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Autoclaved, previously used intravaginal progesterone devices induces estrus and ovulation in anestrous Toggenburg goats

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ABSTRACT

Intravaginal progesterone devices are used worldwide for estrus induction in goats. Reused devices are able to induce estrus; however, this can be a health risk within a flock. The objective was to compare new and previously used (and autoclaved) progesterone-releasing intravaginal devices for induction of estrus and ovulation in seasonally anestrous Toggenburg goats. Anestrous goats ($n = 42$) received new intravaginal devices containing 0.3 g progesterone (CONTROL), or similar devices previously used for either 6 (USED6) or 12 d (USED12) and subsequently autoclaved. All goats received 5 mg dinoprost at device insertion and 200 IU eCG 5 d later, and all devices were removed after 6 d. After device removal, estrus was monitored and females displaying signs of estrus were mated by fertile bucks. Transrectal ovarian ultrasonography was performed after device removal until detection of ovulation. Blood samples were collected for determination of plasma progesterone concentration at different times. There was no difference ($P > 0.05$) among groups CONTROL, USED6 or USED12 for: estrus response (87, 100 or 100%, respectively); duration of estrus (32.3 ± 2.3, 25.2 ± 3.4 or 27.3 ± 4.1 h); ovulation rate (100, 88 or 100%); number of ovulations (1.5 ± 0.2, 1.9 ± 0.3 or 1.7 ± 0.3); and pregnancy rate (60, 58 or 67%). Plasma progesterone (P4) concentrations were greater ($P < 0.05$) in CONTROL than in USED6-treated and USED12-treated goats (7.2 ± 1.2, 4.7 ± 0.7 and 4.3 ± 0.6 ng/mL, respectively) at 6 h after device insertion; these differences were maintained until 4 d after device insertion (3.4 ± 0.4, 2.3 ± 0.2, and 2.5 ± 0.2 ng/mL). Overall, plasma progesterone concentrations were greater ($P < 0.05$) in nulliparous than in lactating goats (3.1 ± 0.8 compared to 2.4 ± 0.6 ng/mL, respectively). In conclusion, autoclaved, previously used intravaginal progesterone-releasing devices resulted in significant lesser plasma progesterone concentrations than new devices, but were similarly effective in inducing estrus and ovulation in anestrous goats.

1. Introduction

Depending on latitude and breed, goats are seasonal breeders. Thus, due to physiological, commercial or technical reasons, estrous induction of goats is justifiable. Many hormonal treatments have already been described,

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varying the dose, type and/or duration of the progesterone/progestagens used, whether of gonadotropin and prostaglandin are used, as well as its time of administration (Gordon, 1997). Ungerfeld and Rubianes (1999) reported that short term (6 days) progestagen priming, that results in greater progestagen concentrations at the time of device removal, is at least as effective as traditional priming (12–14 days) to obtain an out-of-season estrus. Since these earlier publications, many papers were published using this method in goats (Fonseca et al., 2005; Menchaca et al., 2007). Thus, hormonal treatments have the advantage of efficiently inducing an earlier onset of estrus (in about 1 week). However, costs are relatively greater and this sometimes may hinder widespread use of these treatments. As a consequence, the development and/or refinement of efficient techniques that brings about cost reduction could be deemed appropriate in the case of induction.

As protocols for estrous induction shortened the time of exposure to progesterone, devices were able to be reused. Although not recommended by the manufacturer, device reuse is a common practice in dairy herds. Reuse of progesterone intravaginal devices have been reported in cows (Colazo et al., 2004), ewes (Ungerfeld, 2009) and goats (Oliveira et al., 2001; Carvalho et al., 2006; Vilariño et al., 2011), usually without decreasing fertility rate. Goats that are expressing estrous cycles in typical patterns receiving new or reused CIDR showed similar estrous response and pregnancy rates with second (Oliveira et al., 2001) or third uses (Nogueira et al., 2008). In another trial, one group of 20 goats received reused devices for 9 days and a second group of 20 received new devices. Progesterone concentrations measured every 24 h from device insertion to removal were similar in both groups, and the authors concluded that the use of reused devices was feasible (Guido et al., 2007). Similarly, Carvalho et al. (2006) cited that in anestrous goats it was possible to reuse devices three times.

