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SUPEROVULATION OF TROPICAL MILKING CRIOLLO FEMALES

Superovulación de hembras criollas Lechero Tropical

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ABSTRACT

Reproductive biotechnologies are used for the conservation, expansion and genetic improvement of cattle, and to facilitate the creation of germplasm banks of elite animals and endangered breeds. The objective of this study was to compare the effects of two different doses of follicle stimulating hormone (FSH) on the superovulatory behavior of Tropical Milking criollo (LT) females. The study was conducted in Veracruz, Mexico, in a hot climate. Twenty-five LT donors (47 ± 4 months of age and 423.9 ± 12 kg BW) were used. Two levels of follicle stimulating hormone of 260 (FSH1) and 210 mg (FSH2) were used for each treatment. The response variables were analyzed with a linear fixed effects model. The data were processed using generalized models with a Poisson distribution. The weight and age influenced the number of *corpora lutea*. The weight influenced the number of unfertilized oocytes, degenerate embryos and the total of collected structures. Only in unfertilized oocytes, statistical difference (P ≤ 0.004) was found between treatments, but not so in *corpora lutea*, degenerate embryos, blastocyst, grade one embryos, transferable embryos and the total of collected structures (P > 0.05). The FSH1 and FSH2 treatments induced the same number of transferable embryos (2.7 ± 0.6 and 3.5 ± 0.7) and grade one embryos (2.5 ± 0.6 and 3.4 ± 0.7); the 210 mg dose of FSH1 would be the most appropriate for superovulating LT females.

Key words: Embryos; FSH; hot climates; local breeds.

RESUMEN

Las biotecnologías reproductivas se utilizan para la conservación, expansión y mejora genética de bovinos y facilitan la creación de bancos de germoplasma de animales élite y razas en peligro de extinción. El objetivo del estudio fue comparar el efecto de dos dosis de hormona folículo estimulante en el comportamiento superovulatorio de hembras criollas Lechero Tropical (LT). El estudio se realizó en Veracruz, México, en un clima cálido. Se utilizaron 25 donadoras LT (47 ± 4 meses de edad y 423,9 ± 12 kg de peso vivo). Se utilizaron dos niveles de hormona folículo estimulante (FSH) de 260 (FSH1) y 210 mg (FSH2) por tratamiento. Las variables de respuesta se analizaron con un modelo lineal de efectos fijos. Los datos fueron procesados utilizando modelos generalizados con una distribución de Poisson. El peso y la edad influyeron en el número de cuerpos lúteos. El peso influyó en ovocitos no fertilizados, embriones degenerados y total de estructuras recolectadas. Solamente en ovocitos no fertilizados se encontró diferencia estadística (P ≤ 0,004) entre tratamientos, pero no en cuerpos lúteos, embriones degenerados, blastocisto, embriones calidad uno, embriones transferibles y total de estructuras recolectadas (P > 0,05). Los tratamientos FSH1 y FSH2 indujeron similar número de embriones transferibles (2,7 ± 0,6 y 3,5 ± 0,7) y embriones calidad uno (2,5 ± 0,6 y 3,4 ± 0,7), por lo que la dosis de 210 mg de FSH1 sería la más recomendable para superovular hembras LT.

Palabras clave: Embriones; FSH; climas cálidos, razas locales.

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INTRODUCTION

Several reproductive biotechnologies are used for conservation, expansion and breeding of cattle [3, 18, 47]. Multiple Ovulation and Embryo Transfer (MOET) shorten the generation interval and exploit the genetic potential of females and genetically superior sires [18, 26].

In Latin America, there are Criollo livestock breeds adapted to local environmental conditions, as a result of natural and artificial selection [44]. The Tropical Milking Criollo breed (LT, by its Spanish acronym) are *Bos taurus* which comes from cattle introduced to the New World in the fifteenth century, adapted to hot tropical climates [13, 37, 38]. The LT can feed in grasslands of tropical grasses and produce high quality milk in 305 days of lactations [40, 41]. The LT livestock population in Mexico is less than 1000 animals [16] and most farmers do not have pure breed females [2]. In order to genetically improve the LT, reproductive technology is used such as Artificial Insemination (AI); however, MOET has not been used extensively because of the lack of knowledge of the reproductive protocols that make feasible the use of this technology with the LT female.

