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Re-used progesterone devices efficiently synchronise oestrus and ovulation after autoclaving process in Toggenburg goats during the breeding season

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Abstract. This study compared new and previously used (and autoclaved) progesterone devices for synchronisation of oestrus during the breeding season in Toggenburg goats. Nulliparous ($n = 17$) or lactating ($n = 50$) received new intravaginal devices containing 0.3 g progesterone (CONTROL), or similar devices previously used for either 6 (USED6) or 12 (USED12) days and subsequently autoclaved. All goats received 5 mg dinoprost at device insertion and 200 IU equine chorionic gonadotropin 5 days later and all devices were removed after 6 days. After device removal, females were mated by fertile bucks. Ovarian ultrasonography was performed every 12 h after device removal until ovulation detection. Blood samples were collected for determination of plasma progesterone concentration in different moments and intervals (from 7 days before device insertion to 3 days after its removal). There was no difference ($P > 0.05$) among groups CONTROL, USED6 or USED12 for: oestrus response [75% (18/24), 77% (17/22) or 71% (15/21), respectively]; duration of oestrus (30.7 ± 3.4, 31.8 ± 1.7 or 32.8 ± 3.4 h), percentage of ovulating goats [67% (6/9), 78% (7/9) or 56% (5/9)], ovulation rate (1.3 ± 0.2, 1.4 ± 0.2 or 1.8 ± 0.4 units of corpora lutea), average follicle diameter (6.2 ± 0.1, 6.7 ± 0.1 or 6.8 ± 0.3 mm) and pregnancy rate [54% (13/24), 50% (11/22) or 48% (10/21)]. Plasma progesterone concentrations were not different ($P > 0.05$) for does among treatments and between nulliparous and lactating females. In conclusion, autoclaved, previously used intravaginal progesterone devices are effective in synchronising oestrus and ovulation in cyclic goats during the breeding season.

Additional keywords: CIDR, oestrus synchronisation, ovarian follicular dynamics.

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Introduction

Depending on latitude and breed, goats are seasonal breeders. Thus, due to physiological, commercial or technical reasons, oestrus induction of goats is justifiable during the anoestrous season. However, during the breeding season, the induction of a synchronised oestrus is likewise essential for the use of modern biotechnologies such as timed artificial insemination or multiple ovulation and embryo transfer. Hormonal treatments have the advantage of efficiently inducing an earlier onset of oestrus (in ~1 week), in comparison with other induction treatments (light or male effect). However, costs are relatively high and this sometimes may hinder widespread use of these treatments. As a consequence, the development and/or refinement of efficient techniques which reduce costs could be deemed appropriate in the case of synchronisation.

As protocols for oestrus induction/synchronisation shortened the time of exposure to progesterone (Ungerfeld and Rubianes 1999), intravaginal progesterone devices were able to be reused.

The reuse of progesterone intravaginal devices have been reported in cows (Colazo *et al*. 2004), ewes (Pinna *et al*. 2012; Vilariño *et al*. 2013) and goats (Oliveira *et al*. 2001; Vilariño *et al*. 2011), usually without decreasing fertility rate. Goats expressing typical oestrous cycle received new or reused CIDR for 9 days and showed similar oestrus response and pregnancy rates (Oliveira *et al*. 2001).

Despite the desirable results, reused CIDR devices can be a health risk within a flock. Therefore, alternatives for device disinfections before reuse such as benzalkonium chloride (Vilariño *et al*. 2011) or sterilisation under ultraviolet light (Oliveira *et al*. 2001) have been reported. The autoclaving process was more effective than ultraviolet irradiation when both techniques were compared with achieve sterilisation (Gefrides *et al*. 2010). In cattle, the autoclaving process was reported as a good option when CIDR is reused (Zuluaga and Williams 2008) since no difference was observed in ovarian responses as compared with the use of new inserts (Cerri *et al*. 2009). In goats, our group has already demonstrated that it was possible to induce oestrus after the reuse of autoclaved devices during the anestrous season associated to equine chorionic gonadotropin (eCG), when goats have no effect of progesterone from corpora lutea (Souza *et al*. 2011). Alvarez *et al*. (2013) suggested that the buck effect could effectively substitute eCG administration when previously used CIDR was used to induce oestrus and ovulation in anoestrous goats, even if the period of previous use was as long as 14 days. Later on, the same group demonstrated that autoclaving CIDR previously used for 22 days had no positive effects on oestrus and pregnancy rates when applied as priming for the ram effect during the non-breeding season. The authors highlighted that it would be interesting to test if CIDR previously used for shorter periods of time could be autoclaved to use in breeding animals (Ungerfeld *et al*. 2013).

The progesterone profile of nulliparous versus lactating does receiving reused autoclaved devices was different during the non-breeding season (Souza *et al*. 2011). However, no literature was found regarding the reuse of autoclaved devices for oestrus synchronisation during the breeding season in nulliparous and lactating goats and corresponding progesterone profiles. Considering all this information, our hypothesis was that the synchronised oestrus and fertility rate obtained after the use of a previously used (for 6 or 12 days) autoclaved CIDR is similar to that obtained with new CIDR in goats during the breeding season. Therefore, the aim of the present study was to evaluate the efficacy of reusing autoclaved intravaginal devices on synchronisation of oestrus and ovulation and fertility in nulliparous and lactating Toggenburg goats during the breeding season, as well as to characterise the ovulatory response (percentage of ovulating goats and ovulation rate) and plasma progesterone profile.

