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Estrous synchronization in sheep with reused progesterone devices and eCG

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ABSTRACT - The objective of the present research was to determine the effect of long synchronization protocols based on reused progesterone devices (controlled internal drug release [CIDR]) associated with different doses of equine chorionic gonadotropin (eCG) on reproductive variables in crossbred sheep (Suffolk × Kathadin × Dorset). The CIDR were used for eleven days in a previous study in sheep from the same herd and were washed and disinfected before reusing. Sixty-four sheep, in the reproductive season, were randomly assigned to four experimental groups (n = 16). Treatments consisted of a group with 10 d CIDR and 300 IU eCG; a group with 10 d CIDR and 400 IU of eCG; a group with 12 d CIDR and 300 IU of eCG; and a group with 12 d CIDR and 400 IU of eCG. A completely randomized design was used. There was an estrous presentation rate of 100% in all treatments. The begining of estrous, gestation rate, fertility rate, type of parturition, and prolificacy index were equal between groups. Progesterone serum concentration was higher in sheep from the 10 d CIDR groups. The CIDR, reused for the second time, associated with 300 or 400 IU of eCG for estrous synchronization in sheep, are effective to obtain good pregnancy rates and ensures higher prolificacy rates.

Keywords: CIDR reutilization, ovine, reproductive efficiency

1. Introduction

The most used hormonal treatments in estrous syncronization protocols for sheep are those based on progesterone or its analogues (Abecia et al., 2012). Controlled internal drug release (CIDR) is an intravaginal device impregnated with 0.3 g of natural progesterone (Wheaton et al., 1993) designed for use between 12 and 14 days in sheep (Viñoles et al., 2001). The device inhibits GnRH secretion and consecuently prevents the release of gonadotropins, especially luteinizing hormone (LH; Rubianes, 2003). Once the device is removed, an injection of equine chorionic gonadotropin (eCG) is applied (Abecia et al., 2011), which has an effect of follicle-stimulating hormone (FSH) and LH to enhance ovulation (Quintero-Elisea et al., 2011; Martinez-Ros et al., 2018).

Several studies with reused CIDR have been conducted (Cox et al., 2012; Pinna et al., 2012; Bazzan et al., 2013; Silva et al., 2014) with 6-d protocols reusing CIDR up to three times in sheep, obtaining a good response to estrus (Vilariño et al., 2011; Swelum et al., 2018). The results indicate that the cost of CIDR is reduced when reusing it in long protocols (Swelum et al., 2019), which also

reduces environmental contamination due to the lower amount of residual hormones in the device (Gonzalez-Bulnes et al., 2020).

The characteristics of these devices allow them to be washed, disinfected and reused without significant consequences on reproductive parameters in sheep (Vilariño et al., 2011; Pinna et al., 2012; Swelum et al., 2018). The aforementioned studies were carried out in countries with different weather conditions and different sheep breeds from those produced in México. For this reason, it was considered important to perfom a study with sheep (Suffolk × Kathadin × Dorset) adapted to the weather conditions of the State of Mexico (Partida de la Peña et al., 2017), which is the state with the largest sheep production in the country (SIAP, 2019), to obtain data on reproductive variables that could provide relevant information to producers of the region, allowing them to achieve a greater production of lambs per year at a lower cost, while reducing environmental contamination due to the disposal of the devices with high hormonal contents.

Therefore, the objective of this study was to determine the synchronization treatment with the best response on reproductive variables in sheep from four combinations between the days in which CIDR is reused (10 and 12 days) and eCG doses (300 and 400 IU).

2. Matherial and Methods

The research with the animals was conducted following the specifications of the norm of care and use of animals destined to research, considering the Official Mexican Standard NOM-062-ZOO-1999 (SAGARPA, 2001). The study was performed between April and October of 2019 in Montecillo, Texcoco, State of Mexico, Mexico (19°27'18" N and 98°54'26" W, and 2220 m above sea level), with a subhumid, temperate weather and rains in summer.

We used sixty-four sexually mature ovine females (ewes) with no history of pregnancy (Suffolk × Katahdin × Dorset crosses), with 12 months old, an average weight of 41.4 kg, and a body condition score of 3 on a scale of 1 to 5. They were confined and fed oat grain hay (*Avena sativa*), alfalfa hay (*Medicago sativa*), and 350 g of commercial pelleted feed with 14% crude protein, and *ad libitum* access to water. Before starting the study, all sheep received the normal profilactic procedure and were vitaminized, dewormed, and immunized against common farm diseases, and the absence of pregnancy was confirmed by ultrasound.