Despite the desirable results, reused devices can be a health risk within a flock. Therefore, alternatives for device disinfections before reuse such as the immersion in a solution of benzalkonium chloride (Vilariño et al., 2011) were proposed. The possibility for the sterilization was reported earlier when devices were washed in physiological solution and subsequently sterilized under ultraviolet light (Oliveira et al., 2001; Carvalho et al., 2006). In cattle, the autoclaving process was reported and considered to be the most desirable option when reusing progesterone containing inserts (Zuluaga and Williams, 2008) as no difference was observed in ovarian responses after autoclaving and reuse in comparison to use of a new device (Cerri et al., 2009). The autoclaving process was more effective than ultraviolet irradiation when both techniques were compared to achieve sterilization (Gefrides et al., 2010). However, no literature was found regarding the reuse of autoclaved devices for estrous induction in goats and corresponding progesterone profiles with device reuse. If this method is considered to be functional, the use of reproductive programs could be improved, extending the use of progesterone inserts, with lesser costs and similar results. The aim of the present study was to evaluate the efficacy of reusing autoclaved intravaginal devices on induction of estrus and ovulation and fertility in Toggenburg goats, as well as to characterize the plasma progesterone (P4) profile in these animals.

2. Materials and methods

2.1. Location and experimental conditions

The study was conducted during the seasonal anestrous period in the rural area of Piau, MG, Brazil (latitude 21°35′S and longitude 43°15′W). The average altitude was 435 m with Cwa climate, according to Köppen classification (winters without or with minimum rainfall and summers with high ambient temperatures; Peel et al., 2007).

The goats were kept in an intensive system, within pens 15 m in length and 2 m wide which housed 10 goats each, providing 3 m^2 per animal, allowing an acceptable degree of animal welfare (Ribeiro, 1997). Goats were fed corn silage and *Pennisetum purpureum* as forage. Additionally, for lactating goats, a balanced concentrate supplement was given according to their milk production (NRC, 2007). Mineralized salt (Salminas Goats®, Nutriplan, Juiz de Fora, MG, Brazil) and drinking water were available *ad libidum*. This research was reviewed and approved by the Animal Care Committee of Fluminense Federal University (UFF/0048-08).

2.2. Estrous induction treatment and mating

Toggenburg nulliparous ($n = 20$) or lactating ($n = 22$) goats 8 months to 7 years of age were selected and allocated according to category, body weight (BW) and body condition score (BCS, range 1–5), respectively, into three treatments: CONTROL, USED6 and USED12. Does in the CONTROL group ($n = 17$; 8 nulliparous and 9 lactating; 41.5 ± 2.1 kg, BCS 3.4 ± 0.1) received a new, progesterone releasing device containing 0.3 g progesterone (Eazi-Breed CIDR®, InterAg, Hamilton, New Zealand). Does in the other two groups received similar devices previously used for 6 d (USED6, $n = 13$; six nulliparous and seven lactating; 43.5 ± 3.1 kg, BCS 3.5 ± 0.1) or 12 d (USED12, $n = 12$; six nulliparous and six lactating; 44.9 ± 4.6 kg, BCS, 3.5 ± 0.2). Previously used devices (used once 30 days earlier) had been thoroughly washed with water, air dried, then placed individually in special plastic bags (designed for use in an autoclave), and autoclaved for 15 min (121 °C and 1 atm pressure above standard pressure). After autoclaving, devices were stored at room temperature until use.

Concurrent with device insertion, 5 mg dinoprost (Lutalyse®, Pfizer Animal Health) was given, with a subsequent injection of 200 IU eCG (Novormon 5000®, Sintex Industries Biochemistry, Buenos Aires, Argentina) 24 h before device removal. Both dinoprost and eCG were given as submucosal injections in the latero-vulvar area. In all does, devices were removed after 6 d.

After device removal, estrus was monitored with the use of bucks twice a day (07:00 and 19:00 h) and females were considered to be in estrus when allowed to be mounted. Does displaying signs of estrus were mated by fertile bucks at the onset of estrus and 24 h later if they were still in estrus. Toggenburg bucks ($n = 7$) were used for approximately equal numbers of does from each treatment, with a

buck:doe ratio about ≤1:6. Pregnancy rate was diagnosed 30 days after natural mating by ultrasonography.

