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ORIGINAL

Effect of GnRH and D-Chloprostenol application on pregnancy and prolificacy rates on Pelibuey ewes

Efecto de la aplicación de GnRH y D-Cloprostenol en las tasa de gestación y prolificidad en ovejas Pelibuey

Jaime Martínez T,[1] Ph.D, Oziel Montañez V,[2]* Ph.D, Alejandro Ley De C,[1] Ph.D, Fernando Izaguirre F,[1] Ph.D, María Velazco Z,[3] Ph.D, Juan Aguirre M,[1] Ph.D.

[1]Universidad Autónoma de Chiapas, Facultad de Ciencias Agrícolas, Chiapas, México. [2]Universidad de Guadalajara, Centro Universitario del Sur, Departamento de Desarrollo Regional. Enrique Arreola Silva 883, Ciudad Guzmán, Jalisco. 49000. México. [3]Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Tuxtla Gutiérrez, Chiapas, México. * Corresponding: montanez77@hotmail.com

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ABSTRACT

Objective. Was to evaluate the effect of GnRH and D-Chloprostenol application on pregnancy and prolificacy rates on Pelibuey ewes. **Materials and methods.** Forty five ewes were randomly allocated to one of three treatments: T1(n=15), day 0: sponges with 65 mg medroxyprogesterone acetate (MPA) + 200 IU equine chorionic gonadotropin (eCG) and sponge removal (day 12) + breeding by natural mating (days 12-15); T2 (n=15), day 0: 50 µg gonadotropin releasing hormone (GnRH) + 7.5 mg D-Chloprostenol (day 5) + 50 µg GnRH (day 7) + insemination at fixed time (AIFT) 12 to 14 h after last injection of GnRH; T3 (n=15), 100 µg GnRH (day 0) + 7.5 mg D-Chloprostenol (day 5) + 100 µg GnRH (day 7) + AIFT 12 to 14 h after last injection of GnRH. **Results.** The average concentration of progesterone (P_4) in blood was 1.22 ± 0.74 ng/mL, which was used to verify ovarian activity at the beginning of the treatments. 100% of the T1 ewes presented estrus, beginning at 38.4±9.56 h after sponge removal. There were differences ($p<0.05$) for pregnancy rates, of 60, 33.33 and 46.66% respectively, among the treatments. Prolificacy was no different ($p>0.05$) among the treatments where the values were 1.2, 1.4 and 1.4 lambs/ewe for T1, T2 and T3, **Conclusions.** The results of this study show that the use of GnRH and D-Chloprostenol did improve pregnancy rates but did not improve prolificacy in tropical ewes.

Key words: Estrus synchronization, ewes, pregnancy (*Source: USDA*).

RESUMEN

Objetivo. Fue evaluar el efecto de la aplicación de GnRH y D-Cloprostenol en la tasa de gestación y prolificidad en ovejas Pelibuey. **Materiales y métodos.** Cuarenta y cinco ovejas fueron asignadas aleatoriamente a uno de tres tratamientos: T1(n=15), día 0: esponjas con 65 mg de acetato de medroxiprogesterona (MPA) + 200 UI de gonadotropina coriónica equina (eCG) al retirar las esponjas (día 12), servidas con monta natural (día 12-15); T2 (n=15), día 0: 50 µg de hormona liberadora de

gonadotropinas (GnRH) + 7.5 mg de D-Cloprostenol (día 5) + 50 µg de GnRH (día 7) + inseminación a tiempo fijo (IATF) 12 a 14 h después de la segunda inyección de GnRH; T3 (n=15), 100 µg de GnRH (día 0) + 7.5 mg de D-Cloprostenol (día 5) + 100 µg de GnRH (día 7)+IATF 12 a 14 h después de la segunda inyección de GnRH. **Resultados.** La concentración promedio de progesterona (P_4) en sangre fue 1.22 ± 0.74 ng/mL, que demostró actividad ovárica. El 100% de las ovejas de T1 presentaron estro, iniciando a las 38.4 ± 9.56 h del retiro de esponjas. El porcentaje de gestación fue diferente ($p<0.05$) entre T1, T2 y T3, siendo 60, 33.33 y 46.66%, respectivamente. La prolificidad no presentó diferencias ($p>0.05$) para T1, T2 y T3, siendo 1.2, 1.4 y 1.4 corderos/oveja parida, respectivamente. **Conclusiones.** Los resultados de este estudio indican que bajo condiciones tropicales el uso de GnRH y D-Cloprostenol, mejoró el porcentaje de gestación pero no la prolificidad.

