Colegio de Postgraduados

Use of equine chorionic gonadotropin (eCG) in Mashona heifers, under a J-Synch synchronization protocol, and its effect in pregnancy rate

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ABSTRACT

Objective: To evaluate the J-Synch protocol with two eCG doses in beef heifers during the summer in northeastern Mexico.

Design/Methodology/Approach: 218 heifers (109/treatment) with a body weight of 350±12.1 kg were used. A J-Synch protocol was applied in order to evaluate two eCG doses: T1 (250 IU) and T2 (300 IU). Subsequently, the total number of heifers from both treatments were inseminated at a fixed time (FTAI): 72 h after removing the device.

Results: No significant differences ($p>0.05$) were recorded in the estrous percentage at first service (T1=91%; T2=96%) and in the repeating heifers (T1=25.2%; T2=19.2%). The insemination technician and coat color variables impacted the estrous percentage in repeating heifers. The pregnancy percentage at first service was 64.8% and 70.1% for T1 and T2 ($p>0.05$), respectively. The insemination technician had a variable effect on the pregnancy percentage, from 63.4% (the best-qualified technician) to 48.6% (the technician who obtained the lowest percentage).

Study Limitations/Implications: The pregnancy rate in beef heifers will depend mainly on the experience and skill of the insemination technician.

Findings/Conclusions: The same results were obtained regarding the presence of estrous and pregnancy in beef heifers during the summer season, either with 250 or 300 IU of eCG.

Keywords: Mashona heifers, estrous, artificial insemination, breeding season, GnRH.

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INTRODUCTION

Worldwide cattle raising faces a complex outlook due to the climate change that mainly affects the semi desertic regions, as a result of the lack of rainfall. This phenomenon severely limits the productivity of cow-calf production systems. Consequently, breeds with medium to low body weight, with low management requirements, great adaptation to extreme weather, and which tolerate heat stress during the summertime have been introduced (Ferreira *et al.*, 2011; Fernandez-Novo *et al.*, 2020). One such breed is Mashona (*Bos taurus*), an African breed with black, brown, and reddish coat. In semi desertic regions with a high level of solar radiation, this breed could face reproductive issues, mainly related to pregnancy rates.

Anzures-Olvera *et al.* (2019) reported that dark coat colors absorb more solar radiation, while Bertipaglia *et al.* (2018) recorded an outward transfer of heat energy from the coat, impacting body temperature. Consequently, pregnancy rates diminish when heifers suffer heat stress 42 days before and 40 days after being artificially inseminated (Jordan, 2003).

In cow-calf production systems, genetic improvement through artificial insemination and estrous and ovulation synchronization programs have become widespread throughout the years (Bó and Baruselli, 2014; Yánez-Avalos *et al.*, 2021). Modifications to the estrous and ovulation synchronization protocols have helped to correct the absence of estrous and ovulation and have increased pregnancy and calving rates in beef cattle (Dias *et al.*, 2009). One of the most outstanding improvements is fixed-time artificial insemination (FTAI), which includes a progesterone-based hormonal treatment and the administration of estradiol benzoate (EB) at the start of the protocol. The intravaginal device is removed on the eighth day and a synthetic prostaglandin is applied, along with estradiol cypionate. Alternatively, EB is administered 24 h after the FTAI —on average, 56 h after the device is removed (Bó and Cedeño, 2018; Baruselli *et al.*, 2018). However, during the last decade, estrous synchronization protocols have been developed, focusing on beef heifers (Colazo *et al.*, 2017). These protocols face the following limitations: an extended proestrus stage; an inadequate development of the follicle diameter or the age of the ovarian follicle (Menchaca *et al.*, 2015); and a corpus luteum that can produce enough progesterone which can support pregnancy (Aréchiga-Flores *et al.*, 2019; Bó *et al.*, 2019). These principles were the basis for the development of the J-Synch protocol, a short 5-6 d methodology (Motta *et al.*, 2016; Macmillan *et al.*, 2020), during which equine chorionic gonadotropin (eCG) is administered at the time of the removal of the intravaginal device (Motavalli *et al.*, 2017), replacing the estradiol cypionate and the administration of GnRH, 72 h after the vaginal device is removed (Menchaca *et al.*, 2015; Reineri *et al.*, 2020; Zwiefelhofer *et al.*, 2021; Núñez–Olivera *et al.*, 2022).