2.3. Ultrasonography

Transrectal ovarian ultrasonography was performed in 24 goats (eight per treatment group) daily (by the same operator) during progesterone treatment (Days 0–6) and every 12 h after device removal until detection of ovulation, or until 96 h after device removal (if ovulation was not detected). All examinations were conducted with a B-mode transrectal ultrasonographic scanner with 5 MHz transducer (Aloka SSD 500®, Tokyo, Japan). To facilitate manipulation of the transducer, it was taped to a PVC tube. Does were maintained in a standing position, fecal pellets were removed manually (with a finger), and 20 mL of carboxymethylcellulose gel was placed into the rectum with a syringe. Ovaries were located as previously described (Ginther and Kot, 1994), and the number, diameter, and position of ovarian follicles ≥ 3 mm were recorded. The day of ovulation was defined as the day when the largest follicle, previously identified, was no longer detected. The preovulatory follicle diameter was considered the last measurement obtained before ovulation. Approximately 30 d after breeding, the same equipment was used to conduct ultrasonographic pregnancy diagnosis for all 42 goats.

2.4. Plasma progesterone concentrations

Blood samples were collected in 30 goats (10 per treatment group) by jugular veinpuncture, into tubes containing EDTA at the following times: 7 d before device insertion (Day 7); concurrent with device insertion; (06:00 h; Day 0); 6 and 12 h after device insertion (Days 0.25 and 0.5, respectively); and then daily from Days 1 to 9. Tubes were immediately placed in ice, transported to the laboratory, and centrifuged at 2000 × *g* for 15 min. Plasma was removed and stored at −20 °C pending determination of plasma progesterone concentrations with a commercial solid phase radioimmunoassay (RIA) kit (Coat-a-Count® progesterone kit, DPC, Diagnostic Products Corporation, Los Angeles, CA, USA), used according to the manufacturer's instructions. The mean intra- and inter-assay coefficients of variation were 8.8% and 9.7%, respectively. The control value was 4.3% and the analytical detection limit was 0.08 ng/mL.

2.5. Variables and statistical analyses

End points determined were: estrous response (number of does in estrus/number of treated does × 100); interval to estrus (from device removal to first acceptance of mounting); estrous duration (interval from the first to last acceptance of mounting); interval from device removal to ovulation; interval from onset of estrus to ovulation; ovulation rate (number of does with confirmed ovulation/number of does evaluated by ultrasonography × 100); number of ovulations per doe; largest, second largest and average follicle diameter; pregnancy rate (number of pregnant does/number of does submitted to estrus induction treatment); number of lost devices; and plasma progesterone concentration. Statistical analysis was performed using all tests with $P < 0.05$ considered significant. Parametric variables were submitted to one way analysis of variance and compared by SNK test by the SAEG program (System for Statistical Analysis). Non parametric variables were analyzed by the use of the chi-square test. The results are described as mean ± SE.

3. Results

3.1. Sexual behavior and ultrasonography end points

A lactating goat from USED6 became ill and was removed from the experiment. Two females from CONTROL treatment lost their devices, one nulliparous and the other lactating. There was no difference ($P > 0.05$) among groups CONTROL, USED6-treated or USED12-treated goats for: estrous response [87% (13/15), 100% (12/12) or 100% (12/12), respectively], interval to estrus (35.1 ± 3.7, 32.0 ± 3.1 or 32.0 ± 2.7 h), duration of estrus (32.3 ± 2.3, 25.2 ± 3.4 or 27.3 ± 4.1 h), interval from onset of estrus to ovulation (40.0 ± 2.5, 29.1 ± 3.9 or 41.1 ± 4.4 h), interval from device removal to ovulation (72.0 ± 3.4, 61.7 ± 1.3 or 72.0 ± 6.4 h), ovulation rate [100% (8/8), 88% (7/8) or 100% (8/8)], number of ovulations (1.5 ± 0.2, 1.9 ± 0.3 or 1.7 ± 0.3), largest follicle diameter (7.6 ± 0.4, 7.3 ± 0.2 or 7.4 ± 0.1 mm), second largest follicle diameter (7.1 ± 0.6, 6.9 ± 0.2 or 6.5 ± 0.1 mm), average follicle diameter (7.4 ± 0.4, 7.2 ± 0.2 or 7.1 ± 0.1 mm) and pregnancy rate [60% (9/15), 58% (7/12) or 67% (8/12)]. One goat from USED6 and another one from USED12 presented triple ovulations with the third follicle measuring 7.3 and 6.1 mm, respectively. Ovulation was detected before the end of estrus in 40% of goats (8/20) whereas 60% (12/20) of goats ovulated after estrus, i.e., in metaestrus.

