Reproductive performance of hair ewes and rams implanted with Melatonin previous to the anestrus season in Northwest Mexico

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Abstract

Ovine reproductive behavior depends on annual photoperiodic cycle and its impact on endogenous melatonin secretion. In this regard, exogenous melatonin administration previous to the physiological anestrus period could modify the reproductive performance of sheep. Two independent studies were performed to evaluate such hypothesis in hair sheep implanted with melatonin prior to the anestrus season in northwest Mexico. Study 1 involved 15 rams assigned to one of three treatments receiving 0mg (n=5), 18mg (n=5) or 36mg (n=5) of melatonin subcutaneously. Study variables were measured monthly since implantation (d0) and included testosterone concentration, scrotal circumference, mass motility, individual motility, and sperm concentration. Study 2 included 50 ewes assigned to one of two treatments receiving 0mg (n=25) or 18mg (n=25) of melatonin subcutaneously. In ewes, progesterone concentration and the frequency of females in anestrus were measured during the implantation (-30d), as well as at the beginning (0d) and at the end (45d) of the mating period, while pregnancy rate was determined by ultrasonography 45d after. Continuous variables were analyzed using a mixed effects model considering treatment, time, and the treatment by time interaction as fixed effects. Animal nested within treatment was the random effect. Binary variables were analyzed using the chi-square test. In males, melatonin improved testosterone and sperm concentrations (P<0.05), while in females, a 28% higher pregnancy rate was observed in implanted ewes (P<0.05). Although melatonin enhanced reproductive parameters in both sexes, its exogenous administration previous to the anestrus season in northwest Mexico could be more beneficial and cost effective in rams.

Introduction

Sheep farming represents an interesting and profitable animal production system in northwest Mexico (Retes-López et al. 2012). This geographical region offers the opportunity to commercialize ovine derived products nationally and internationally (Rodríguez-Pérez et al. 2010). Nonetheless, the seasonal anestrus period occurring during spring and summer in these small ruminants poses a challenge to sustain a constant lamb production throughout the year (Malpaux et al. 1997; Arroyo, 2011). Consequently, it has been noted that the adoption of technology and new management practices focused on manipulating sheep's reproductive efficiency are an imperative need in this productive zone (Leyva et al. 2015).

Reproductive seasonality is a natural mechanism limiting reproductive activity of some species to ensure that females have their calvings during the most favorable time of the year (Arroyo, 2011). The main environmental factor controlling such seasonality in sheep is photoperiod (Williams and Helliwell, 1993; Rosa and Bryant, 2003; Correa and Riveros, 2017). During seasons with long days, melatonin secretion is suppressed in ovine pinealocytes and reproductive activity gets disrupted (Lépinay et al. 2021; Malpaux et al. 1999; Ramírez-Ramírez et al. 2021). In rams, testicular size, testosterone levels, daily sperm cell production and libido are reduced (Bustos-Obregón and Torres-Díaz, 2012; Casao et al. 2010; Cevik et al. 2017; Rosa et al. 2012). In ewes, reproductive hormone concentrations decrease or become absent, which in turn ceases estrus and ovulation activities generating a seasonal anestrus (Arroyo et al. 2007; Zarazaga et al. 2011).

As a method to counteract the adverse effects of reproductive seasonality, exogenous administration of melatonin has shown positive effects on sperm motility, testicular volume, and reproductive parameters in rams (Casao et al. 2010; Rosa et al. 2012; Cevik et al. 2017; Deng et al. 2018). In the female side, it has also been reported an improvement in fertility due to the increase in ovarian activity and embryonic viability observed in treated ewes during spring (Malpaux et al. 1999; Forcada et al. 2006; Abecia et al. 2008; Palacín et al. 2011).

Although there is a paucity of studies related to ovine reproductive seasonality in northwest Mexico, it has been noted that calving events severely decrease between July and September. In this region, photoperiod effects have been reported to be variable in hair breeds. For instance, studies conducted with Pelibuey ewes on latitudes 27º N and 32º N suggested that within this breed, some females experience reductions in estral and ovarian activity (Macías-Cruz et al. 2015, Silva-Ávila et al. 2015), while others seem to have a lack of anestrus (Arroyo et al. 2011). Interestingly, no studies related to reproductive seasonality on rams have been reported for this zone. Likewise, the effects of subcutaneous administration of melatonin have not been explored in ewes or rams raised in latitude 25º N. Given the key role of melatonin in ovine reproduction, its exogenous administration prior to the expected seasonal anestrus period may positively influence their reproductive performance. Therefore, the objective of this study was to evaluate the reproductive efficiency of hair sheep implanted with melatonin prior to the spring onset at latitude 25º N.