Palabras clave: Índice de gestación, ovejas, sincronización de estro (*Fuente: USDA*).

INTRODUCTION

Different estrus synchronization systems exist for ewes, which is used to increase the productiveness percentages. It is very common today to use, synthetic progestogens, such as fluorogestone acetate (FGA) and medroxyprogesterone acetate (MPA), for a period of 10 to 16 days, which have proven effective for synchronizing estrus in ewes (1). In addition, application of gonadotropins, such as equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG), have increased the rate of ovulation and fertility when used in combination with progestogens in sheep (2); however, the pregnancy rate, is lower than through their natural estrus cycle.

An estrus synchronization system, known as ovsynch exists for ewes (3). This system combines the gonadotropin-releasing hormone (GnRH) with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to overcome problems and limitations in detecting estrus. With this method, a follicular wave is produced for the increasing GnRH pulses, causing the dominant follicle atresia and a new follicular wave is produced two or three days later (4). After a period of seven to nine days, luteolysis is induced by applying $PGF_{2\alpha}$. A second dosage of GnRH 48 h after $PGF_{2\alpha}$ causes synchronization of ovulation 8 h later. Currently, methods to control the time of GnRH administration to predict ovulation more precisely are sought (3). More research is required, on the control of combined synchronization and ovulation induction to prevent low conception rates in ewes.

This study was conducted to evaluate the effect of sponges with MPA plus eCG with natural mating (as relative control), compared to the administration of two doses of GnRH plus D-Chloprostenol™ and insemination at fixed time (IATF) on the fertility rate in Pelibuey (PEL) ewes under tropical conditions.

MATERIALS AND METHODS

Location. The study was conducted between the months of October and November of 2011, at the facilities of the Centro Universitario de Investigación y Transferencia de Tecnología CUITT (University Center for Research and Technology Transfer), San Ramón, of the Universidad Autónoma de Chiapas, located in the municipality of Villaflores, Chiapas, Mexico at 16º 14' N and 93º 15' W at an altitude of 610 m. The predominant climate is warm sub-humid with an average annual precipitation of 2600 mm distributed between May and November, and an average temperature of 22ºC (5).

Animals. The animals used were 45 PEL second lambing ewes, weighing 32.4±3.8 kg with a minimum of 60 d postpartum and a body condition score of three (6), were used in a completely randomized design with 15 ewes for treatment. Each group was housed in 10 × 10 m pens with fixed feeders, automatic watering devices for *ad libitum* access to water, and sufficient shade. The ewes were subjected to semi-intensive management which included grazing (7:00 to 16:00 h) in African star grass (*Cynodon plectostachyus*) pastures. They were offered 500 g of 16% CP supplement (mineral salt, kernel corn, sorghum, fishmeal, coffee husks, and molasses) per ewe, in metal troughs. Twenty days before the trial started, all of the ewes were dewormed orally (Albendazole 4%, Mexico) and were given an intramuscular injection (IM) of ADE vitamins (Vigantol™, Mexico). Additionally, 2.5 mL of Triangle™ Bac 8V (Bacterin toxoid, Mexico) was administered subcutaneously.

Procedure. Estrus synchronization protocols were based on MPA (Sincro gest™, Ovejero, Spain), eCG (Folligon™, Mexico), GnRH (Fertagyl™, Mexico), and $PGF_{2\alpha}$ (D-chloprostenol™, Mexico). The treatments were as follows: T1) was

considered the control treatment, in which on d 0 a sponge with 65 mg MPA was provided and withdrawn d 12 later when 200 IU eCG was injected IM. During days 12 to 15, ewes were bred through controlled natural mating. T2) was given 50 µg GnRH on d 0, and 7.5 mg D-Chloprostenol™ on d 5, 50 µg GnRH was administered again on d 7, and artificial insemination (AIFT) 12 to 14 h after second injection of GnRH was used. T3) received 100 µg GnRH on d 0, and then on d 5, 7.5 mg D-Chloprostenol™ and 100 µg GnRH again on d 7, plus AIFT 12 to 14 h after the second injection of GnRH.