Of the 37 goats in estrus, 27 (73%) and 10 (27%) were initially identified in estrus at 07:00 and 19:00 h, respectively. There was no difference ($P > 0.05$) between nulliparous and lactating goats on the following end points, respectively: interval to estrus (33.5 ± 2.5; 27.7 ± 3.0 h), estrous duration (32.7 ± 2.9; 29.3 ± 2.6 h), ovulation rate [100%(12/12);92%(11/12)], number of ovulations (1.4 ± 0.2; 1.9 ± 0.2), interval from device removal to ovulation (72.0 ± 5.6; 66.0 ± 3.1 h), interval from estrus to ovulation (40.5 ± 3.2; 34.0 ± 4.1 h), largest follicle diameter (7.5 ± 0.3; 7.4 ± 0.1 mm), second largest follicle diameter (7.3 ± 0.6; 6.7 ± 0.1 mm), average follicle diameters (7.4 ± 0.3; 7.1 ± 0.1 mm) and pregnancy rate [53%(10/19);70%(14/20)].

3.2. Plasma progesterone concentration

All 30 goats had subluteal concentrations (<1 ng/mL) on Day 7 and Day 0. Plasma progesterone concentrations were greater in CONTROL than USED6-treated and USED12-treated goats (7.2 ± 1.2, 4.7 ± 0.7 and 4.3 ± 0.6 ng/mL, respectively) at 6 h after device insertion and these differences were last significant 4 d after device insertion (3.4 ± 0.4, 2.3 ± 0.2, and 2.5 ± 0.2 ng/mL, respectively). Concentrations superior to 1 ng/mL at the moment of device removal were detected in all females. On the following days

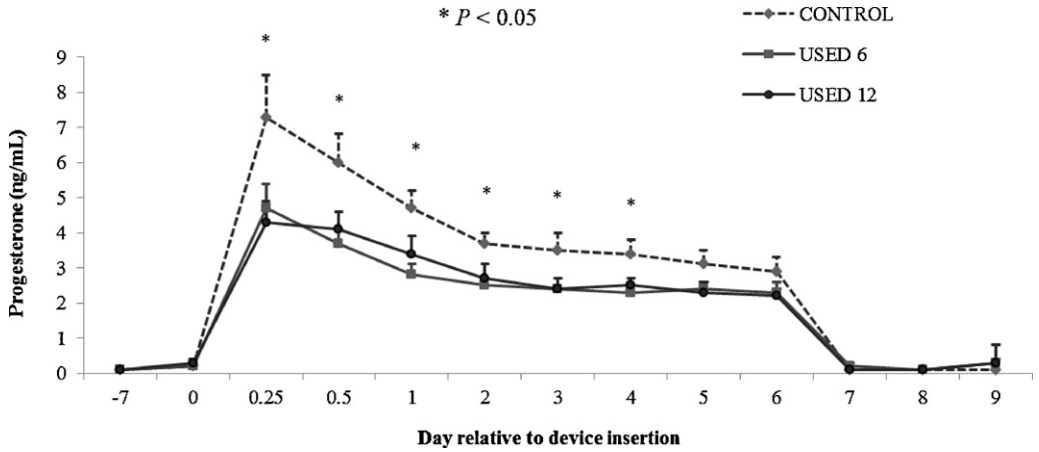

Fig. 1. Plasma progesterone concentration in Toggenburg goats submitted to estrous induction (anestrous season) receiving new CIDR (CONTROL), autoclaved CIDR previously used for 6 d (USED6) or 12 d (USED12).