Materials And Methods

The study was performed from February to June of 2020 in two commercial flocks located in Guasave (25°42'1N; 108°43'W) and Culiacan (24°47'N; 107°23'W), municipalities of Sinaloa, Mexico. Research efforts were divided into two independent studies to evaluate the effect of exogenous melatonin in both rams, and ewes. The ethics committee of the Department of Agronomic and Veterinary Sciences of the Instituto Tecnológico de Sonora (ITSON) supervised and approved animal procedures performed in this investigation.

Study 1: animals, management, experimental design, and measurements in rams

A total of fifteen (12-Pelibuey, 3-Dorper) mature rams (3-to-4 years old) with an average body weight of 64 ± 3.4 5 kg and mean body condition score of 3.5 on a scale of 1 to 5 (where 1 is emaciated and 5 is obese; McHugh et al. 2019) were involved in the study. None of the rams presented reproductive disorders at the enrollment moment. Rams were housed in individual pens (2.5 m^2) where they had free access to water and were fed with a mixture of alfalfa hay, ground maize, and provided with macro and microminerals supplements to cover their nutritional requirements. Experimental units were assigned under a randomized complete block design to one of three possible treatments (considering breed as the blocking factor). Using an intradermal applicator after a disinfection process with 2% povidone-iodine, randomly, five rams received in their left ear pavilion two 18-mg melatonin (M36) subcutaneous implants

(Dermatonin, Melatek LLC, Prairie du Sac, WI 53578 USA) Other five rams received only one 18-mg melatonin subcutaneous implant (M18), while the five remaining rams were not implanted (0-mg of melatonin) and were considered as the control group (mCTRL). To challenge the animals against the reproductive season, the period from March to June was considered the season of low fertility for the region (Arroyo, 2011). Implants were administered in February (0d) and at 30d, 60d, 90d and 120d post-implantation were performed andrological tests and blood collections to determine testosterone levels (Fig. 1).

Starting at 30d post-implantation, scrotal circumference (ScrotC), mass motility (MassM), individual motility (IndM), sperm concentration (CONC) and testosterone levels (TEST) were measured. For the ScrotC determination, testicles of the rams were pulled toward the lower part of the scrotum while rams were on a stand position, and then the widest part of the testicles was measured with a flexible metric tape (Cevik et al. 2017).

Semen collection

To evaluate seminal parameters (MassM, IndM and CONC) on each sampling date, semen samples were collected (8:00–10:00h) using an electroejaculation equipment (Standard Precision Elecronics; Arvada, Colorado 80001, USA). Previous to the semen collection, males were brought into a pen in front of a group of ewes in estrus to facilitate sexual stimulation. At each collection, preputial area was cleaned, and hairs were trimmed. Randomly per group, each ram was removed from the pen to receive a digital prostate massage (applied using nitrile gloves and lubricant gel) for 30-to-60 seconds until relaxation. Subsequently, the lubricated probe of the electroejaculation equipment was utilized for a second massage without electrical stimulation. Finally, when the penis became externalized electrical pulses started to be applied in a rhythmic and gradual fashion (from gentle to moderate intensity). After discarding the pre-seminal fluid, the ejaculate was collected using a 300 ml plastic bag placed in the collector device of the equipment. Once each sample was collected, it was immediately divided into a sub-sample of fresh semen and a sub-sample that was diluted 1:2 with non-fat commercial milk and kept in a water bath at 37 ºC for later analysis.

Semen evaluation

Using the sub-sample of fresh semen, a drop (~ 0.5ml) of such sample was placed on a previously warmth slide (37ºC) to evaluate MassM using an optical microscope (10x) observing the edge of the drop. The MassM was categorically classified from 0 to 5 according to Vera and Ricarte (2009), considering zero as the absence of motile spermatozoa and five as the maximum velocity for extremely high wave formations (> 90% motile spermatozoa).

