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Reproductive response of postpartum multiparous and nulliparous ewes to GnRH and prostaglandin $F_{2\alpha}$ injections

Respuesta reproductiva de ovejas multíparas postparto y nulíparas a inyecciones de GnRH y prostaglandina $F_{2\alpha}$



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ABSTRACT

The objective was to compare the reproductive responses of postpartum multiparous and nulliparous ewes to GnRH and PGF_{2a} injections. The ewes in G1 (*n*=14) were lactating, non-pregnant and with 68.21±13.48 days postpartum. The ewes in G2 (*n*=13) were lactating, non-pregnant and with 93.76±7.25 days postpartum. The ewes in G3 (*n*=14) were non-pregnant and non-lactating. Transrectal ultrasonography was carried out nine days apart to assess ovarian activity. Then, two GnRH injections were given nine days apart to ewes. An injection of PGF_{2a} and a third injection of GnRH was administrated to ewes seven and nine days after the second injection of GnRH. The number of ewes bearing a corpus luteum was higher after the injections with GnRH (*P*≤0.05). Follicle population was higher (*P*≤0.05) in ewes from G1 than in ewes from G2 and G3. The diameter of the largest ovarian follicle was larger (*P*≤0.05) in G2 than in ewes from G1 and G3. Estrus presentation and pregnancy rate were not different among groups (*P*>0.05). In conclusion, two GnRH injections increased the incidence of corpus luteum and equalized estrus presentation and pregnancy rate after PFG2a injection in postpartum multiparous ewes compared to nulliparous ewes.

Keywords: anestrus, gonadotropins, lambing, luteolysis, ovarian follicles, pregnancy, prolificacy.

RESUMEN

El objetivo fue comparar la respuesta reproductiva de ovejas multíparas postparto y nulíparas a inyecciones de GnRH y PGF_{2α}. Las ovejas en G1 (*n*=14) estaban lactando, vacías con 68.21±13.48 días posparto. Las ovejas en G2 (*n*=13) estaban lactando, vacías con 93.76±7.25 días posparto. Las ovejas en G3 (*n*=14) estaban vacías y no lactando. Ultrasonografía transrectal fue llevada a cabo, nueve días aparte, para medir actividad ovárica. Posteriormente, las ovejas recibieron dos inyecciones, nueve días aparte, de GnRH. Una inyección de PGF_{2α} y una tercera de GnRH fueron proporcionadas a las ovejas el día siete y nueve, después de la segunda inyección de GnRH. El número de ovejas con cuerpo lúteo fue mayor después de



las inyecciones con GRH ($P \le 0.05$). La población folicular fue mayor ($P \le 0.05$) en ovejas del G1 que en las del G2 y G3. El diámetro del folículo más grande fue mayor ($P \le 0.05$) en ovejas del G2 que en las del G1 y G3. La incidencia de celos y tasa de gestaciones no fueron afectados (P > 0.05). En conclusión, dos inyecciones de GnRH incrementaron la incidencia de cuerpos lúteos, e igualó la incidencia de celos y la tasa de gestaciones después de la inyección con PGF2 α en ovejas multíparas postparto en comparación con ovejas nulíparas.

Palabras clave: folículos ováricos, gonadotropinas, luteolisis, parición, preñez, prolificidad.

INTRODUCTION

Animal production systems support human welfare (Salmon et al., 2020). However, they are currently producing food products under pressure due to the increasing demand for animal commodities. In addition, there is political pressure that demands sustainable production to avoid environmental degradation (Kusch-Brandt, 2020). Therefore, the intensification of production and application of novel approaches to preserve our living environment are the current challenges of the global community of animal production scientists (Mayberry et al., 2020). Regarding sheep production, intensification of the system requires more than one lambing per year (Miguel-Cruz et al., 2019). This might be achieved by improving reproductive management and applying hormonal and natural (male effect) treatments, mainly during the seasonal and postpartum anestrum. In this regard, progesterone, prostaglandin $F_{2\alpha}$ (PGF_{2 α}), GnRH and equine chorionic gonadotrophin (eCG) are the most common hormones used to synchronize ewe reproductive events. However, there has been an increasing concern about the use of progesterone and eCG, and they are not widely available (Gonzalez-Bulnes et al., 2020). Thus, there is an urgency to focus on the strategic use of GnRH and $PGF_{2\alpha}$ in estrus synchronization protocols to improve sheep reproductive performance.