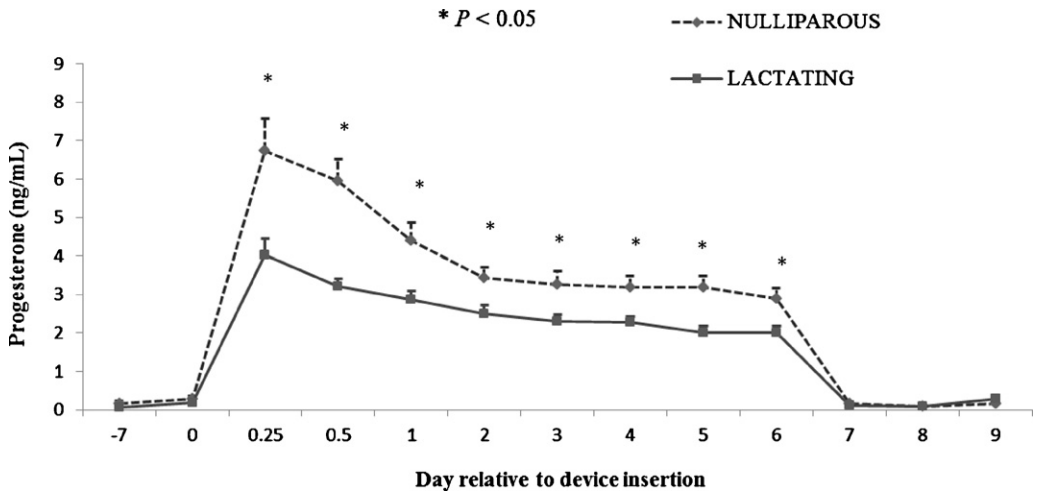

Fig. 2. Plasma progesterone concentration in nulliparous or lactating Toggenburg goats submitted to estrous induction (anestrous season) receiving new CIDR (CONTROL), autoclaved CIDR previously used for 6 d (USED6) or 12 d (USED12).

(Days 7–9) progesterone decreased to subluteal concentrations in all goats of all treatments (Fig. 1). Nulliparous goats had greater ($P < 0.05$) progesterone concentrations than lactating goats throughout the period devices were inserted (Fig. 2).

4. Discussion

As no statistical difference was detected in any reproductive end point among all treatments, averages were assessed. Estrous response rate was on average 95% (37/39). This value is slightly inferior to 100% recorded for Saanen and Nubian goats (Regueiro et al., 1999) and superior to 87% (Fonseca et al., 2005) and 75% (Zambrini, 2006), described for the same breed used in this study. These data indicate that the reuse of devices after the autoclaving process does not negatively influence the estrous response. Does were initially detected in estrus in the morning (73%) more frequently than in the afternoon (27%). This finding is in agreement with Fonseca et al. (2005) who, using the same breed and location, observed 85% of females exhibiting estrus in the morning. These reports suggest that the onset of estrus in goats is a phenomenon that occurs predominantly at night. Timing of onset of estrus and its influence in the ovulation time should be considered in establishing the optimal time to artificial insemination.

The interval from device removal to estrus was on average 33.1 ± 1.9 h. This value was shorter than 53 h cited for Boer breed (Greyling and Van der Nest, 2000) and similar to 33 h for dairy goats (Regueiro et al., 1999). Estrous duration was on average 28.6 ± 1.9 h. It was observed that in Boer goats the average estrous duration was 31 h (Greyling and Van der Nest, 2000), in Alpine, 25 h (Fonseca et al., 2008), in Saanen, 58 h (Regueiro et al., 1999) and in Toggenburg, 32 h (Zambrini, 2006). Thus, treatments used in the present study did not affect interval to estrus or duration as compared with results from previous studies in the same breed.

Pregnancy rate of goats receiving new or reused autoclaved devices averaged 62% (24/39). This is in agreement

with a previous report when Saanen goats received reused intravaginal devices, since the authors observed that this practice did not affect the reproductive parameters evaluated (Oliveira et al., 2001). In another trial, dairy goats were treated with intravaginal devices that had been previously used for 6, 12 or 18 days and were submitted to sterilization under ultraviolet light. Similarly, no loss in reproductive efficiency was detected (Carvalho et al., 2006). Average percentage regarding exclusively USED6 and USED12 devices in this study (63%) was comparable to that obtained in literature for second and third use (65%) also in anestrous goats (Vilariño et al., 2011). Although no differences were detected in the present study, given the small number of goats used, the fertility rate should be better evaluated in another trial using a higher number of animals to confirm the current results.

According to the literature, the present study is the first report of which we are aware in anestrous goats treated with progesterone intravaginal devices after undergoing sterilization by autoclaving process. Different approaches have been used to clean, disinfect or sterilize devices in studies reporting CIDR reuse, but the autoclaving may be the best option when re-using CIDR inserts for estrous synchronization because it considerably reduces the risk of disease transmission and results in greater blood progesterone concentrations when compared to a disinfectant solution (Zuluaga and Williams, 2008).