Sheep were randomly assigned to four experimental groups (n = 16/group) based on hormonal progesterone treatment with reused CIDR® intravaginal devices (330 sheep & goat insert, ZOETIS) and an injection of equine chorionic gonadotropin (eCG; NOVORMON, Virbac 5000®). The treatment groups were 10 days with CIDR and 300 IU of eCG, 10 days with CIDR and 400 IU of eCG, 12 days with CIDR and 300 IU of eCG, and 12 days with CIDR and 400 IU of eCG (Figure 1).

The CIDR were previously used for 11 days in sheep from the same herd. Upon removal, they were washed and disinfected, dried, and refrigerated for up to 24 h prior to reuse. The devices were inserted intravaginally with an applicator disinfected with nitrofurazone; likewise, the perivulvar area of the females was cleaned and disinfected with a florfenicol/oxytetracycline solution. Once the applicator was removed, the device was verified to be correctly positioned, and its permanence *in situ* was reviewed every 24 h. At the time of CIDR removal, intramuscular injections with 300 and 400 IU of eCG were applied.

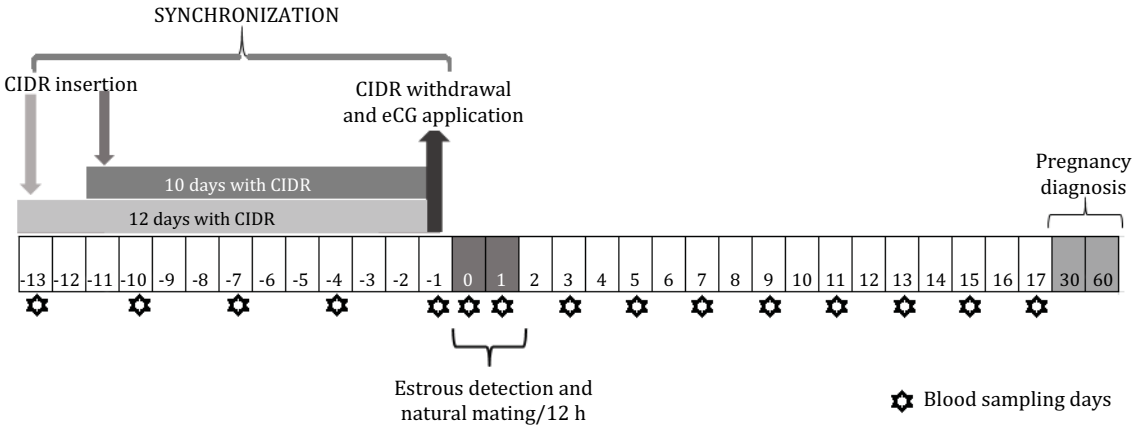
Estrus detection started 24 h after CIDR removal (day 0). Controlled natural mating was used (three matings per ewe with 12-h interval). For this process, 19 rams of proven fertility were used, which were randomly assigned to each female that showed estrus. Females were considered in estrus as soon as they allowed mating.

The gestation rate (pregnant ewes/treated ewes × 100) was determined by ultrasonography with a Sonovet 600 equipment with a 7.5 Mhz transrectal transducer, 30 and 60 days after mating. Fertility

rate (farrowed sheep/treated sheep × 100), prolificacy index (total lambs born/total ewes lambing), and type of farrowing (single or double) were determined by recording lambing.

Blood samples from the jugular vein were collected to determine progesterone concentrations in 10 ewes from each group (Figure 1). The first blood sample was collected on the day of the insertion of CIDR (d −13) on treatments with 12 days and the last sample was collected on day 17 post estrus. The samples were collected in 5 mL polypropylene tubes, which were transported to the laboratory, where the serum was separated by centrifuging at 1500 *g* for 20 min at 4 °C; the serum obtained was transferred to 1.5-mL microtubes and stored at −20 °C until analysis. Progesterone concentrations were determined by Radioimmunoassay (RIA) using the commercial kit PROGESTERONE [I-^{125}] RIA® with a sensitivity of 0-37.7 ng/mL. The intra and inter-assay coefficients of variation were 7.6 and 8.2%, respectively.