Ewes have two periods of anestrus, which naturally occur after lambing and during the long-day season of the year. A rapid resumption of reproductive activity after these periods is essential to lambing intensification. However, ewes face several physiological challenges after lambing, such as lactation and lamb suckling, which are known to delay the resumption of the reproductive axis function by suppressing the neurological network involved in the release of GnRH and gonadotropins (Hernández-Hernández *et al.*, 2021). In addition, silent ovulations and abnormal luteal phases (short and long duration) that compromise ewe fertility are observed during the postpartum period (Ascari *et al.*, 2013).



GnRH and PGF_{2α} have been extensively used to improve reproductive performance during the breeding season (Fernandez *et al.*, 2019; Martínez T *et al.*, 2013) and seasonal anestrus in non-pospartum ewes (Miguel-Cruz *et al.*, 2019; Mirzaei *et al.*, 2017). However, there is a lack of information exploring the effect of the combined use of these hormones to improve the reproductive performance of ewes during the postpartum period, probably because during the early postpartum ewes have limited response to GnRH injection and they have an abnormal secretion of PGF2α (Crowder *et al.*, 1982; Schirar *et al.*, 1989), but their response to these hormones are expected to improve as the postpartum period progresses. Thus, this study aimed to compare the reproductive response of postpartum ewes to nulliparous ewes after multiple injections of GnRH and PGF_{2α}.

MATERIALS AND METHODS

Location and animal care

The study was conducted during the breeding season (December-January) at the Instituto de Ciencias Agricolas of the Universidad Autonoma de Baja California, Mexico. The animals used in the present study were handled following the guidelines of the Canadian Council of Animal Care (CCAC, 2009). In addition, the study was approved by the "Comité de Ética y Evaluación de la Investigación y el Posgrado" from the Instituto de Ciencias Agrícolas (File No. 0942/2022-1).

Animal and experimental procedure

The experimental units (n=41 Dorper × Pelibuey × Katahdin crossbred ewes) were assigned to one of three experimental groups (G1, G2, and G3) according to their reproductive status (non-pregnant and lactating or non-pregnant and nulliparous) and days postpartum (\leq 77 days postpartum or >77 days postpartum. The multiparous ewes in G1 (n=14; 68.21 ± 13.48 days postpartum, 57.86 ± 10.82 kg of body weight) were \geq 2 years old, non-pregnant and nursing at least one lamb. The multiparous ewes in G2 (n=13; 93.76 ± 7.25 days postpartum, 57.57 ± 11.69 kg of body weight) were \geq 2 years old, non-pregnant, and nursing at least one lamb. The ewes in G3 (n=14, 44.70 ± 4.78 kg of body weight) were >1 year old, nulliparous, non-pregnant and non-lactating.

Reproductive management

The experimental units were kept in separate pens, according to their experimental group, during ovary ultrasonography, application of the estrus synchronization protocol and estrus detection. The animals were subjected twice to transrectal ovarian ultrasonography (Handscan V8, Sunway Medical, 7.5 Mhz linear transducer) with a nine-day interval before the first injection of GnRH to determine follicle population and the presence of corpus luteum. Three days after the last ultrasonography scan, the first injection of GnRH



(270 µg of gonadorelin acetate ewe⁻¹, GnRH-Sanfer) was given to all experimental units. Nine days after the first injection, the second injection of GnRH was given to all ewes. The size of the largest ovarian follicle and corpus luteum was determined by ultrasonography seven days after the second injection of GnRH. An injection with PGF₂(12.5 mg dinoprost ewe⁻¹, Lutalyze-Zoetis) was given to all animals immediately after the last ultrasonography scan, and the lactating ewes were weaned. The last injection with GnRH was given to all ewes 48 h after the injection with dinoprost.