Six days before the beginning of the experiment, 3 mL blood samples were taken from the jugular vein. Samples were centrifuged at 1560 × g for 15 min and stored at 2ºC until analysis. The serum was sent to the reproduction physiology laboratory of the School of Veterinary Medicine and Animal Production of the Universidad Nacional Autónoma de México (UNAM) to determine serum concentrations of P_4 by solid phase radioimmunoassay (RIA); concentrations below 1 ng/mL were considered indicators of an anoestrus stage, and concentrations above 1 ng mL^{-1} were considered indicators of a reproductive stage. In domestic species, physiological status it can be defined easily by an analysis of P_4 in blood or milk (7). The onset, distribution and percentage of estrus, were only measured in T1 ewes, between sponge removal and natural mating, and these were not measured in T2 and T3 due to the use of fixed time artificial insemination.

Pregnancy rate. The pregnancy rate was determined 50 d after the last mount or AITF, with a real time B mode ecograph and a 3.5 MHz transabdominal convex transductor. The percentage of estrus occurrence during the 4 days after withdrawing the intravaginal devices was quantified. The ewes were mounted by three, previously tested for health and fertility rams, 12 h after withdrawing the devices to ensure they were mounted. The onset of estrus was determined when the ewes were mating and was measured as the time interval, in hours, between removal of the sponges and mounting of the ram. Prolificacy was quantified after lambing in all of the treatments by dividing the number of lambs born by the number of ewes that gave birth.

Statistical analysis. The data obtained on pregnancy rates and prolificacy was analyzed by the chi-square test by the software MINITAB 14 (8).

RESULTS

The onset, distribution and estrus percentage were only measured in ewes T1, between sponge removal and natural mating, we found that all ewes in T1, presented estrus during the first 72 h after removing the sponges. In the first 24 h interval from estrus onset to the last application of eCG, one T1 ewe presented signs of estrus. In the interval of 24 h to 36 h, three ewes (20%) were in estrus; between 36 h and 48 h, nine ewes (53.3%) were in estrus. After 48 h, three ewes (20%) were in estrus. In T1, of the 15 ewes bred through natural mating, 100% initiated estrus in an average time of 38.4 ± 9.56 h after sponge removal.

There were differences ($p>0.05$) in pregnancy rates between the treatments. The results obtained were 60%, 33.33% and 46.66% for T1, T2 and T3, respectively. No differences in prolificacy were found among the treatments ($p>0.05$; Table 1).

Table 1. Pregnancy rate and prolificacy in ewes treated with different hormonal protocols.

	Treatments[1]		
	T1	T2	T3
Pregnancy rate	60.0a	33.3b	46.6a
Prolificacy	1.2a	1.4a	1.4a

[1]T1: Application of 65 mg MPA, plus 200 UI eCG with controlled mounting; T2: Application of 50 µg GnRH, plus 7.5 mg D-Chloprostenol™, plus 50 µg GnRH and AIFT. T3: Application of 100 µg GnRH, plus 7.5 mg D-Chloprostenol™, plus 100 µg GnRH and AIFT. [a, b] values with different literal are significant ($p<0.05$).

The results of the P_4 concentration indicate that three ewes (one in T1 and two in T2) were cycling only at the beginning of the experiment with P_4 average value above 1.1 ± 0.7 ng/mL in the blood.

DISCUSSION

Estrus induction results are similar to those reported by Martínez-Tinajero et al (9) and Zarakawi (10), who stated that when they applied sponges impregnated with MPA and eCG, estrus began after removing the sponges. In the study conducted by Martínez-Tinajero et al (11), when estrus was synchronized with the controlled internal drug releasing (CIDR) device impregnated with 0.3 g natural P_4 for 12 d, combined with 200 IU eCG, they found 100% of the F1 ewes (Damara x Merino) in estrus after removing the CIDR. Likewise, Ortega (12) observed that 89.5% of Katahdin ewes treated with intravaginal sponges containing 65 mg MPA for 14 d, plus IM application

of 250 IU eCG on day 12 of the treatment, began estrus. Karaca et al (13) reported a percentage of estrus induction of 90% on Polwarth ewes synchronized with intravaginal sponges with 65 mg MPA plus 250 IU eCG.

Estrus synchronization in ewes is possible using sponges inserted in the vagina for 12 d. When removed, they induce estrus by simulating the effect of a drastic reduction in P_4 levels in the blood stream and luteolysis, followed by re-initiation of follicle activity and appearance of estrus 1 to 2 d later. Some authors have reported this previously on wool ewes (9,10) as well as in hair sheep (14,15).