Follicle Stimulating Hormone (FSH) has been widely used for superovulating large size milk producing females, such as the Holsteins, at doses up to 400 mg of Folltropin-V [23]; 1 mg of Folltropin-V is equivalent to 1 mg of NIH-FSH-P1 Bioniche Animal Health Inc., Belleville, Ontario, Canada, reference standard. LT females are small with an adult body weight (BW) of about 420 - 450 kg [12] and their superovulatory physiological response to different doses of FSH is unknown. Criollo Limonero cows from Venezuela were superovulated using 260 mg of Folltropin-V [19]. The use of MOET is expected to increase the number of pure LT females, facilitate genetic improvement and allow the establishment of a germplasm bank. Therefore, the objective of this study was to compare the effect of two doses of FSH on the superovulatory response of LT females.

MATERIALS AND METHODS

Location

The study was conducted in Veracruz, Mexico, at 19° 11´ N and 96° 20´ O, at an altitude of 23 meters, with an average annual rainfall and temperature of 1060 mm and 26.4 °C. The climate is sub humid hot with summer rains [Aw(w)(i´)g] [17].

Experimental females

Twenty-five LT donors were used with at most two calvings, of 47 ± 4 months and 423 ± 12 kg BW. All donors had regular estrous cycles and transrectal palpation was implemented in order to detect the presence of ovarian cysts or other abnormalities in accordance with the International Embryo Transfer Society (IETS) [30]. The donors' average body condition was 3.2 ± 0.4 on a scale of 1 (emaciated) to 5 (obese) [48]. Genetically superior bulls and elite females for 305 days (d) milk production were used as AI sires and donors. The straws used containing 20 x 10^6 sperm, they were thawed and the semen was inspected with a microscope (Axiostar Plus, ZEISS®, LLC, New York, USA); it showed individually progressive motility higher than 60 %. Three LT sires were randomized to cover the females, according with the structure of six families set out in the selection nucleus, in order to avoid inbreeding in the offspring.

Donor management

The donors were fed with star grass (*Cynodon plectostachyus*), pará (*Brachiaria mutica*) and native grass (*Paspalum* spp.); they were supplemented with a concentrate with 18 % crude protein, at 2 kg d^{-1} $animal^{-1}$, and corn silage (*Zea mays*), at a rate of 15 kg d^{-1} $animal^{-1}$, they were also given mineral salts and water *ad libitum*. They were internally dewormed orally (Paraxane 10%, Biogenesis Bago, SA, Monte Grande, Buenos Aires, Argentina, 5 mL per 100 kg BW) and externally (Bovitraz 12.5%, Bayer Laboratories, SA, Mexico City, Mexico; 40 mL per 20 L of water) by spraying every 20 d, they also received two applications of selenium (Mu Se®, Schering-Plough Laboratories, Friesoythe, Germany, 1 mL per 50 kg) and 15 mL of organic phosphorus (Catosal, Bayer Health Care Laboratories, Shawnee, KS, USA,) 30 and 10 d before the start of the superovulation protocol. The donor behavior was observed 1 hour (h) h in the morning and 1 h in the evening, for two cycles, and was found that all had regular estrous cycles, in an interval of 18-24 days (d).

Superovulation protocol and treatments

A superovulation protocol at fixed time (FIG. 1) was used. On d zero each donor received a progesterone-releasing intravaginal device (CIDR-B®, 1.9 g Dec. Manufacturing, Hamilton, New Zealand) and additionally 2.5 mg of estradiol benzoate (BE, Estrol, Loeffler, Institute Agrobioquímico SA de CV, Mexico) and 50 mg of Progesterone (P_4, Lapisa, SA de CV for Zoetis Mexico S de RL de CV, Mexico) were injected intramuscularly. Decreasing doses of FSH were administered daily from d four to seven in the morning and afternoon (Folltropin-V, NIH-FSH-P1, Bioniche Animal Health Inc., Belleville, Ontario, Canada) which completed total levels of 260 (FSH1, n = 13) and 210 (FSH2, n = 12) mg, corresponding to the two treatments, each assigned randomly to females. Intramuscular doses of 25 mg of Prostaglandin F2α (PGF2α; Lutalyse, Dinoprost-tromethamine, Pharmacia & Upjohn, Inc., Michigan, USA) were applied on d six in the morning and afternoon. The CIDR was removed 24 h after the PGF2α was applied and every donor received intramuscular doses of 200 UI of equine Chorionic Gonadotropin (eCG; Folligon, Intervet International, BV Boxmeer, Netherlands) in the morning and afternoon. On d eight, during the morning, 24 h after removing the CIDR, an intramuscular dose of 0.25 mg of Gonadotropin-releasing Hormone (GnRH; Gonadorelin Fertagyl, Intervet

International, Germany) was applied. The first AI was performed at 36 h of removing the CIDR and the second 12 h later, using two straws each time.