In order to determine the IndM, a drop of the diluted sample was taken and placed on a pre-warmth slide (37°C) to be observed at 40x. Fifty cells were observed in 4 or 5 fields of the microscope, spermatozoa showing a straight, energetic, and active movement were considered as with good individual progressive motility. Each sample was rated in percentage basis (from 0-100%) in relation to the number of

spermatozoa with good progressive motility out of the 50 considered gametes (Vera and Ricarte, 2009). The remaining portion of the fresh semen sub-sample was preserved at 5°C to determine CONC 24h later. To determine CONC, the undiluted semen sub-samples were first tempered at 25–27 ºC, then, aliquots containing 0.5 ul of semen were taken and diluted (1:400 = 0.0025ml) in 2 ml of physiological saline solution also at 25–27 ºC. After 3-to-5 minutes within this solution, the Neubauer chamber was loaded and placed on the stage of the optical microscope to count the number of cells per ml of semen in five quadrants of the chamber (40x). Determination of the number of spermatozoa per ml of ejaculate was performed according to the methodology explained by Vera and Ricarte (2009). Briefly, the CONC was calculated as follows:

CONC= (Num. of espermatozoa in 5 quadrants) * (Common factor)

Common factor used to express the result as millions of spermatozoa per ml = 1 million / 0.000,000,05 = 20,000,000 sperms (2 x 10^{8}). The means and standard errors of CONC were expressed in x10^{8} .

Study 2: animals, management, experimental design, and measurements in ewes

Fifty multiparous (≥ 3 calvings) and healthy Pelibuey ewes with an average body score of 3.5 were included in the study. All ewes were confirmed as non-pregnant by ultrasonography in order to be enrolled in the experiment. Using a completely randomized design, ewes were randomly allocated within one of two treatments: 1) f-MEL (n = 25) receiving one subcutaneous implant with 18-mg of melatonin or 2) f-CTRL (n = 25) control group of females not receiving subcutaneous implants (0-mg of melatonin). For ewes in the f-MEL group the application of the subcutaneous melatonin implants was performed as described for males in study 1. Figure 2 illustrates that treatments application started in February of 2020 thirty-five days before (-35d) the beginning of the mating period (0d). Mating period lasted 45 days and, within it, each experimental group allocated in different pens was exposed simultaneously to not implanted but proven fertility rams (n = 2).

Fertility evaluation in females

Females of both treatments were subjected to a pregnancy rate (PREG) determination using transrectal ultrasonography (Sonoscape®; 3.5MH linear transducer) 45d after the end of the mating period (90d). Furthermore, at each sampling date the percentage of females in anestrus (ANEST) was determined by examining serum progesterone (P4) concentrations and classifying as anestric ewes to those having P4 concentrations < 1ng/ml.

Hormone analyses for both studies

Blood serum collection and hormone concentration determinations

Blood sample collections were performed similarly in both studies by puncture of the jugular vein using BD Vaccutainer® blood sampling kits (blood collection needles 21G x 32 mm; and 10 ml tubes without anticoagulant). In the rams' study, samples were collected at 0, 30, 60, 90 and 120d (Fig. 1); while in the females' study blood was drawn at the implantation date (-30d), as well as at the beginning (0d) and the end (45d) of the mating period (Fig. 2). Each sample was left to stand at room temperature (~ 27ºC) for 40 min to allow detachment of the clot. Subsequently, tubes were transported (5ºC) to the Molecular Biology Lab of the Department of Agronomic and Veterinary Sciences of ITSON, in Cd. Obregón, Sonora. Serum was obtained by centrifugation at 3500 g for 10 min (Beckman coulter® centrifuge, model Allegra™ X-22). Then, serum was collected in Eppendorf tubes (2 ml) and used for hormone determinations. Commercial ELISA kits were used to determine TEST (Monobind Inc., Lake Forest CA, USA) and P4 (Beckman Coulter France, Roissy CDG, France). In both cases, instructions of the ELISA kits manufacturers were followed. Finally, an ELISA reader (Kontrol Lab®; ELIRead; Guidonia, Rome, Italy) along with an automatic plate washing equipment (Kontrol Lab®;Eli-Wash; Guidonia, Rome, Italy) were used to obtain hormone concentrations in ng/ml.

