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Follicular population at the onset of a superovulatory treatment and ovarian response in hair ewes

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Abstract

The objective of this study was to assess ovarian follicular status as defined by the follicular populations in the ovary before a treatment of superovulatory stimulation and to establish a correlation between ovarian follicular status and the level of response to a superovulatory treatment. Estrus was synchronized in 48 ewes using intravaginal sponges containing FGA for 14 days and two injections of 15 mg of Luprostiol® seven days apart. Ewes received decreasing doses of 200 mg of FSHp (40, 40, 30, 30, 20, 20, 10 and 10 mg) at 12 hours intervals, initiating on day 11 of sponge insertion. From 48 ewes under treatment, 41 responded, with a mean of 18.10 ± 1.3 follicles observed by ultrasonography before FSH treatment and a mean of 8.08 ± 0.8 corpus luteum (CL) found by endoscopy on the seventh day after sponge withdrawal. A positive correlation (P<0.05) was found between both variables. Dorper ewes had the greatest (P<0.05) response to treatment (11.8 ± 1.41 CL), as compared to Blackbelly (9.0 ± 1.4 CL), Katahdin (7.5 ± 1.5 CL), and Pelibuey (5.6 ± 1.3 CL), with no differences among the latter three. We conclude that a positive correlation exists between ovary status prior to superovulatory treatment and ovarian response and that Dorper present a greater superovulatory response than Katahdin, Blackbelly and Pelibuey ewes.

Keywords: Follicles, corpus luteum, hair ewes, superovulation.

Abbreviations: FGA – fluorogestone acetate; FSHp – porcine follicle stimulating hormone; CL – corpus luteum

Introduction

Superovulatory treatments are nowadays an important tool in production systems to obtain higher rates of pregnancy; factors affecting superovulatory response are related to breed, age, nutrition and reproductive status (Hahn [1]. Also, genetics has been identified as a factor that could contribute to ovulatory variability; in general, prolific breeds show better superovulatory response due to their genetic profile (González-Bulnes [2]. The objective of superovulation protocols is to increase numbers of descendants of selected females, by increasing the number of ovulations and viable embryos in response to the use of hormonal treatments (Baril [3]. These protocols usually include administration of a gonadotropin treatment, during the last days of estrus synchronization protocols through intravaginal progestins administration, to synchronize ovulation (Ramón [4]. The response to superovulatory treatments in sheep, as in other ruminant species, is associated with a high incidence of alterations in follicular development, oocyte maturation and ovulation mechanisms (González [5]; Kafi [6]. Some of these alterations are common to all treatments of superovulation but there are other factors such as the source of commercial preparations of gonadotropin (Donaldson [7]; Driancourt [8], their purity (Cognie [9]; López-Sebastian [10] and the management protocol (Navarrete [11]; Ramón [4]; Palacin [12], that influence the final response. The aim of this study was to assess ovarian follicular status as defined by the follicular populations in the ovary before a treatment of superovulatory stimulation and to establish a correlation between ovarian follicular status and the level of response to a superovulatory treatment.

Materials and methods

The study was carried out in the Biotechnology Laboratory at the Centro de Selección y Reproducción Ovina (CeSyRO) of Instituto Tecnologico de Conkal, located in the municipality of Conkal, northeast of the state of Yucatan, located at 20º 29' North latitude and 98º 39' West longitude, with an altitude of 9 meters above sea level, and a warm-dry climate (AWo), with average annual temperature of 26.5°C and annual precipitation of 700-900 mm (García [13].

Forty eight adult ewes aged 2 to 8 years old, open and none lactating, with a body condition score of 3 or greater (Russel [14] of different tropical breeds, (Dorper, n=12; Pelibuey, n=12; Kathadin, n=12; and Blackbelly, n=12) were included in the study. All animals were managed under rotational grazing (6 hours/day) on pastures of star grass (*Cynodon nlenfuensis*) and a commercial supplement (300 g/hd/day) with 14% crude protein, with free access to water and mineral salts.

All ewes were cycling and had their estrus synchronized with intravaginal sponges impregnated with 20 mg of fluorogestone acetate (FGA; Chronogest®, Intervet International B.V.) for 14 days (Scaramuzzi [15]. Two applications of 15 mg of luprostiol (Prosolvin®, Virbac Salud Animal, Mex.) per ewe were given at the time of the intravaginal device insertion and seven days later. As superovulatory treatment, all ewes received 200 mg of decreasing doses (40, 40, 30, 30, 20, 20, 10, 10 mg) of FSHp (Folltropin-V®, Bioniche Animal Health, Canadá) at 12 h intervals initiating on day 11 of insertion of the sponges. Before starting the superovulatory stimulation treatment (on day 10), ovarian follicular status was assessed by real time ultrasonography (DP-6600 Shenzhen VET. Mindray® ***Mindray*** North América, USA). This was performed with a 7.5 MHz linear array transrectal transducer (Ravindra [16], in order to count all follicles larger than 2 mm of diameter present in the ovaries. Seven days after sponge withdrawal (Sponge withdrawal = Day 0) all ewes were subjected to endoscopy (Karl Storz® ***Karl Storz Endoscopia México,*** S.A. de C.V.) and numbers of corpora lutea present in the ovaries were registered (Fig. 1).