Under the J-Synch protocol, the eCG will boost the increase of the circulating estradiol concentrations (De la Mata *et al.*, 2015; De la Mata *et al.*, 2018), the diameter of the ovarian follicle (Souza *et al.*, 2009; Pessoa *et al.*, 2016), and the progesterone concentration after the ovulation in beef heifers (Sales *et al.*, 2016; Ferraz *et al.*, 2019; Núñez-Olvera *et al.*, 2020). Based on this scheme, 300 IU (Núñez-Olvera *et al.*, 2020; Yanet-Avalos *et al.*, 2021) or 500 IU (Mahdavi-Roshan *et al.*, 2020) doses have been used to induce the ovulation of the codominant follicle. These eCG doses have been used in medium and large size cows; however, their effect in medium to small size heifers (such as Mashona cows) has not been evaluated yet. Therefore, the objective of this study was to determine the effect of two eCG doses on the estrous and the pregnancy percentage of 13-month-old heifers, during the summer in northeastern Mexico. Recent studies carried out with dairy cattle established that the use of two eCG doses (0 and 300 IU) does not affect the follicle dynamic or the pregnancy rate (Sanz *et al.*, 2022); consequently, this study proposes the hypothesis that using two doses of eCG (250 and 300 IU) will have a similar effect on the estrous and the pregnancy rate of beef heifers.

MATERIALS AND METHODS

The experimental work was carried out in the Rancho Río Salado, located in the municipality of Vallecillo, Nuevo León, México (26° 53' 25.1" N and 99° 52' 15.6" W), at 274 m.a.s.l., with a 580-mm mean annual precipitation. The experiment was developed from June to August 2021, during the summer in northeastern Mexico, when the average temperature was 35.3±2.1 °C.

Animal handling

The study was carried out according to the NOM-062-ZOO-1999 official standard, complying with the care and welfare of the animals during all the stages of the 12-week research. Eighteen-month-old heifers (n=218) from the Mashona (*Bos taurus*) breed were used for the experiment; on a scale from 1 to 9, they had a body condition (BC) of sex and body weight (BW) of 350±22.5 kg. All the heifers grazed in a marvel grass (*Dichanthium annulatum*) prairie, and they also had free access to mineral salt (Fosminsal 9%, Agronutrientes del Norte, General Escobedo, N.L., Mexico).

Treatments

Before the establishment of the experimental work, a reproductive evaluation was carried out using an Eco2 real time ultrasound with a 7.5 MHz transrectal probe (Sonoscape, USA). The aim of this evaluation was to exclude pregnant animals or animals with poor development of their reproductive system. Consequently, out of 232 animals, n=218 were available for the study. In order to assign the treatments and to improve the artificial insemination (AI) process, the heifers were randomly divided into two groups: Group I=114 heifers and Group II=104 heifers.

Estrous synchronization and ovulation

The J-Synch protocol was applied to a total of 218 heifers, which were inseminated a week apart, according to the group to which they had been assigned. The protocol consisted of the application of 2 mg of EB (Internacional Prode, Jalisco, Mexico) and a Dispocel Max intravaginal device (ID) (Internacional Prode, Jalisco, Mexico), with 1.2 g of synthetic progesterone on day 0. The device was removed on day 5 and 0.15 mg of cloprostenol (DC) (Internacional Prode, Jalisco, Mexico) was applied. The heifers of each group received one of the two eCG treatments: T1=250 IU and T2=300 IU. The hormone was applied at the moment of the removal of the intravaginal device (Figure 1). Regardless of the eCG treatment, all the heifers were subjected to fixed-time artificial insemination (FTAI), 72 h after the removal of the device; 0.5-mL straws of frozen semen from a bull of the same breed and of proven fertility were used for the FTAI. The heifers were inseminated by four experienced technicians (two technicians per group).

Each heifer received 10.5 μg of GnRH (Buserelin-acetate, Biogenesis Bago, Mexico) at the moment of the AI. In order to guarantee a 72-h AI within each group (consisting in an average of 25-50 heifers), the device was inserted and removed at 2 h intervals. The same intravaginal devices of the first service were used for the resynchronization of the estrous. The devices were washed, disinfected (with Antibenzil©, Altamirano,

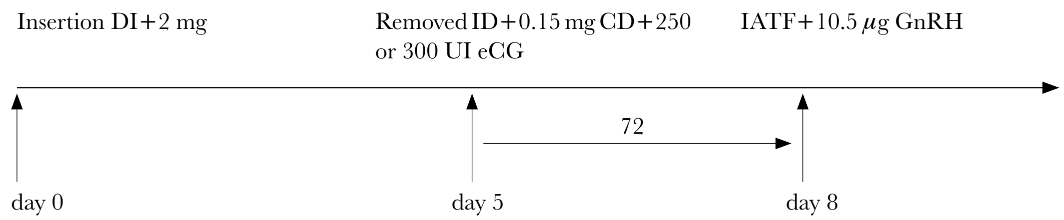

Figure 1. Arrangement of the J-Synch protocol used for the estrous synchronization and the ovulation, applied to Mashona heifers during the summer in northeastern Mexico. ID: intravaginal device; DC (CD): cloprostenol; FTAI (IATF): fixed-time artificial insemination; eCG: equine chorionic gonadotropin; GnRH: gonadotropin-releasing hormone.