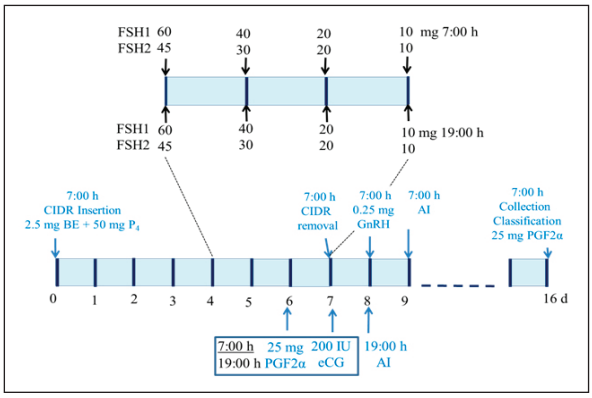

FIGURE 1. SUPEROVULATION PROTOCOL USED IN TROPICAL MILKING CRIOLLO FEMALES.

Embryo collection and classification

Embryos were collected by the nonsurgical method [45] seven d after performing the AI. The number of *corpora lutea* (CL) was determined by transrectal ovarian palpation before starting the washing. The epidural and perineal areas and the labia were disinfected with iodine foam, iodine germisin (Pharmaceuticals Altamirano of Mexico, SA de CV, Mexico) and 70 % alcohol; the epidural area was anesthetized with 5-10 mL of 2% lidocaine (Astra Zeneca, SA de CV, Mexico). The embryos were collected with a two-way Foley catheter of 14 or 20 fr with 30 cc balloon (CR Bard, Inc., Covington, GA, Malaysia); for the washing 1 L animal^{-1} of the collection medium was used (Vigro Complete Flush Solution, Bioniche Animal Health, Inc., Pullman WA, USA). The embryos were recovered from both horns with an EmCon (Agtech, Inc., Wisconsin, USA) filter. After the collection, 25 mg de PGF2α animal^{-1} were applied to lyse the existing CL and avoid a possible pregnancy. The embryos were located using a stereoscope (ZS-6 Plus, Bausch & Lomb, New York, USA), they were placed in a maintenance medium (Vigro Holding Plus, Bioniche Animal Health, Inc., Washington, USA) and were classified according to their degree of embryonic development [6].

Response variables

The response variables were measured according to classification described by Bó and Mapletoft [6]. It was considered that the donor responded to treatment if it presented two or more CL identified by transrectal ovarian palpation, when collecting embryos. The number of CL was registered when doing a transrectal palpation in each ovary just before starting the embryo collection. The number of unfertilized oocytes (UO), number of degenerated embryos (DE), number of blastocyst stage embryos (B), number of embryos grade one (EG1) –morulae and blastocysts-, number of transferable embryos (TE) –grade one and two, morulae and blastocysts- and total number of collected structures (TCS) –different stage embryos, including unfertilized oocytes- were studied.

Statistical analysis

The response variables were analyzed with a fixed effect linear model:

$$y_{ij} = \mu + T_i + \beta_1 (x_{1ij} - \bar{x}_{1...}) + \beta_2 (x_{2ij} - \bar{x}_{2...}) + \beta_3 (x_{3ij} - \bar{x}_{3...}) + \epsilon_{ij}$$

Where;
y_{ij} = j-th observation of i-th treatment.

μ = constant that characterizes the population.

T_i = fixed effect of the i-th treatment, i = 1, 2.

β_1 = regression coefficient relating the live weight with the response variable.

x_{1ij} = live weight associated with the response variable.

$\bar{x}_{1...}$ = average live weight.

β_2 = regression coefficient relating age with the response variable.

x_{2ij} = age associated to the response variable.

$\bar{x}_{2...}$ = average age.

β_3 = regression coefficient relating body condition with the response variable.

x_{3ij} = body condition associated to the response variable.

$\bar{x}_{3...}$ = average body condition.

ϵ_{ij} = random error. $\epsilon_{ij} \sim IIP(\lambda)$.

Initially, the bull effect was included in the model, but had no significant effect on the response variables, and was removed. The data were processed using generalized models with the procedure GENMOD of SAS [42]. A Poisson distribution of errors with logistic link function was used.

RESULTS AND DISCUSSION

The estimated means of the response variables are shown in TABLE I. No treatment effect (P > 0.05) for CL was observed. However, the covariates weight (P ≤ 0.006) and age (P ≤ 0.001) affected the number of CL with $\beta \hat{=} 4.4 \times 10^{-3} \pm 1.6 \times 10^{-3}$ and $\beta \hat{=} -0.3 \times 10^{-3} \pm 0.9 \times 10^{-3}$, respectively.