Statistical analysis

Given the distributions of the original records for CONC, MassM and IndM were not normal, observations for these variables underwent through a normalization process (square rooting them) before their formal statistical analysis. All continuous variables (ScrotC, TEST, P4, CONC, MassM and IndM) were analyzed using a mixed model with repeated measures over time using PROC MIXED. Within such model, fixed effects were treatment, time (sampling day) and treatment-by-time interaction (in the case of the rams' study the fixed effect of block was also included, considering breed as the blocking factor). Animal nested within treatment corresponded to the random effect. Different variance-covariance structures were tested, and the variance-component (VC) structure resulted as the most appropriated according to the AIC and BIC criteria. Means comparisons were performed with the Tukey test considering significant differences when α was < 0.05. In the case of the categorical variables PREG and ANEST, a chi-square test using PROC FREQ was performed for treatment effects. All statistical procedures were performed in SAS software, version 9.3 for Windows (SAS, 2014).

Results And Discussion

Study 1 (rams' assay)

Table 1 shows the results of the reproductive parameters measured in rams. No statistical difference ($P > 0.05$) was found for treatment, time, and treatment-by-time interaction for ScrotC, MassM and IndM. In the case of TEST and CONC, the treatment-by-time interaction was not significant ($P > 0.05$) but a statistical difference ($P < 0.05$) was detected for treatments in both variables. Figure 3 depicts the behavior of TEST and CONC for all treatments throughout the study. With respect to TEST, it is possible to note that the M36 group showed numerically superior hormone concentrations from 60d and onwards in comparison to M18 and mCTRL groups. The M18 group showed TEST levels that were numerically

higher than mCTRL between the 0 and 60d, although this behavior was reversed at 90 and 120d with the mCTRL group showing higher hormonal concentrations. Regarding CONC, again the M36 had higher sperm concentrations from 60d onwards over the rest of the experimental groups. For the M18 animals, sperm concentrations were higher than mCTRL counterparts between 60 and 90d; however, results between these two groups were slightly opposite in favor of mCTRL group by 120d.

Table 1
Means and standard errors of reproductive variables in males implanted with melatonin previous to the onset of the anestrous season in northwest Mexico.

	Treatments					
Variable	mCTRL	M18	M36	Probability		
	Mean ± EE	Mean ± EE	Mean ± EE	*Treat.*	*Time*	*Interac*
ScrotC (cm)	35.41 ± 0.64^{a}	36.61 ± 0.64^{a}	36.89 ± 0.79^{a}	0.191	0.916	0.958
MassM	1.88 ± 0.07^{a}	1.93 ± 0.07^{a}	2.03 ± 0.08^{a}	0.209	0.096	0.588
IndM (%)	80.59 ± 0.02^{a}	81.85 ± 0.02^{a}	88.27 ± 0.03^{a}	0.061	0.115	0.153
TEST (ng/ml)	4.07 ± 0.92^{a}	5.59 ± 0.94^{ab}	7.59 ± 1.13^{b}	0.023	0.705	0.154
CONC (x10^{8})	32.0 ± 6.08^{a}	35.5 ± 5.84^{ab}	57.5 ± 6.00^{b}	0.007	0.169	0.281

^{a,b} In the same row indicates statistical difference between treatment means ($P < 0.05$). ScrotC = scrotal circumference; MassM = mass motility; IndM = individual progressive motility; CONC = sperm concentration; TEST = testosterone concentration.

As part of the strategy for this study, the exogenous melatonin administration was intended to stimulate melatonin receptors in the reproductive tract of rams to prevent the expected decline in reproductive activity during the non-breeding season. In males, melatonin receptors have been found in the epididymis, prostate, and seminiferous tubules (González-Arto, et al. 2017). Even when melatonin concentrations were not assessed in this study, it has been reported that at 37 days post-implantation, exogenous melatonin levels reach high blood levels and remain high until 97 days after (Zúñiga et al. 2002). Consequently, it was considered that melatonin administration through subcutaneous implants caused an increase on its blood levels altering the reproductive physiology of the rams. It is widely known that melatonin in rams stimulates the pulsatile secretion of GnRH at the pituitary level, which in turn, steams up the secretion of FSH and LH by the adenohypophysis. These gonadotropins travel systemically to the testes where they exert action on Sertoli and Leydig cells, respectively. Sertoli cells support spermatogenesis and contribute to the regulation of FSH secretion through inhibin and activin, as well as other testosterone-binding proteins known as ABPs. For their part, Leydig cells initiate testosterone synthesis and secretion by LH (Bustos-Obregón and Torres-Díaz, 2012; Correa and Riveros, 2017).