Estrus detection was carried out with 10 rams of proven fertility for 34 days at 12-h intervals during the first 72 h after injection with dinoprost and every 24 h after that. One or two rams were used at the same time to detect estrus in each pen. The rams were randomly rotated to each experimental group every day and allowed to mate with the ewes in estrus. The ewes detected in estrus and mated by a ram were temporally removed from the pen. Rams and ewes mated during the entire period of standing estrus. Therefore, in some cases, a single ewe was bred more than one time by more than one ram during one standing estrus episode. The ewes returning to the estrus before the end of the estrus detection period were allowed to mate with a ram. Pregnancy diagnosis was carried out by transrectal ultrasonography 30 days after the last ewe was detected in estrus and mated by a ram.

Nutrition and feeding

Ewes were fed a 1.8 kg ration (12.7% protein, 3.2% calcium, 2.6% phosphorus) containing 49.2% wheat straw, 30% wheat, 20% soybean meal, and 0.8% limestone as fed. However, at the end of estrus detection period, the ewes were moved to a single group and fed ryegrass (*Lolium multiflorum*) ad *libitum* until pregnancy was diagnosed. All animals had ad *libitum* access to fresh clean water.

Response variables

The response variables were follicle population, size of the largest ovarian follicle and corpus luteum, ewes bearing a corpus luteum before and after two applications of GnRH, as well as the number of ewes that showed estrus after $PGF_{2\alpha}$ injection, the number of ewes that were mated by a ram >1, but ≤3 times during the standing estrus period, pregnancy rate, and the number of ewes lambing two or more lambs. The following considerations were taken into consideration. The follicle population was determined by count of the number of ovarian follicles ≥ 1mm in diameter in both ovaries. The diameter of the largest ovarian follicle and corpus luteum was calculated by the average of horizontal and vertical measurements in ewes where the ovaries were visible. The ewes were declared in estrus when they stood to be mounted by a ram.



Statistical analysis

The normality test of the residuals was performed in all the continuous variables and follicle population. A data log transformation was carried out when the normality test was not approved. The follicle population was analyzed by PROC MIXED considering repeated measurements. The diameter of the largest ovarian follicle and corpus luteum were analyzed by PROC GLM. The means among experimental groups were compared with the Tukey test. The results are reported as the mean ± standard error. The categorical variables were analyzed by a Chi squared test using PROC FREQ. However, if the Chi squared test conditions were not satisfied, then a Fisher exact test was applied to the data. Regarding the number of times a ram mated a ewe, this response variable was analyzed considering the following: if a ewe exhibited estrus more than once during the estrus detection period, then the average number of times that a ram mated her was used for the statistical analysis. The number of ewes bearing a corpus luteum before and after the two injections of GnRH was compared by a McNemar test using PROC FREQ. SAS 9.4 was the statistical software used to perform the statistical analysis.

RESULTS

Values of the measured response variables are shown in table 1. The number of ewes bearing a corpus luteum before the first injection of GnRH tended to depend on the experimental group (p=0.0594); the largest numerical difference was observed in G3, relative to groups G1 and G2. However, regardless of the experimental group, a higher number (p≤0.05) of ewes bearing a corpus luteum was observed after the two injections of GnRH. In addition, despite the significant differences (p≤0.05) in follicle population and diameter of the largest ovarian follicle, a similar pregnancy rate was achieved among groups (p>0.05). The number of times (>1, but ≤3) that a ram mated the ewes during the standing estrus period was not dependent on the evaluated groups (p≥0.05; 5, 4 and 5 ewes in groups G1, G2 and G3 mated more than one time, respectively). The number of ewes lambing more than one lamb depended on the experimental group (p≤0.05), the largest difference was observed against group G3 (6, 7 and 1 ewe gave birth to more than one lamb in groups G1, G2 and G3, respectively).