In the present study, overall ovulation rate was 96% (23/24), a greater percentage when compared to 80% in the Alpine breed (Fonseca et al., 2010). This rate indicates the application of reused autoclaved devices does not adversely affect estrous synchronization success. The number of ovulations averaged 1.7 ± 0.1 (34/20). A greater ovulation rate was reported by Cruz et al. (2008) when working with a FGA sponge in Nubian (3.4) and Saanen (2.5) breeds. However, ovulation rates in the present study were similar to that reported by our group in Saanen goats receiving MAP sponges for 5 (1.6) or 6 days (1.8; Souza et al., 2007), in Alpine (1.7; Fonseca et al., 2010) and Nubian breed (1.2; Souza et al., 2010). There is a linear relationship between dose of eCG and ovulation number, but 200 IU eCG is considered to be sufficient to stimulate ovulation without inducing an acceptable incidence of multiple ovulations (Ritar, 1993). The average interval from device removal to ovulation was 68.4 ± 2.9 h, which is longer than that obtained for Saanen goats receiving MAP sponges for 5 (62 h) or 6 days (58 h), during the nonbreeding season (Souza et al., 2007), as well as in the Alpine breed (59 h; Fonseca et al., 2010). Conversely, similar results were attained for the Nubian breed (67 h; Souza et al., 2010) in protocols where goats received a progestagen and 200 IU eCG.

The largest and second largest follicle diameters averaged 7.4 ± 0.1 and 6.8 ± 0.1 mm, respectively. This is in agreement with a previous report, two or more follicles in each wave reach > 5 mm diameter in goats (Ginther and Kot, 1994). Gonzalez-Bulnes et al. (2004), using Murciano-Granadina goats, determined that the pre-ovulatory follicles had a mean diameter of 7.8 mm, whereas Tenório Filho et al. (2007) found much lower value (5.5 mm) in Nubian goats. Castro et al. (1999) reported the ovulatory wave mean follicle diameter was 7.0 mm. Thus, follicle diameter value seems to vary greatly depending on the breed evaluated.

The minimal initial progesterone concentrations (ng/mL) in all treatments 7 days before or immediately before device insertion may be interpreted as a consequence of reproductive seasonality. This shows that in Southeastern Brazil during long day photoperiods (spring/summer) period, Toggenburg goats appeared to remain in anestrus, because it was assumed ovulations(functional CL) are present when progesterone concentrations are ≥ 1.0 ng/mL (Thimonier, 2000). Maffili et al. (2006) detected one Toggenburg goat of 12 evaluated with a progesterone concentration greater than 1 ng/mL at the time of device insertion at a similar latitude as that in which the present study was conducted.

In cattle, the application of re-used autoclaved devices appears to markedly increase serum concentrations of progesterone during the first 8 h after insertion compared to a non-autoclaved, new CIDR (Zuluaga and Williams, 2008). However, in the present study, greater concentrations were detected until Day 4 in the CONTROL treatment when compared to the others—USED6-treated and USED12-treated does. It is interesting to note that supraluteal concentrations were maintained up to device removal in all goats, which may indicate that the autoclaving process did not adversely affect progesterone availability, as it sustained sufficient concentrations of this hormone to block a LH surge. This corroborates with Guido et al. (2007) who found used devices are able to maintain progesterone concentrations similar to the new devices in goats up to the time of device removal. After device removal (Days 7–9) progesterone concentrations were less than 1.0 ng/mL in all goats, which is in agreement with Maffili et al. (2006) in Toggenburg and Motlomelo et al. (2002) in Boer goat serum progesterone concentrations. This value is desirable because it will allow the occurrence of estrus.

The significant difference identified in the progesterone profile in nulliparous compared to lactating goats is the first report in the literature of which we are aware, because this was only previously described in cattle. It is well known that lactating females generally receive a diet with a greater intake of dry matter compared with nulliparous ones, which may adversely affect reproductive performance (Dunne et al., 1999). An inverse relationship between dry matter intake and plasma progesterone concentrations in ewes (Parr et al., 1993) and cows (Vasconcelos et al., 2003) has been described, due to increased blood flow to the hepatic portal vein. As the liver is the site of the greatest metabolism of progesterone, with an efficiency of 96%, probably the greater dry matter intake elevates the metabolism rate of this hormone (Parr et al., 1993; Sangsritavong et al., 2002). Therefore, this could be the explanation for this effect in the present study.

5. Conclusions

Autoclaved, intravaginal progesterone devices that were previously used for either 6 or 12 days were able to maintain progesterone above concentrations necessary to synchronize and induce estrus in anestrous does.

Autoclaving progesterone devices prior to reuse provides minimal health risk, decreases the cost of estrous synchronization programs, and makes such programs more economically feasible for producers. Across treatments, lesser progesterone concentrations were observed in lactating versus nulliparous does, suggesting precaution in that previously used devices may be marginally effective in lactating does.

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