Endocrinologically, this study evaluated the reproductive activity of rams through TEST levels and this variable was indeed affected by treatments. Kokolis et al. (2000) reported a peak in testosterone levels 45 days post-implantation of melatonin in rams, which is similar to our findings since the M18 and M36 groups reached their maximum TEST levels between 30-60d and 60-120d post-implantation, respectively (Fig. 2). Although it was expected that increments in TEST were followed by an enlargement of ScrotC and, thus, a raise in CONC, MassM and IndM; only an increase in CONC was observed in rams receiving exogenous melatonin. These results partially agree with Kaya et al. (2000) whose reported an increase in testosterone levels 70 days after melatonin implantation and associated it with benefits in individual sperm motility and sperm morphology. Perhaps the origin of studies discrepancies relates to differences in implantation timing, since this has been reported as an important factor for the achievement of good stimulation for melatonin receptors in testes. For instance, Rosa et al. (2012) implanted melatonin (18mg) in late spring and reported that while the treatment successfully increased testosterone and libido in rams, due to the moment when implants were given, there was not enough time to stimulate testicular growth and sperm production. Conversely, Cevik et al. (2017) implanted Charollais and Kivircik rams with 54mg of melatonin during the non-breeding season and reported that 45d post-implantation an increase in scrotal diameter was observed for both breeds (5.05 and 1.15 cm in Charollais and Kivircik rams, respectively). Similarly, Egerszegi et al. (2014) reported an improvement in scrotal circumference and seminal production, with the highest peaks in these parameters observed between 30 and 60 days after the first implantation of a double dose of 18mg of melatonin during the non-breeding season. Results of this study for CONC align with Kaya et al. (2000), since authors reported a higher sperm concentration (as well as a better sperm motility) in rams implanted with melatonin seasonally. Finally, our study detected a tendency ($P = 0.061$) for treatment effects in IndM, which agrees with the findings of Casao et al. (2010), whose reported a positive effect on the percentage of progressive motility from 45 to 75 days post-implantation, which according to the authors, conferred a greater ability of spermatozoa to penetrate the zona pellucida of the oocyte.

Study 2 (ewes' assay)

The P4 concentration (Table 2) was not affected by the treatment-by-time interaction or the individual effect of treatment ($P > 0.05$). Conversely, there was a significant effect of treatment ($P < 0.05$) in PREG, with the f-MEL group showing a 28% higher PREG than the f-CTRL group. For ANEST, no statistical differences ($P > 0.05$) were detected at implantation (-35d) between experimental groups (32 vs 40%, in f-MEL and f-CTRL, respectively). During the following two sampling dates (0 and 45d), no statistical differences ($P > 0.05$) were found between treatments for this variable which decreased to 0% in both groups.

Table 2. Progesterone levels, pregnant rate, and percentage of females in anestrus in females implanted with melatonin previous to the anestrous season onset at the northwest of Mexico.

	Treatments				
	f-CTRL	f-MEL	Probability		
Variable	*Mean ± SE*	*Mean ± SE*	*Treat*	*Time*	*Interaction*
P4 (ng/ml)	4.06 ± 0.53 [a]	3.49 ± 0.54[a]	0.330	< 0.001	0.48
PREG %	88[a]	60[b]	0.024	-	-
ANEST % (-35d)	32[a]	40[a]	0.555	-	-
ANEST % (0d)	8[a]	4[a]	0.551	-	-
ANEST % (45d)	0[a]	0[a]	0.999	-	-

[a,b] In the same row indicate statistical difference (P < 0.05) between treatments. f-MEL = females with 18mg melatonin. f-CTRL = females with 0mg melatonin. P4 = Progesterone levels (ng/ml); PREG = Percentage of pregnant ewes at 45d of the assay. ANEST = Indicate the percentage of females in anestrus (< 1ng/ml) at -35d (implantation), 0d (Male income) and 45d (male outcome) of study.