Mexico), and dried at room temperature. The devices were reinserted 13 days after the first service and were removed 7 days later. In order to detect the estrous, the base of the tail of the heifers was painted; afterward, the heifers were divided into groups depending on the time that the estrous was detected. Heifers were inseminated 12 h later. The intravaginal devices that fell at the removal, during the insertion and the reinsertion periods, were counted. The devices that were not found inside the vagina at the moment of the removal were considered lost.

A Chi-square (χ^2) test was used to determine the discrete variables —such as percentage (%) of estrous, repeating heifers, pregnancy at the first and second service, and devices lost during the first and second service. A lineal model was used to determine the removal-artificial insemination interval (h) and the time used to inseminate the heifer (s). The factors evaluated by this model were the eCG=250 and 300 IU doses, the group number (I and II), the insemination technician, and the color of the coat of the heifer (black, brown, and reddish). The SPSS software, version 22, was used.

RESULTS AND DISCUSSION

Table 1 shows that there were not differences regarding the percentage of estrous and the eCG doses applied, and the time after the removal of the devices, as well as the time required to carry out the AI. Regarding the evaluated factors, only the inseminator technician and the color of the coat impacted the percentage of the repeating heifers in the second service. The A and F technicians obtained a >30% estrus. These technicians were in charge of inseminating the first group of heifers. Consequently, Table 1 shows that Group I had a higher percentage of estrus than Group II ($P<0.001$). Additionally, regarding the coat of the heifers, brown coat heifers recorded the highest estrus percentage, doubling the percentage (36.9%) obtained by the reddish and black heifers. Regarding the removal-AI period, the parameter of the J-Synch protocol was fulfilled: in average the insemination was carried out 72 h after the removal of the intravaginal device. Table 1 also shows the differences in the pregnancy % of the first and second services related to the insemination technician. The best insemination technician recorded a 14.8% difference in the pregnancy %, compared with the technician that recorded the lowest pregnancy % ($P<0.05$). The other factors did not record statistically significant differences ($P>0.05$).

Table 1. Percentage of estrus during the second service, average time of insemination, time required to inseminate each heifer, and total pregnancy % of the first and second services (mean±SE).

Effect	Repeater heifers (% / estrous)	Interval removal-AI (h)	Time AI (s)	Pregnancy (%)
Inseminator technician				
A	13/35 (37.1)^{a}	73.5±0.2	80.8±15.5	48.6 (17/35)^{b}
B	4/58 (6.8)^{b}	72.3±0.1	90.4±14.3	59.5 (34/58)^{ab}
C	32/79 (40.5)^{a}	73.3±0.1	88.1±10.5	51.9 (41/79)^{b}
D	2/47 (4.2)^{b}	72.4±0.2	77.9±17.8	63.4 (30/47)^{a}
Treatment: eCG				
250 IU	28/111 (25.2)	72.9±0.1	81.5±10.2	64.8 (72/111)
300 IU	21/107 (19.2)	72.8±0.1	87.0±10.6	70.1 (75/107)
Color coat:				
Reddish	3/19 (15.7)^{b}	72.7±0.1	74.3±17.0	63.1(12/19)
Brown	17/46 (36.9)^{a}	72.8±0.1	88.2±13.0	63.0 (29/46)
Black	29/153 (18.9)^{b}	73.0±0.0	90.2±5.3	59.4 (89/153)
Lot:				
I	45/114 (39.4)^{a}	73.4±0.0	84.6±9.3	60.5 (69/114)
II	4/104 (3.8)^{b}	72.3±0.1	84.1±11.4	67.3 (70/114)

[a,b] The subscript of letters inside the same column are statistically different ($P<0.05$).

Table 2 indicates the values of the lost device percentage, both for the first insertion and the reinsertion of the devices. No significant differences were recorded ($P>0.05$). A high number of intravaginal devices were lost during the reinsertion of the devices, regardless of the groups or the color of the coat.

In this study, the hypothesis was that 250 or 300 IU of eCG will not result in differences in the estrus and pregnancy % of heifers, applying a J-Synch protocol. Given the lack of differences between both doses, the Mashona breed would respond to a lower dose. Remarkably, the pregnancy percentage was higher that the results reported by Macmillan *et al.* (2020), who used a J-Synch protocol with 300 IU of eCG and obtained a 48.7% pregnancy. However, this percentage can be the result of the presence of the corpus luteum at the moment when the eCG was applied.