DISCUSSION

Disturbed gonadotrophin secretion and abnormal luteal function are responsible for the depressed reproductive activity of lactating ewes after lambing. Early research on the physiology of the anestrus postpartum showed that responsiveness of the pituitary to GnRH was limited during the first weeks after lambing (Crowder *et al.*, 1982), and lamb suckling diminished gonadotropin secretion. In addition, prolonged uterine production of PGF_{2α} after lambing is responsible for the short luteal phases observed after estrus in the



postpartum period (Schirar *et al.*, 1989). Therefore, the first postpartum estrus, followed by a normal cycle, might not be observed until 53 days after lambing (Schirar *et al.*, 1989). In the present study, nulliparous ewes (G3 group) were used as a control because the postpartum factor was absent. Therefore, these animals expected normal responses to GnRH and PGF2 α injections. The multiparous ewes in G1 and G2 had different postpartum lengths because a differential response to hormone injection was expected (Crowder *et al.*, 1982). Attempts to restore reproductive activity by increasing gonadotropin secretion were performed by providing external stimuli to GnRH secretion in postpartum ewes (Hernández-Hernández *et al.*, 2021). However, there is a lack of information about the effect of GnRH on estrus response and fertility during postpartum anestrus in ewes.

Table 1. Reproductive responses of postpartum multiparous and nulliparous ewes injected v	vith
GnRH and prostaglandin PGF _{2α}	

Response variable ^β	G1 ⁸	G2 8	G3 [∂]	P value
Ν	14	13	14	
Follicle population (mean \pm EE)	6.96±0.33 ^a	5.23±0.34 ^b	5.71±0.33 ^b	0.0028
Ewes bearing a corpus luteum before GnRH injection	4 ^a	5ª	10ª	0.0594
Ewes bearing a corpus luteum after two injections of GnRH [*]	14 ^a	7 ^a	11 ^a	0.5758
Corpus luteum diameter at $PGF_{2\alpha}$ injection (mm, mean ± EE)	8.67±0.57 ^a	8.85±0.80 ^a	6.90±0.64 ^a	0.0864
Largest ovarian follicle diameter at PGF _{2α} injection (mm, mean \pm EE) ^{Ψ}	5.28±0.67 ^{ab}	6.41±0.51ª	4.63±0.53 ^b	0.0437
Ewes that showed estrus after $PGF_{2\alpha}$ injection	12 ^a	10 ^a	11 ^a	0.8918
Ewes that showed estrus within 72 h after $PGF_{2\alpha}$ injection	7 ^a	7 ^a	8 ^a	0.8101
Pregnant ewes	8 ^a	10 ^a	10 ^a	0.5927

^g Multiparous ewes with \leq 77 days postpartum. ^a Multiparous ewes with >77 days postpartum. ^a Nulliparous, non-pregnant and non-lactating ewes. ^{*}Ewes that were scanned to detect the presence of corpus luteum were 7, 14 and 12 for groups G1, G2 and G3, respectively. ^wEwes that were scanned and detected with ovarian follicles were 7/7, 12/14 and 11/14 for groups G1, G2 and G3, respectively. ^βValues of the response variables with different superscripts within a row are statistically different between groups.

In this study, postpartum and lactating ewes with corpus luteum before GnRH injections tended to be fewer than nulliparous ewes with corpus luteum. This was not surprising because the lack of corpus luteum is a characteristic of anestrus ewes after lambing. However, after two injections of GnRH, given nine days apart, there was an increase in the number of ewes bearing a corpus luteum. Ovarian follicles were observed in all experimental groups before GnRH injection. Even though, follicle diameter was not measured at the moment of the first two GnRH injections, it is feasible that an ovarian



follicle ovulated in response to GnRH injections giving rise to a corpus luteum (Hashem *et al.*, 2015). The latter is highly probable because follicles larger than 5 mm in diameter have been observed in ewes after 31 days postpartum (Ascari *et al.*, 2013), and it is known that GnRH is able to induce ovulation in follicles larger than 4.7 mm in diameter (Rubianes *et al.*, 1997).