Given the natural influence of pineal melatonin on circadian activity (Lépinay et al. 2021; Ramírez-Ramírez et al. 2021), the exogenous administration of melatonin mimics the actions of the endogenous version of this hormone and influences follicular development and ovulatory rate through the control of the hypothalamus-pituitary-ovarian axis (Zarazaga et al. 2011). A meta-analysis of 105 studies reported a lambing rate similar to the pregnancy rate of the present study (Palacín et al. 2011). Additionally, the same study indicated that in mating's performed between March and July, exogenous melatonin increased fertility and prolificacy by 29% (e.g., 0.08 lambs/lambing) compared to females not supplemented with this hormone. Forcada et al. (2006) observed higher ovulation rates in melatonin-implanted ewes during March. For its part, Vásquez et al. (2009) reported higher embryo quality in the anestrus season in melatonin-implanted ewes. In the present study, mating's were conducted between March and April, a period of the year recognized as the lowest in ovine reproductive activity within the geographic zone of latitude 25º N. According to the melatonin implants manufacturer's recommendation, we considered strategic to implant the ewes in February, 35 days prior to the beginning of the mating period. The reason for this was that it was expected that in February melatonin receptors were still naturally active (Lépinay et al. 2021) and, consequently, the exogenous melatonin would be able of preventing the ewes' sensitivity to photoperiod and their entry into anestrus. Melatonin receptors (MT1) have been identified in various parts of the mammalian reproductive tract such as the ovary (Zhao et al. 2002) and uterus (Tamura et al. 2009).

At the moment of placing melatonin implants (third week of February, latitude 25º N), a percentage of ewes in both treatments were observed in anestrus (32% and 40% in f-MEL and f-CTRL, respectively). This

indicates that within the Pelibuey breed exists a group of ewes susceptible to photoperiod as suggested by other studies performed in northern Mexico at latitudes of 27º N (Silva-Ávila et al. 2015) and 32º N (Macías-Cruz et al. 2015). Slightly different results have been documented in latitude 19º N by Arroyo et al. (2007) since authors reported that between 22.3% and 33.4% of Pelibuey ewes were found in anestrus from January to March of 2004; nonetheless, during 2003 and considering the same group of ewes, the percentage of females in anestrus during the same period of the year was zero. In the case of the reduction in ANEST in the f-CTRL group, a plausible explanation for this phenomenon could be the well-known male effect brought about by the introduction of rams at the beginning of the mating period (Zúñiga et al. 2002).

In both control groups of the present study (f-CTRL and mCTRL), reproductive activity was observed even though animals were managed during the non-breeding season and were not implanted with exogenous melatonin. In this regard, it has been mentioned that in tropical and subtropical zones (latitudes < 23º N), reproductive seasonality tends to disappear, and most species are able to breed all year around (Correa and Riveros, 2017). Our study was conducted between latitudes 24 and 25ºN and represents the first study related to reproductive seasonality of sheep in this particular area. Results of this investigation agreed with Arroyo et al. (2007) and Arroyo (2011) regarding the existence of individuals apparently not affected by photoperiod. Such physiological particularity opens the opportunity to investigate molecular markers and potentially select against seasonality within the Pelibuey breed.

Even when exogenous melatonin enhanced reproductive parameters in both sexes, it might result more attractive and cost effective to restrict its use only to rams. In this scenario, less implants would be required, and the benefits of its usage would not only be exhibited by rams but also indirectly in females through the male effect. Physiologically, the increase in TEST would enhance the rams' libido, which has been reported as capable of induce cyclicity of females in anestrus (Veliz et al. 2006; Ángel-García et al. 2015). In this sense, the implantation of rams with 36mg melatonin 35d previous mating (January-February) will increase testosterone levels and sperm concentration showing peaks between 60 to 120d post implantation. Consequently, it would be possible to control different mating groups with a duration of 30 to 40 days using the male effect with rams supplemented with melatonin during the anestrous season at the northwest Mexico.