A completely randomized experimental design was used with four treatments and 16 repetitions per treatment. The results obtained from variables at the onset of estrus and prolificacy index were analyzed with a normality Shapiro-Wilk test and a variance homogeneity Levene test. Since they did not show normality or homocedasticity, a Kruskal Wallis test was applied. In dichotomous variables, such as the presence of estrous (showed or not estrous), pregnancy rate (percentage of pregnant ewes), fertility rate (percentage of ewes that gave birth), and type of lambing (single or twins), the χ^2 test was carried out using contingency tables. The variable concentration of P4 was analyzed by repeated measures over time using the PROC MIXED procedure. Least square means were calculated with the Tukey-Kramer test. For all analyses, a significant difference was considered ($\alpha = 0.05$). The data was analyzed with SAS program (Statistical Analysis System, version 9.4) for Windows.



CIDR - controlled internal drug release; eCG - equine chorionic gonadotropin.

Figure 1- Experimental protocol.

3. Results

Twenty-four hours after CIDR removal, 37.50% (6/16) of the ewes from the 10dCIDR-400 IU eCG group (Figure 2) and 18.75% (3/16) of the ewes from 10dCIDR-300 IU eCG and 12dCIDR-400 IU eCG groups showed onset of estrus, comparted with 12.5% (2/16) of the ewes from 12dCIDR-300 IU eCG group. After 30 h, a higher concentration of the presence of estrus (87.5%) was observed, and almost all ewes (100 and 93.75%) of the treatments with 400 IU of eCG had already shown estrus. Mean time in hours for onset of estrus in these groups was 28.13±3.61 and 28.88±2.42 h for treatments with 10- and 12dCIDR, respectively, compared with ewes from groups which received 300 IU of eCG (30.00±3.79 and

30.75±3.79 h) for treatments with 10- and 12dCIDR, respectively (Table 1). At 36 h after CIDR removal, all ewes showed estrus, without differences (P>0.05) between treatments (Figure 2).

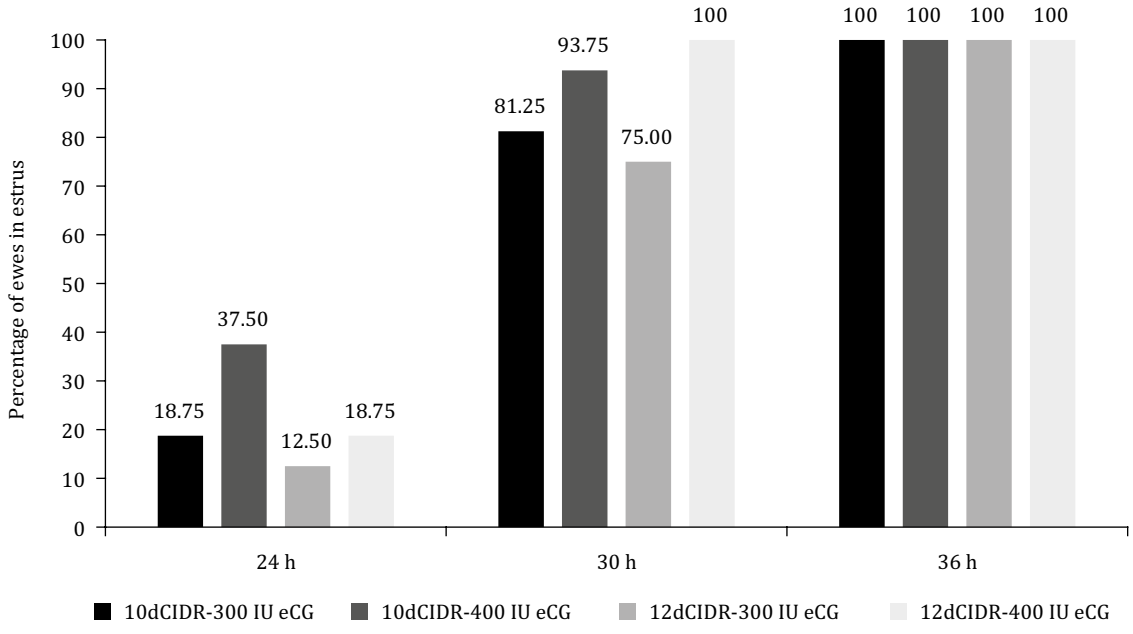
In gestation and fertility rates, no differences (P>0.05) were observed among groups. The total gestation rate was 84.38% and total fertility rate was 82.8%. In the 10dCIDR-300 IU eCG group, 93.75% (15/16) of the sheep were pregnant, although one sheep from this group had an abortion, which reduced the fertility rate to 87.5% (Table 1).