TABLE I
SUPEROVULATORY RESPONSE TO TWO DIFFERENT DOSES OF FSH (FOLLTROPIN-V) IN DONOR TROPICAL MILKING CRIOLLO (least square means ± standard error).

Treatment	CL	UO	DE	B	EG1	TE	TCS
FSH1 (n=13)	12.0±1.0a	1.4±0.5a	1.3±0.4a	2.9±0.6a	2.5±0.6a	2.7±0.6a	6.1±0.9a
FSH2 (n=12)	11.4±1.0a	0.3±0.2b	1.8±0.4a	3.3±0.7a	3.4±0.7a	3.5±0.7a	5.7±0.9a

a, b Different literal per column indicates statistical difference (P < 0.05). FSH1 = 260 mg, FSH2 = 210 mg, CL = corpora lutea, UO = unfertilized oocytes, DE = degenerated embryos, B = blastocyst, EG1 = embryos grade one, TE = transferable embryos, TCS = total number of collected structures.

In an empirical experience with LT cows, in which a dose of 160 mg of Folltropin-V was used for inducing superovulation, it was observed that the animals did not respond to treatment because of the low dose. Females of different genotypes have been superovulated using different doses of Folltropin-V. The number of CL is affected by the donor's age, females with advanced ages show lower ovulation [24] because older cows have fewer small ovarian follicles recruited in a follicular wave [28] and less large follicles after ovary overstimulation [29]. In contrast, very young donors superovulated with high FSH doses exhibit ovarian overstimulation and consequently poor ovulation [8]. The CL obtained in FSH1 and FSH2 were higher than 8.2 ± 0.6, 8.6 ± 0.6 and 8.7 ± 0.9 obtained in Sistani, native cattle from Bangladesh and Kamphaen Saen (*Bos indicus*) from Iran, Bangladesh and Tailand with the application of low doses of 120, 200 and 200 mg of Folltropin-V [1, 4, 32], respectively. The high response of zebu breeds to the application of low doses of FSH is attributable to a high degree of sensitivity of *Bos indicus* to exogenous applications [5]. However, for superovulation in big frame *Bos taurus* breeds such as Holstein Friesian and Brown Swiss from Canada and Turkey, high doses of 400 mg of Folltropin-V [10, 35] have been used, obtaining 8.7 ± 2.0 and 9.1 ± 1.8, lower results than those found in this study (TABLE I). Although the number of CL obtained between treatments in this study was similar, it was observed that LT females respond positively to the application of a moderate dose of FSH.

The Folltropin-V level affected (P ≤ 0.004) the UO number. The highest number of UO was observed with FSH1. The live weight covariate affected (P ≤ 0.03) the UO with $\beta \cong 1.4 \times 10^{-2} \pm 7.1 \times 10^{-3}$. The UO number obtained in FSH1 treatment was almost five times higher than in FSH2. Lopes da Costa et al. [27] obtained 0.7 ± 0.3 UO in Mertolenga native cattle from Portugal when applying 400 mg of Folltropin-V. Dong-Soo et al. [14] in Korean native cows with porcine FSH doses of 28 and 24 mg (Artrin-R10) obtained 2.1 ± 0.5 and 2.2 ± 0.5 UO. The largest UO number obtained with FSH1 treatment is associated with high doses of Folltropin-V because of the amount of FSH/Luteinizing Hormone (LH) contained in the product. Increasing the dose of products containing FSH increases the LH content equally, which causes luteinisation of follicles and premature ovulation [21].

The DE were not different between FSH1 and FSH2 (P > 0.05). The live weight covariate affected (P ≤ 0.02) the DE with $\beta \cong 1.1 \times 10^{-2} \pm 5.1 \times 10^{-3}$. The DE values obtained in this study (TABLE I) were lower than 2.7 ± 1.7 and 3.2 ± 0.2 obtained in Gyr and Brahman cows from Colombia which were superovulated with low doses of 200 and 240 mg of Folltropin-V [31, 39]; but, similar to 2.8 ± 0.9 and 1.4 ± 0.7 obtained in Mertolengo cows from Portugal and Brown Swiss cows from Turkey superovulated with high doses of 400 mg of Folltropin-V [9, 34]. Dong-Soo et al. [14] with Korean native cows receiving 28 and 24 mg of porcine (*Sus scrofa*) FSH (ARtrin-R10) obtained 0.8 ± 0.2 and 1.0 ± 0.3 DE. Although no differences between treatments were observed, the amount of DE obtained in this study was very low.