In conclusion, the use of exogenous melatonin prior to the anestrous season in pelibuey sheep raised in northwest Mexico (latitudes 24 to 25º N) increased testosterone levels and sperm concentration in rams, as well as the pregnancy rate in ewes. Additionally, it was confirmed the presence of pelibuey ewes sensitive to photoperiod in the study area. The usage of 36mg of melatonin in haired rams represents a practical and cost-effective option for controlled mating when combined with the male effect without the need of melatonin supplementation in ewes.

Declarations

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Author contribution. José Clemente Leyva Corona: conceptualization, investigation, methodology, data analyses, funding acquisition, and writing-original draft. **Norberto Ismael Angulo Valenzuela**: conceptualization, and data collection. **Blanca Margarita Laborin Escalante**: data analyses, methodology, data collection, review and editing. **Miguel Ángel Gastelum Delgado**: Technical support. **Nidia Jahzeel SilvaÁvila:** methodology and data collection. **Pablo Luna Nevarez:** review and editing. **Carlos Eduardo Aragón López**: laboratory support. **Miguel Ángel Sanchez Castro**: data analyses, writing original draft, review and editing. **Marcela Ivone Morales Pablos**: conceptualization, data analyses, resources, supervision, funding acquisition, and writing-original draft. All authors read and approved the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Ethical standards and statement of animal rights

The Research Committee of the Department of Agronomic and Veterinary Sciences of the Instituto Tecnológico de Sonora approved the procedures used in this study, which were in accordance with the Mexican Official Standard NOM-062-ZOO-1999 for the production, care and use of experimental animals.

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Figures

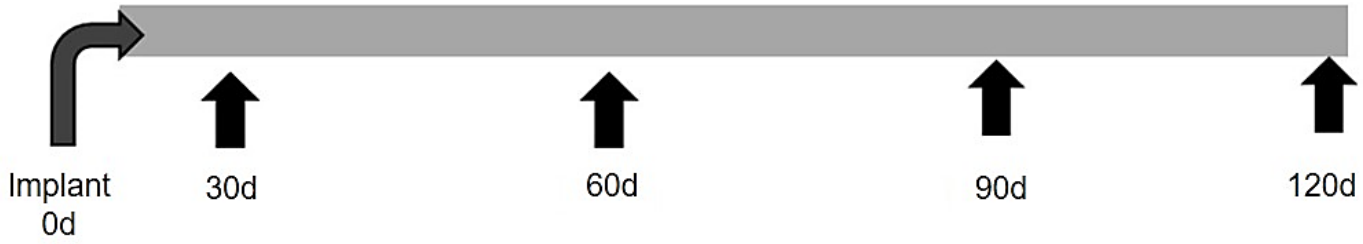

Figure 1

Scheme of days for implantation and sampling post-implantation for andrological tests and blood collections for testosterone levels determination in the ram's study.

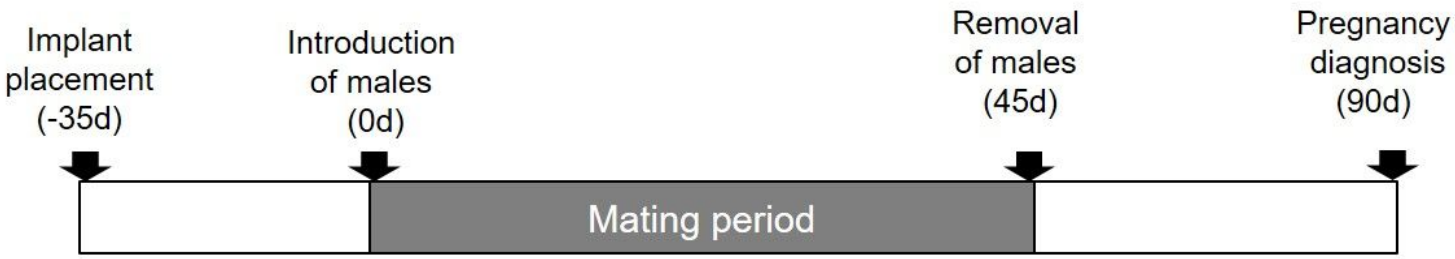

Figure 2

Scheme showing the days of melatonin implantation, beginning, and ending of the mating period, as well as the pregnancy diagnosis in the ewe's study.