In the variables of prolificacy index and number of lambs born (single or twins), there were no differences (P>0.05) among groups. The prolificacy index was 1.60, with no diference among treatments (P>0.05); however, with 300 IU of eCG, the prolificacy index was below 1.5 (mean prolificacy), while in treatment 10dCIDR-400 IU eCG, the value was near 2 (high prolificacy) (Table 2). There were differences (P≤0.05) in P4 concentration (Table 3), and treatments with higher (P≤0.05) concentrations during the sampling period were those with 10 days of CIDR permanence, while doses of eCG did not influence the concentration of serum P4 (P = 0.4713) (Figure 3).

Table 1 - Response in variables of CIDR treatments (10 and 12 days) and different doses of eCG (300 and 400 IU) on the reproductive variables estrus onset, pregnancy rate, and fertility rate of ewes

Group	Variable		
	Estrus onset (h)	Pregnancy rate (%)	Fertility rate (%)
10dCIDR-300 IU eCG	30.00±3.79	93.75 (15/16)	87.50 (14/16)
10dCIDR-400 IU eCG	28.13±3.61	81.25 (13/16)	81.25 (13/16)
12dCIDR-300 IU eCG	30.75±3.79	75.00 (12/16)	75.00 (12/16)
12dCIDR-400 IU eCG	28.88±2.42	87.50 (14/16)	87.50 (14/16)
χ^2-value	0.1583	0.4992	0.7512

CIDR - controlled internal drug release; eCG - equine chorionic gonadotropin.
No difference among groups (P<0.05).



CIDR - controlled internal drug release; eCG - equine chorionic gonadotropin.

Figure 2 - Percentage of females detected in estrus from 24 h after CIDR withdrawal and application of eCG doses.

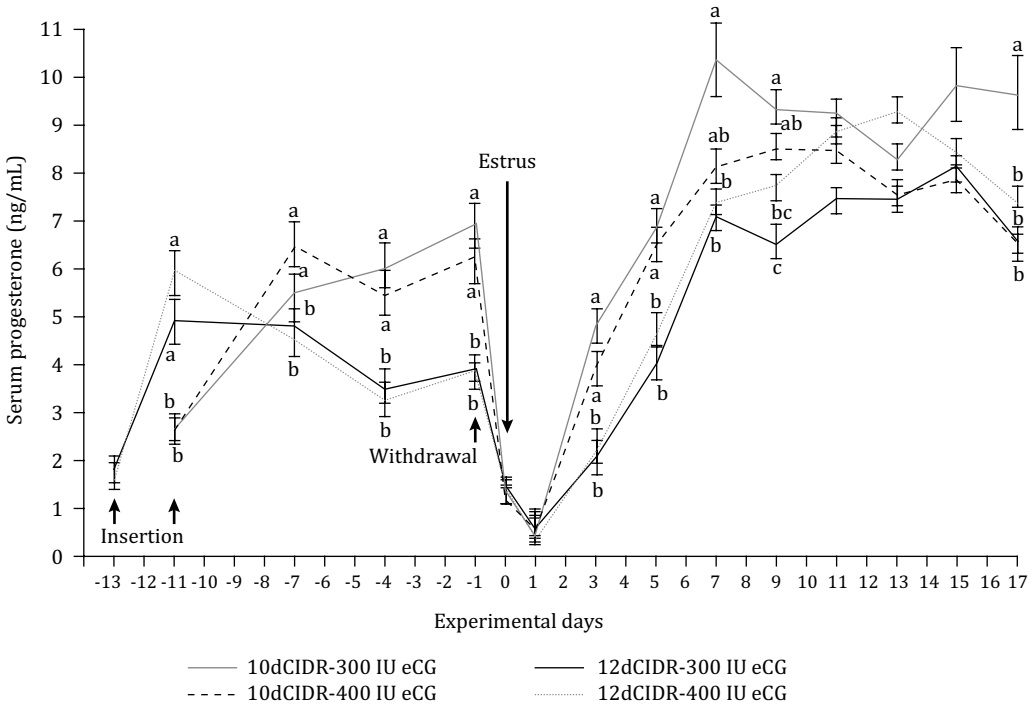
Table 2 - Results obtained in the variables prolificity index and percentage of lambs born single or in twins

Group	Variable		
	Prolificacy index	Lambs born	
		Single (%)	Twins (%)
10dCIDR-300 IU eCG	1.43	57.14	42.86
10dCIDR-400 IU eCG	1.82	23.08	76.92
12dCIDR-300 IU eCG	1.36	58.33	41.67
12dCIDR-400 IU eCG	1.69	28.57	71.43
χ^2-value	0.0904	0.1332	0.1332

CIDR - controlled internal drug release; eCG - equine chorionic gonadotropin.
No difference among groups (P<0.05).