The distribution of the data was checked using normal probability plots and the Kolmogorov-Smirnov test. Data of numbers of follicles registered on ultrasound and numbers of corpus luteum registered on laparoscopy were analyzed with the GLM procedure of SAS 9 (SAS Corp). Differences between breeds were determined by least significant difference at P <0.05. The Statistical analysis included a linear model of fixed effect of breeds for a completely randomized. $Y_{ij}= \mu+G_i+\varepsilon_{ij}$ Where: $Y_{ij=\ ij-}$ th observation**;** μ= general mean; G= random effect of j-th genotype of ewe; ε= random error associated to the ij-th observation. Mean of studied variables, were compared through the multiple comparisons Tukey Test.

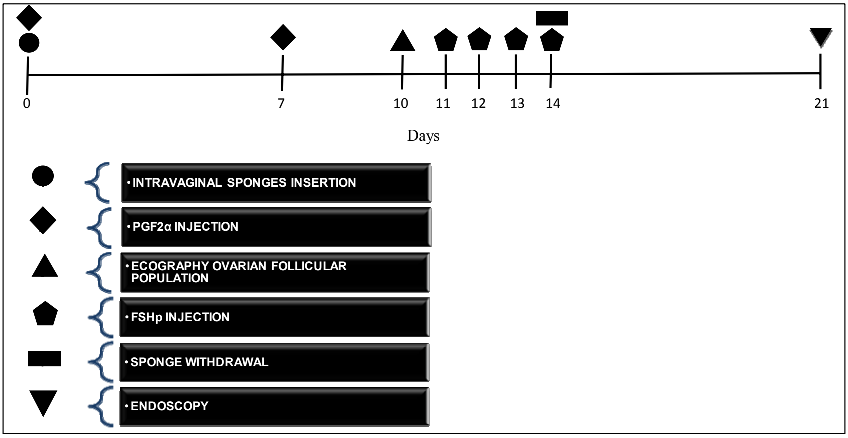

Figure 1. Experimental Plan

Results and discussion

Results of the 48 sheep included in the study presented a positive correlation ($P<0.05$) between follicles seen on ultrasound with corpora lutea observed at endoscopy, with a mean of 18.10±1.3 follicles observed on ultrasound and an average of 8.08± 0.8 corpora lutea found on endoscopy. A correlation between the state of ovarian follicular populations prior to superovulatory treatment and response to the latter was established in the present study. Similar results were observed by Roche [17], who found a positive correlation ($r=0.37$ to 0.51, $P<0.05$ to 0.005) between the numbers of follicles at the onset of treatment with the number of corpora lutea and the recovery rate, such a correlation was also observed in the number of viable embryos, similarly with the results described by ultrasound (González-Bulnes [18] and endoscopy (Cognie [19] in the present study. Forty one of 48 ewes treated in the study responded to treatment, Table 1 illustrates numbers of follicles and corpora lutea observed by ultrasound and endoscopy, respectively. Results in numbers of follicles observed by ultrasound and average corpora lutea found by endoscopy, were consistent with those reported with ovarian stimulation protocols similar to this experience. Results in the present study were lower than those presented by Roche [17], with an average of 15.5 CL, Olivera [20] with an average of 16 CL and Cocero [21] with an average of 15.9 CL, but they were higher than those observed by Rubianes [22] who reported a mean of 10.8 follicles and 2.8 CL. Moreover, Cocero [21] mentioned an average of 13.6 CL in ewes treated with constant doses of FSH, administered differently to the present study.

Table 1. Numbers of follicles observed by ultrasonography and corpora lutea observed by endoscopy in hair ewes receiving a super ovulatory treatment

	Follicles	Corpus lutea
No. of ewes	48	48
Means	18.1	8.08
Standard Error	8.6	0.83
Variation Coefficient	47.91	71.31
Minimum	5	0
Maximum	36	20

Differences were observed among breeds (Table 2) in the numbers of corpora lutea found by endoscopy. Dorper ewes had a greater (P<0.05) response to superovulatory treatment as compared to all other breeds, Pelibuey ewes showed the lowest response of all. The superovulatory response to hormonal treatments varied between 0 and 30 CL per ewe in this study. Some authors indicate that such variability could be determined, among other things, by the existing population of follicles in the ovary at the beginning of the FSH treatment (González-Bulnes [23] in the case of low prolificacy breeds such as Merino (González-Bulnes [24], and medium-high prolificacy such as Ile de France (Brebion [25]. However, in the absence of information related to tropical breeds of sheep, this work indicates a greater response in Dorper ewes (low prolificacy) than in Blackbelly (high), Katahdin (low) and Pelibuey (upper middle) ewes, which should be confirmed in further studies. The response to 58.4, 49.5, 60.0 and 49.1% of ovulation rates for Dorper, Pelibuey, Katahdin and Blackbelly, respectively, showed different levels of follicular development, maturation and atresia, in relation to the genetic background between composite (Dorper and Katahdin) and pure (Pelibuey and Blackbelly) breeds (Rastogi [26].

Table 2. Follicular populations (F) and numbers of corpora lutea (CL) by breed of ewes receiving a super ovulatory treatment

Breed of ewe	No. of ewes	F (means±SE)	CL (means±SE)
Dorper	12	20.25±2.40	11.84±1.41^{a}
Pelibuey	12	11.31±2.68	5.60±1.38^{b}
Katahdin	12	12.56±2.56	7.54±1.54^{b}
Black Belly	12	18.41±2.97	9.04±1.47^{b}

Different letters (a, b) within columns indicate significant differences (P<0.05).

Conclusions

The scanning of the ovary through real-time transrectal ultrasonography allows knowing with certainty the follicular status before superovulatory stimulation treatment. With this approach, a correlation between the state of ovarian follicular populations prior to superovulatory treatment and level of response can be established. In the present study, Dorper ewes had a greater superovulatory response as compared to Katahdin, Blackbelly and Pelibuey ewes.

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