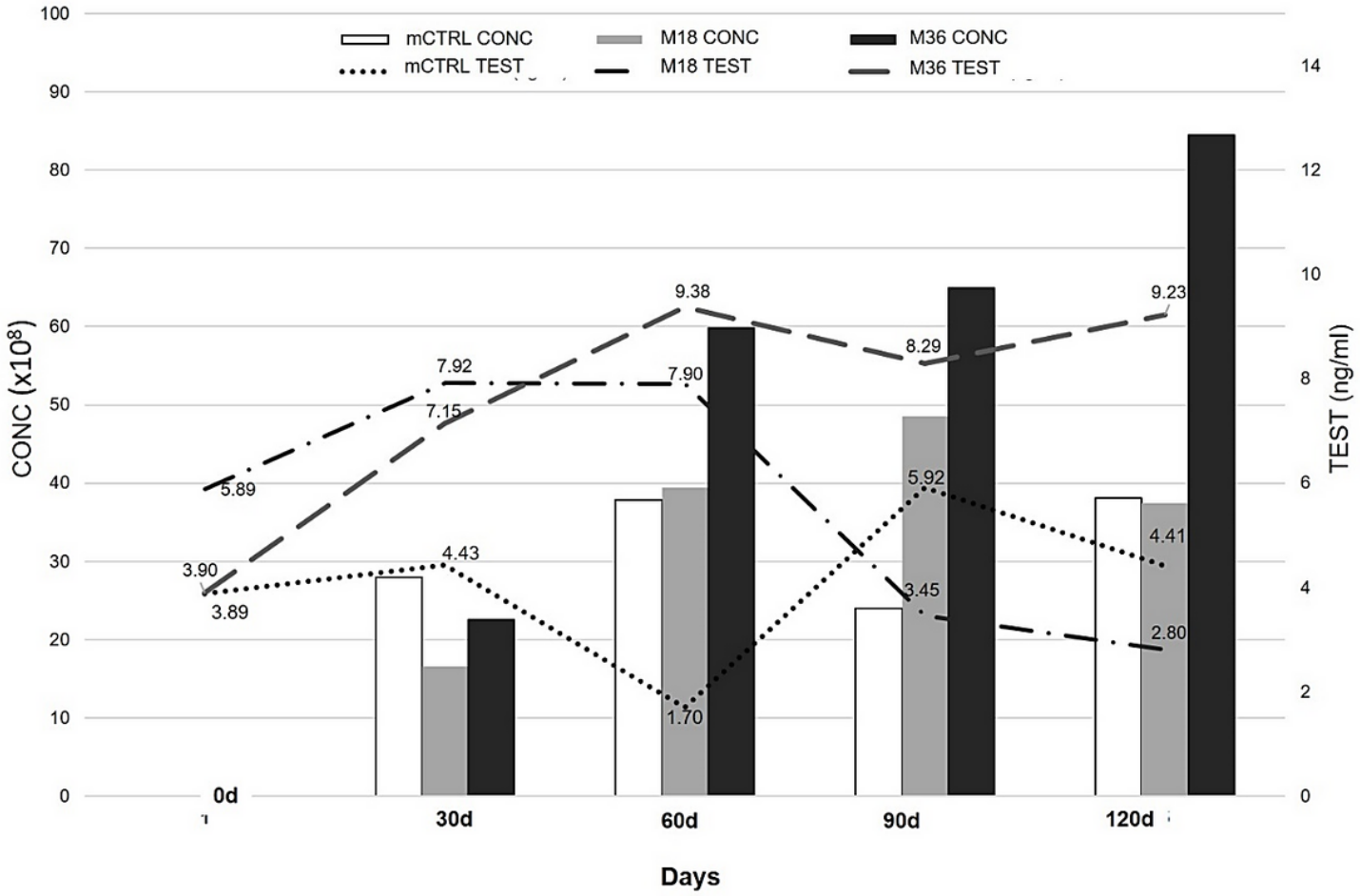

Figure 3

Sperm concentration (CONC) and serum testosterone levels in rams implanted previous to the onset of the anestrous season in northwest Mexico.