus, assuring a larger corpus luteum, that is able to produce higher amounts of progesterone which subsequently increases pregnancy rates. SOUZA et al. (2009), SÁ FILHO et al. (2010a) and BUTLER et al. (2011) reported these benefits from application of eCG in Holstein and Nelore cattle after synchronization protocols that include CIDR, estradiol and $PGF_{2\alpha}$. However, in the present study it was observed that the maximum diameter of the pre-ovulatory follicle, in both cows and heifers, did not increase after the application of eCG, when compared to females treated with estradiol.

Conversely, eCG increased the proportion of heifers with small pre-ovulatory follicles (<10.7 mm). In general, it was observed that follicles in Criollo cows and heifers treated with eCG or estradiol were considerably smaller than those observed by others authors in studies performed with other cattle breeds (JAISWAL et al., 2009; TOWNSON et al., 2002). Perhaps these results are due to the body condition of the females at the time of the experiments. BUTLER et al. (2011) reported that the correct function of the ovary after the application of a protocol that includes CIDR, estradiol, $PGF_{2\alpha}$ and eCG depends directly on the body condition of the females.

It should be noted that GINTHER et al. (2003) defined as dominant a follicle that has reached a diameter of ≥ 10 mm, which is contrary to that observed in the present study. Criollo cows and heifers in our study had follicles <10 mm (8 and 9 mm), which were considered as dominant, because they ovulated after estradiol or eCG application. This may suggest that, even though they are smaller, dominant follicles in Criollo cattle may be capable of responding to estradiol or eCG with ovulation in the same manner as larger follicles from females of other *Bos taurus* (SARTORI et al., 2001) and *Bos indicus* (GIMENES et al., 2008) breeds.

In cows, application of eCG 24 h after CIDR removal increased pregnancy rates, when compared to estradiol (60 and 27.3 % of treated females, respectively, and 75 and 27.3 % of females that showed estrus, respectively). This is caused by the high synchrony between the events related to estrus and ovulation and AI, in cows treated with eCG. This result may be observed easily considering that most cows showed onset of heat (87.5 %) between 24 and 36 h after the removal of the CIDR and they were inseminated 12 h after, which means that AI was performed between 36 and 48 h after treatment, and that most cows (70 %) that were treated with eCG ovulated between 48 and 72 h after treatment. Previously, ZARATE-MARTÍNEZ et al. (2010) recommended the AM-PM rule to inseminate Criollo cows after a synchronization protocol similar to the one with estradiol in the present study. However, the results in the present study indicate that this technique is not appropriate to increase the pregnancy rate after estradiol treatment, in contrast to eCG. It should be mentioned that according to these results, eCG treatment with the synchronization protocol in the present study may be accompanied by fixed-time AI. In the heifers, the overall pregnancy rate was not high, but when it was calculated from treated animals, it was observed that estradiol (31.6 %) yielded a higher pregnancy rate, when compared to eCG (10 %). These results were observed because only heifers that showed estrus were inseminated and eCG did not produce a good estrus response.

Conclusions

In conclusion, under the conditions in which the present study was conducted, application of eCG in substitution of estradiol 24 h after removal of the CIDR, proved to

be more effective in cows than in heifers, since eCG increased the pregnancy rate in cows with estrus and ovulation occurrence similar to estradiol. In Criollo heifers, substitution of estradiol by eCG reduced estrus occurrence, and consequently the pregnancy rate, since only those females that showed estrus were inseminated. Studies may be required in the future of the optimum dose of eCG in estrus synchronization protocols for Criollo females, taking into account the high percentage of heifers that were treated with eCG and had silent estrus in the present study. Finally, considering that both estradiol and eCG induced ovulation favorably in Criollo cows and heifers but eCG yielded ovulations in a more grouped manner and a higher pregnancy rate, it is feasible to develop a hormonal treatment with fixed-time AI in these cattle.

Conflict of Interest

The author declares no conflicts of interest.

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SAŽETAK

Provedena su dva pokusa sa svrhom procjene učinka korionskog gonadotropina konja (eCG) na estrus, ovulacijski odgovor i stopu gravidnosti u krava Rodeo Criollo pasmine. Korionski gonadropin je korišten kao zamjena za estradiol i primijenjen je 24 sata nakon uklanjanja intravaginalnoga progesteronskog sredstva kao dijela protokola za sinkronizaciju (estradiol + CIDR + $PGF_{2\alpha}$) s otkrivanjem estrusa i umjetnim osjemenjivanjem. Protokol je primijenjen u krava (pokus 1, n = 21) i junica (pokus 2, n = 39). Sve su one bile osjemenjene 12 sati nakon otkrivanja estrusa. Estrus, ovulacijski odgovor i maksimalni preovulacijski promjer folikula bili su slični (P>0,05) u skupinama krava kod kojih je bio primijenjen korionski gonadrotropin ili estradiol. Međutim, u skupini kod koje je bio primijenjen estradiol vrijeme do estrusa je bilo kraće (P<0,05), dok je korionski gonadropin konja povećao (P<0,05) grupiranje estrusa i ovulacije kao i stopu gravidnosti. Kod junica, estradiol je povećao (P<0,05) estrusni odgovor i skratio (P<0,05) vrijeme do estrusa, bez razlike (P>0,05) u stopi ovulacije. Oba su postupka dovela do niže stope gravidnosti, pri čemu je smanjenje kod junica kojima je bio primijenjen korionski gonadotropin konja bilo signifikantno (P<0,05). Zaključno, primjena korionskog gonadotropina konja, kao zamjene za estradiol nakon uklanjanja sredstva za otpuštanje progesterona, dovela je u sklopu protokola za otkrivanje estrusa i umjetno osjemenjivanje do povećanog grupiranja estrusa i ovulacije te posljedično veće stope gravidnosti u krava, ali ne i u junica.

Ključne riječi: autohtono govedo, sinkronizacija estrusa, korionski gonadotropin konja