Table 3 - Fixed effects test of P4 concentration in sheep serum during the experimental days

Effect	F value	Pr<F
Treatment	7.04	0.0006
Time	61.30	<0.0001
Treatment × time	4.45	<0.0001



CIDR - controlled internal drug release; eCG - equine chorionic gonadotropin.
a,b,c - Mean ± standard error; different letters among groups differed (P<0.05).

Figure 3 - Effect of CIDR reuse in 10- and 12-day protocols on serum P4 concentrations (ng/mL) during experimental days in sheep.

4. Discussion

In the present study, the presence of estrus in all ewes (100%) was similar to that found by Pinna et al. (2012), who worked with Santa Inês sheep, evaluating reused CIDR in a five-day treatment and obtained 92.9% presence of estrous with new and first-use CIDR and 100% with second-use CIDR. Similar results

were also obtained by Bazzan et al. (2013), using seven-day treatments in Texel and Ile de France ewes, obtaining 93.7 and 95.8% in the first and second reuses, respectively. Our results coincide with those of Vilariño et al. (2013) and Pinna et al. (2012), who mentioned that reused CIDR are as effective as new devices to synchronize estrus and ovulation.

Regarding the onset of estrus, results were consistent with those reported by Martínez-Ros et al. (2018), who obtained estrus presentation at 33.8±4.0 h with an interval of 24-40 h when using seven-day protocols with new CIDR associated with 5 mg of prostaglandin and 400 IU of eCG. Similar results were obtained by Biehl et al. (2019), who used 300 IU of eCG and reported a mean of 34.9 h, with the highest concentration of presence of estrus between 36 and 41.9 h.

The use of eCG reduces the interval to ovulation by enhancing the onset of estrus (Cox et al., 2012) by stimulating the recruitment and maturation of follicles and oocytes (Manes and Ungerfeld, 2015). With lower eCG doses, gonadotropin stimulation is reduced. This led Cox et al. (2012) to notice the beginning of estrus at 32.8±3.2 h when using 350 IU of eCG, whereas in sheep with no doses of eCG, they had a wider interval (45.3±2.8 h). Bazzan et al. (2013) observed onset of estrus between 24 and 72 h using 200 IU of eCG with an average of 48 h.

In regards to gestation rate, in this study we found higher values than those obtained by Silva et al. (2014), who reported gestation rates of 73.3% with new CIDR, 72.7% with the first, and 64.7% in the second reuse using natural mating, while Gastal et al. (2013) obtained 68% of pregnant sheep in the second use of CIDR with artificial insemination. Both studies followed a six-day protocol with CIDR and applied 250 IU of eCG and sodium cloprostenol (0.133 mg or 0.263 mg).

Despite the fact that long protocols based on P4 and eCG have been related with alterations in the quality of oocytes (development of persistent follicles, premature nuclear maturation) that determine low fertility rates and deteriorated embryonic development (Viñoles et al., 2001; Berlinguer et al., 2007; Swelum et al., 2019), in the present study, we obtained an adequate response in gestation rate and fertility with the association of long P4 protocols and 300 or 400 IU of eCG, although a higher gestation rate was obtained (93.75%) when using 10dCIDR and 300 IU eCG.

A study evaluating the use of seven- and 11-day protocols with reused CIDR was conducted by Biehl et al. (2019) in Santa Ines ewes; they found that the 11-day protocol tended to increase ($P = 0.07$) gestation rate (33%) compared with the seven-day protocol (24%) using fixed-time artificial insemination. Gestation rate and fertility are associated with the efficiency of the devices reused for 10 and 12 days by inhibiting GnRH secretion, which induce folicular dynamics at device removal and enhance LH and FSH secretion in ewes (Arbués et al., 2018). This result can also be explained for the use of 300 and 400 IU of eCG applied, since this hormone improves folicular development and increases ovulation and conception rate (Quispe et al., 1995; Boscos et al., 2002).

A prolificacy index of 1.58 was obtained, which indicates a good ovulation rate, since Dorset and Katahdin sheep are breeds with a prolificacy index of 1.2 to 1.66 (Wildeus, 2012). The 400 IU dose of eCG favored the percentage of double births and the prolificacy index when modifying the ovulatory rate. A high dose of eCG in estrus induction and synchronization protocols causes a superovulatory effect in ewes and, therefore, an increase in prolificacy (Zeleke et al., 2005; Azawi and Al-Mola, 2010; Quintero-Elisea et al., 2011).