No differences between treatments (P > 0.05) were observed for B and a reduced number of morulae embryos and blastocyst expanded was found, which might be related to the degree of development of the embryos for the day since the AI until day seven of collection. The development degree of the embryos is related to the day elapsed since the AI until the collection [6, 25]. Callesen et al. [11] in dairy cows superovulated with FSH and eCG obtained 1.4 ± 0.6 B and Bono et al. [7] in beef cows superovulated with Human Menopausal Gonadotropin (hMG) obtained similar values to 3.3 ± 0.8 B, those results are similar to the observed in this study.

There was no treatment effect in EG1 (P > 0.05). Although no statistical differences were observed in EG1, a greater number was observed in FSH2. In Brown Swiss cows receiving 400 mg of Folltropin-V, 2.9 ± 1.3 EG1 [9] were obtained. Singh et al. [46] obtained 4.1 ± 0.7 EG1 in Simmental heifers with the application of 9.0 mg of ovine FSH-17 (Ovagen). The application of FSH2 low dose made it possible to induce a similar number of EG1 compared to FSH1 in this study.

No differences between treatments were observed for TE (P > 0.05). However, a higher number was observed with FSH2, the maximum number of TE was seven, in FSH1, and nine, in FSH2; and the minimum was zero, in both treatments. TE is the most important response variable of a multiple ovulation since the higher number of transferable embryos is more likely to produce a successful pregnancy [43]. The estimated TE values (TABLE I) were similar to 3.2 ± 0.2, 2.7 ± 1.1, 7.2 ± 7.4, 4.0 ± 2.4 and 4.4 ± 3.9 obtained in Native cattle from Bangladesh, Sistani, Gyr, Kanphaeng Saen and Brahman cows (*Bos indicus*), superovulated with low doses of 200, 120, 200, 200 and 400 mg [1, 4, 31, 32, 39] and Brown Swiss, Mertolengo and Holstein Friesian cows (*Bos taurus*) superovulated with high doses of 400 mg of Folltropin-V, respectively [10, 34, 35]. However, Roa et al. [36] superovulated Criollo Limonero cows in Venezuela and

obtained 2.2 ± 0.4 TE, similar results to those in this study (TABLE I). Estrada et al. [15] superovulated cows of different Colombian native breeds with doses of 36 and 24 mg of Folltropin-V and the highest value obtained was 3.0 ± 2.3 TE. The results of this study are greater than 1.3 ± 1.2 TE obtained with *Bos taurus* dairy genotypes heifers of 20-28 months age and of 196-265 kg, not adapted to tropical conditions and superovulated with different gonadotrophins [22]. However, no differences between treatments were observed, it is desirable to obtain a similar number of TE using a low dose of FSH.

The TCS was not different between treatments (P > 0.05). The covariate weight influenced (P ≤ 0.04) TCS with β = 5.7 x 10^{-3} ± 2.8 x 10^{-3}. TCS is affected by donor's weight because in an underweight female, alteration occurs in the pulsatile secretion of LH that disrupts the process of follicular development to the preovulatory phase [20]. The results (TABLE I) are similar to 3.8 ± 0.4, 4.7 ± 1.4, 9.8 ± 9.0, 6.7 ± 1.2 and 9.1 ± 5.6 TCS obtained in *Bos indicus* superovulated with low doses of 120, 200 and 240 mg of Folltropin-V [1, 4, 31, 32, 39]. However, Nilchuen et al. [33] in Kamphaeng Saen cows obtained 14.3 ± 3.6 TCS with doses of 200 mg of Folltropin-V. *Bos taurus* cows were superovulated with high doses of 400 mg of Folltropin-V [10, 34, 35] and obtained 6.3 ± 1.4, 10.0 ± 2.2 and 5.0 ± 2.1 TCS, similar to this study (TABLE I). However, Roa et al. [36] superovulated Criollo Limonero cows using low doses of 240 mg of Folltropin-V and obtained 3.1 ± 0.5 TCS, lower than in this study (TABLE I). The moderate response to the application of the 210 mg FSH2 doses in this study is reflected in the similar number of TCS regarding FSH1.

CONCLUSIONS

FSH2 treatment with doses of 210 mg of Folltropin-V produced the same number of transferable embryos and embryos grade one as FSH1 treatment, with doses of 260 mg. In turn, with the FSH2 treatment the number of unfertilized oocytes was almost five times lower than with FSH1, so the FSH2 treatment would be the most suitable to superovulate LT females.

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