Table 2. Loss of the intravaginal devices inserted in Mashona heifers, during the first and second services, using FTAI with a J-Synch protocol ($P>0.05$).

	Losses device first insertion (%)	Losses device reinsertion (%)
Lot		
I	9/114 (7.8)	11/114 (9.6)
II	12/104 (11.5)	18/104 (17.3)
Color coat		
Reddish	1/19 (5.2)	3/19 (15.7)
Brown	3/46 (6.5)	2/46 (4.3)
Black	17/153 (11.1)	24/153 (15.6)

The pregnancy rate was 20% lower than the results for Angus heifers recorded by De la Mata *et al.* (2015). In our study, reducing the time of insertion of the progesterone device extended the proestrus period, which could have improved the pregnancy %. An additional factor of improvement is to carry out the insemination 12 h after the estrus is detected. This study provides another alternative: a fixed time insemination, carried out 72 h after the removal of the device (Bó *et al.*, 2016).

In this regard, the impact of eCG on the pregnancy rate seems to extend the duration of the proestrus, improving the ovarian follicle diameter and the estradiol content, as well as the luteum function. Consequently, insemination 72 h after the removal of the device increased the pregnancy %, which matched the results of Nuñez-Olvera *et al.* (2020) and Sanz *et al.* (2022). In contrast to heifers, the use of eCG with or without the estrus did not change the pregnancy rate of adult cows (Mion *et al.*, 2019). The detection of the estrus during the first service was not a limitation in this study; however, the second service recorded low estrus percentages. This phenomenon could be the result of the adaptation of this breed to the negative effects of heat stress. Most of the studies about heifers have been carried out in favorable environments. However, this study was carried out during summer, with a range of temperatures from 30 °C to 35 °C. The above-mentioned conditions match the findings of Anzures-Olvera *et al.* (2019), who carried out their study in similar areas and found that the dark color of the Holstein heifers did not impact their reproductive yield and, instead, the temperature conditions affected milk production. The heat stress that prevailed during summer could have jeopardized the potential quality of the oocytes. Nevertheless, the breathing rate and the rectal temperature of the dark color coat heifers tend to raise, increasing the skin temperature and reducing the quality of the oocytes (Ferreira *et al.*, 2011). However, these results are different from those reported by Bertipaglia *et al.* (2018), who found that cows with $<$2 mm dark pigmentation recorded 0.3 conception services less than cows with $>$3 mm dark pigmentation areas. Although these studies are the first to be conducted using heifers under a J-Synch protocol, the pregnancy results of this study are acceptable, considering that they were carried out during a summer of extreme temperatures in northeastern Mexico. Nevertheless, further studies are required to determine if the J-Synch protocol has a growing rate that matches the dominant follicle after ovulation. That rate could result in a higher progesterone concentration and, therefore, a higher pregnancy percentage, particularly in heifers exploited under extreme summer conditions (Bó *et al.*, 2018).

This study determined that the insemination technician plays an important role in the process. Four expert technicians carried out the AI on the two groups, with 14 days of difference between procedures. The pregnancy rate difference between the technician who recorded the highest pregnancy % and the technician who recorded the lowest percentage was 16%. These differences could be the result of the amount of knowledge and the animal handling experience and, above all, the years of experience that the best technician has, given that the technician constantly puts the procedure in practice, manipulating the reproductive system of the animals. In the particular case of the Mashona heifers, we found out that the structure of the cervical canal obstructs the introduction of the insemination gun (Anzar *et al.*, 2003). García-Ispierto *et al.* (2007) reported that the inseminator technician contributed to the fertility improvement of dairy cows, because 23% of the technicians were experts and professionals. In our study, 25% of the inseminator technicians achieved a pregnancy improvement of at least 15%. Additionally, the percentage of intravaginal devices lost during the insertion and the reinsertion was higher than the figure reported by Hernández *et al*. (2008) in their study with adult Brangus (2.3%). The number of devices lost in our study was higher than the number recommended by Islam (2011) for cattle (<5%). This high percentage of lost intravaginal devices can mainly result from the narrow space in which the device can be inserted into the heifers: a third part of the device remained outside the vagina.

CONCLUSIONS

The J-Synch protocol is a reproduction management alternative to synchronize the estrus and the ovulation of heifers under the summer conditions of northeastern Mexico. Two-hundred fifty IU of eCG can be used without compromising the pregnancy rates of heifers, considering that the skill of the insemination technician will play an important role in the pregnancy in beef cattle. In this study, the Mashona breed obtained appropriate pregnancy rates regardless of the color of the coat of the heifers.

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