The injection of GnRH has been effective in improving reproductive performance when is given to ewes at estrus (Dursun, 2019) and on day seven post-mating (Hashem *et al.*, 2015) because it ensures ovulation and the formation of accessory corpus luteum. However, the formation of corpus luteum in response to single or multiples injections of GnRH was not reported by Jordan and colleagues in seasonal anestrus ewes (Jordan *et al.*, 2009). This is because follicle atresia might also occur if responsive follicles are not present when GnRH is given to animals (Twagiramungu *et al.*, 1995), which complete avoids the possibility of corpus luteum formation (Ayaseh *et al.*, 2021).

The formation of a corpus luteum and exposure of the reproductive axis to progesterone is essential to induce estrus behavior in response to follicular estradiol. This explains the success of estrus synchronization protocols that use intravaginal pessaries to deliver exogenous progesterone into the animal organism. In this regard, the incidence of estrus in lactating and weaned ewes increases 45 to 50% when progesterone is part of the estrus synchronization protocol compared with other protocols, such as a double injection of prostaglandin given 10 days apart (Ronquillo et al., 2008). In addition, progesterone protocols are better at inducing estrus expression in ewes during the anestrus season than estrus synchronization protocols using injections of GnRH and prostaglandin F_{2a} (Garoussi et al., 2020; Miguel-Cruz et al., 2019). However, during the breeding season, estrus behavior can be expressed in a high proportion of synchronized ewes (91%) with two injections of prostaglandin F_{2α} given 15 days apart (Olivera-Muzante et al., 2020). The presence of a corpus luteum is mandatory to manipulate the estrus cycle with prostaglandin F_{2q} injections. The corpus luteum is normally detectable during the breeding season, but not during the anestrus periods (seasonal or postpartum). This is the reason for greater estrus expression after prostaglandin injection during the breeding season than in the anestrus periods. It also explains the high incidence of estrus behavior (76-78%) among experimental groups in the present study, similar to that reported in postpartum and lactating ewes (50 days postpartum) synchronized with intravaginal progesterone release devises (Biehl et al., 2019).

The time between prostaglandin injection and estrus presentation ranges from 60 to 96 h (Mekuriaw *et al.*, 2016; Ramírez *et al.*, 2018). This was taken into consideration before execution of the present study, and the third dose of GnRH was applied to synchronize ovulation and the reappearance of estrus in animals experiencing abnormal luteal



phases, which occurred in only two, four and one of the ewes from groups G1, G2 and G3, respectively. These ewes exhibited estrus three to 12 days after GnRH injection. All the ewes that showed estrus were mated by a ram, obtaining a pregnancy rate of 68%, similar to that reported in ewes bred after synchronization with exogenous progesterone (40-68%) (Kuru *et al.*, 2020; Martinez-Ros & Gonzalez-Bulnes, 2019). This is relevant because it indicates that an acceptable pregnancy rate can be obtained shortly after lambing without using exogenous progesterone. In addition, the protocol used in G1 and G2 was effective to achieved new pregnancies rapidly after lambing, which is beneficial when technicians and farmers want to intensify lamb production. Finally, prolificacy was dependent on the experimental group. The lower incidence of ewes delivering more than one lamb was observed in G3, which is unsurprising because they were nulliparous.

CONCLUSION

Two GnRH injections increased the incidence of corpus luteum and equalized estrus presentation and pregnancy rate after PFG2 α injection in postpartum multiparous ewes compared to nulliparous ewes.

Conflict of interest

The authors have no conflicts of interest to declare.

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