Concentrations of P4 >1.0 ng/mL in all four treatments previous to CIDR insertion (experimental days −13 and −10, Figure 1) confirm luteal activity in ewes (Uribe-Velásquez et al., 2011). On days −7, −4, and −1, P4 concentrations >4 ng/mL were found in all groups, which indicates that the P4 content of reused CIDR is able to increase the P4 seric profile and mimic the activity of the corpus luteum. Higher P4 concentrations ($P<0.5$) were found in 10dCIDR treatments.

Progesterone concentrations decreased to <2 ng/mL 24 h after CIDR removal (day 0), while on day 1, subluteal concentrations <1 ng/mL were obtained from 100% of sampled ewes; this agrees with Wheaton et al. (1993), who stated that P4 concentrations decrease dramatically after CIDR removal. This decrease in P4 is required to disinhibit GnRH secretion and trigger the release of gonadotropin,

inducing the onset of estrus and ovulation (Fabre-Nys and Gelez, 2007; Abecia et al., 2011; Amiridis and Cseh, 2012).

On experimental days 3 and 5, there was an increase in the concentration of P4 (>1 ng/mL) in all ewes, showing that ovulation was induced in the four treatments, although P4 concentrations were higher ($P<0.05$) in 10dCIDR treatments. The highest P4 concentration was observed on day 7 in the 10dCIDR 300 IU eCG ($P<0.05$) ewes and then reached a plateau on days 11, 13, and 15 with no difference among groups ($P>0.05$). On day 17, an evident reduction in the mean concentration of P4 was observed in ewes from both 12dCIDR treatments and 10dCIDR-400 IU eCG treatment. Bazer (2013) stated that 12 to 13 days after mating is a critical period in sheep gestation due to the beginning of corpus luteum regression. The implantation and manteinance of the corpus luteum, and therefore this stage of gestation, depend on the secretion of interferon-tau (IFTN) from the embryo (Arosh et al., 2004).

According to the behavior of the P4 profile, it is possible to say that 100% of the sheep presented estrus, ovulated, and subsequently increased P4 concentrations to levels that indicated the development of at least one functional corpus luteum, but 15% of the sampled ewes presented no maternal recognition of pregnancy; this statement is supported by the fact that these ewes were identified as non-pregnant when a pregnancy diagnosis was performed by ultrasound 30 days after the breeding period.

The CIDR devices were effective in maintaining concentrations of P4 similar to those that take place in the luteal period, thus simulating a functional corpus luteum during the 10- and 12-day treatment periods. Higher P4 concentrations were reported in the 10dCIDR-300 IU eCG.

5. Conclusions

Long protocols with reused controlled internal drug release associated with equine chorionic gonadotropin in cyclic ewes are efficient in the synchronization of estrous; the addition of different doses of equine chorionic gonadotropin at the time of withdrawal of controlled internal drug release causes a positive effect on the main reproductive variables (onset of estrus, prolificacy index, and type of parturition). With doses of 400 IU of equine chorionic gonadotropin, the results of these variables improve, the onset of estrus is shortened, and the prolificacy index and percentage of twin lambing increase.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: S. López-García, M.T. Sánchez-Torres, J.L. Cordero-Mora, J.L. Figueroa-Velasco and J.A. Martínez-Aispuro. Data curation: M.T. Sánchez-Torres and J.L. García-Cué. Formal analysis: S. López-García, M.T. Sánchez-Torres, J.A. Martínez-Aispuro, J.L. García-Cué and M. Cárdenas-León. Funding acquisition: M.T. Sánchez-Torres. Investigation: S. López-García, M.T. Sánchez-Torres, J.L. Cordero-Mora, J.L. Figueroa-Velasco, J.A. Martínez-Aispuro and I. Martínez-Cruz. Methodology: S. López-García, M.T. Sánchez-Torres and I. Martínez-Cruz. Project administration: J.L. Cordero-Mora. Software: S. López-García, J.A. Martínez-Aispuro and J.L. García-Cué. Supervision: J.L. Cordero-Mora, J.A. Martínez-Aispuro and I. Martínez-Cruz. Visualization: J.L. Figueroa-Velasco. Writing-original draft: S. López-García, J.L. Figueroa-Velasco, J.A. Martínez-Aispuro, J.L. García-Cué and M. Cárdenas-León. Writing-review & editing: M.T. Sánchez-Torres and J.L. Figueroa-Velasco.

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