The estrus distribution results were similar to those found by Perez et al in goats (16) using sponges impregnated with 60 mg MPA. Martínez-Tinajero et al (9) also obtained intervals between 36 and 60 h in adult ewes treated with intravaginal sponges impregnated with 65 mg MPA and eCG, while Karaca et al (13) reported no differences in estrus response time in ewes synchronized with MPA. Fierro et al (17) mentioned that provoking CL regression and inducing estrus in ewes with two injections of $PGF_{2\alpha}$ separated by 8 d is not very efficient since intervals of up to 138±13.7 h after the last application of the treatment can be achieving due to luteus regression failure in a high proportion of ewes after the last injection. Pregnancy rates for T1 were similar to those reported by Fonseca and Torres (18), who obtained 59.9% using 200 IU eCG; Cueto and Gibbons (19) also reported a pregnancy rate of 47% with MPA, while Catalano et al (20), using a higher dosage of eCG (500 IU) plus sponges with MPA, obtained a pregnancy rate of 54% in cross-bred ewes (Frisona x Corriedale).

Kridli and Al-Khetib (21) state that the use of sponges for estrus synchronization allows controlled lambing in a high percentage of ewes (95.7%) in a short period of time (10 days), with no negative effects in pregnancy rates (86.6%). In addition, Vázquez et al (2) mention that injecting 400 IU eCG 48 h before removing the MPA sponges increases ovulation rates and, consequently, pregnancy rates. Powell et al (22) report that ovulation synchronization with analogues of the hormone GnRH has been used with better results in cattle. The application of GnRH in cattle causes ovulation of the dominant follicle at the moment of treatment, whether in the growth phase of the static phase, causing atresia of the follicles that are not in condition to ovulate and then a new wave of follicle development occurs two or three days after treatment. Boggio (23) states that the use of GnRH in ovines aims to group estrus in shorter intervals and induce estrus and ovulation in periods in which fertility is reduced because of the season of the year, additional to the time of year or to their nutritional state at the time of treatment (24), or perhaps because ovulation did not occur in all of the animals after treatment. Nagatani et al (25) indicate that different environmental stimuli and nutrition were considered among the most important factors in regulating the reproductive function of the animals. For this reason, it is possible that the fertility rates obtained in this experiment were affected by the time of year in which the ewes were treated. Furthermore, Hopkins et al (26) mentions that ewes kept at high environmental temperatures exhibit a reduction in their reproductive and productive behavior.

The ovsynch method has been applied and studied more in cattle than in ovines; Chevel et al (27) determined the effects of re-synchronizing after using the ovsynch protocol, administering GnRH (100 µg) on day 21 after AITF in Holstein cows, which reported a pregnancy rate of 70.9% 21 days later. Stevenson et al (28) used the ovsynch protocol in cows plus application of CIDR (1.9 g P_4) before the first injection of GnRH. The sponge was removed 1 to 2 h before the $PGF_{2\alpha}$ injection. They found a conception rate of 50% 28 d later. There is not much information in the literature on reducing the dosage of GnRH in cattle under the ovsynch protocol, but Pursley et al (29) reduced the dosage to 50 µg and reported percentages of synchronization similar to those when 100 µg GnRH were used. They mention, however, that the complete recommended dosage of $PGF_{2\alpha}$ should be used for the AITF protocols.

Akif and Kuran (30), state that the used of gonadotropins in ewes stimulates the release of FSH and LH by the anterior hypophysis, which results in an increase in the ovulation rate, and therefore, in the pregnancy rate. Induction of multiple ovulation is transcendental in the smaller ruminants since higher dividends can be obtained if the ewes produce more than one offspring at a time.

In conclusion the use of 50 or 100 µg GnRH, combined with D-Chloprostenol ($PGF_{2\alpha}$) and AIFT, did improve pregnancy rates but did not increase prolificacy percentages in PEL ewes when compared to the MPA protocol plus eCG and natural mating. The combination of GnRH and $PGF_{2\alpha}$ in AIFT protocols facilitates artificial insemination at a predetermined hour without the need of detecting estrus. There is not sufficient information on the ovsynch protocol in ewes, and we recommended that further studies be conducted on a larger number of animals, in order to obtain more information about this topic.

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