Materials and methods

Location and experimental conditions

This research was reviewed and approved by the Animal Care Committee of Fluminense Federal University (UFF/0048–08). The study was conducted during the breeding season in the rural area of Piau, MG, Brazil (21°35′S, 43°15′W). The average altitude was 435 m with Cwa climate, according to the Köppen classification (winters without or with minimum rainfall and summers with high ambient temperatures).

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. 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). Mineralised salt (Salminas Goats, Nutriplan, Juiz de Fora, MG, Brazil) and drinking water were available *ad libitum*.

Oestrus induction treatment and mating

Toggenburg nulliparous (n = 17) or lactating (n = 50) goats 8 months to 8 years of age were selected and allocated according to category, bodyweight (BW) and body condition score (BCS, range 1–5), respectively, into three treatments: CONTROL, USED6 and USED12. Does in the CONTROL group (n = 24; 6 nulliparous and 18 lactating; 48.8 ± 2.3 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 days (USED6, n = 22; 6 nulliparous and 16 lactating; 48.8 ± 2.8 kg, BCS 3.5 ± 0.1) or 12 days (USED12, n = 21; 5 nulliparous and 16 lactating; 47.7 ± 2.4 kg, BCS, 3.4 ± 0.1). Previously used devices (used once 45 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, New York, NY, USA) 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 days.

Oestrus was evaluated once per day while the devices were placed. After device removal, oestrus was monitored with the use of bucks twice a day (0700 hours and 1900 hours) and females were considered to be in oestrus when allowed to be mounted. Does displaying signs of oestrus were mated by fertile bucks at the onset of oestrus and 24 h later if they were still in oestrus. Toggenburg bucks (n = 9) were used for approximately equal numbers of does from each treatment, with a buck : doe ratio about $\leq$1 : 8.

Ultrasonography

Transrectal ovarian ultrasonography was performed in 27 goats (nine 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. The same equipment was used to conduct ultrasonographic pregnancy diagnosis for all 67 goats ~30 days after the last natural mating by ultrasonography.

Plasma progesterone concentrations

Blood samples were collected in 27 goats (same does submitted to ultrasonography) by jugular venipuncture, into tubes containing EDTA at the following times: 7 days before device insertion (Day –7); concurrent with device insertion; (0600 hours; Day 0); 6 h 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 on ice, transported to the laboratory, and centrifuged at 2000*g* for 15 min at 4°C. Plasma was removed and stored at −20°C pending determination of plasma progesterone concentrations with a commercial solid phase radioimmunoassay 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.

Variables and statistical analyses

End points determined were: oestrus response (number of does in oestrus/number of treated does × 100); interval to oestrus (from device removal to first acceptance of mounting); duration of oestrus (interval from the first to last acceptance of mounting); interval from device removal to ovulation; interval from onset of oestrus to ovulation; percentage of ovulating goats (number of does with confirmed ovulation/number of does evaluated by ultrasonography × 100); ovulation rate (units of corpora lutea); largest follicle diameter; pregnancy rate (number of pregnant does/number of does submitted to oestrus induction treatment); and plasma progesterone concentration. Statistical analysis was performed using all tests with $P < 0.05$ considered significant. Parametric variables (interval to oestrus, duration of oestrus, interval from device removal to ovulation, interval from onset of oestrus to ovulation, largest follicle diameter and plasma progesterone concentration) were submitted to one-way ANOVA and compared by SNK test by the SAEG program (System for Statistical Analysis). Non-parametric variables (oestrus response, ovulation rate, percentage of ovulating goats and pregnancy rate) were analysed by the use of the Chi-square test. The results are described as mean ± s.e.

Results

Oestrus percentage and ultrasonography end points

No doe lost any device nor showed oestrus while intravaginal devices were *in situ*. There was no difference ($P > 0.05$) among groups CONTROL, USED6-treated or USED12-treated goats for: oestrus response [75% (18/24), 77% (17/22) or 71% (15/21), respectively], interval to oestrus (39.3 ± 3.2, 32.7 ± 2.7 or 40.8 ± 5.3 h), duration of oestrus (30.7 ± 3.4, 31.8 ± 1.7 or 32.8 ± 3.4 h), interval from onset of oestrus to ovulation (38.0 ± 4.8, 34.3 ± 4.8 or 38.4 ± 4.5 h), interval from device removal to ovulation (78.0 ± 5.1, 72.0 ± 5.2 or 84.0 ± 6.6 h), percentage of ovulating goats [67% (6/9), 78% (7/9) or 56% (5/9)], ovulation rate (1.3 ± 0.2, 1.4 ± 0.2 or 1.8 ± 0.4 units of corpora lutea), largest follicle diameter (6.3 ± 0.1, 6.9 ± 0.1 or 7.1 ± 0.3 mm) and pregnancy rate [54% (13/24), 50% (11/22) or 48% (10/21)]. One goat from USED12 presented triple ovulation. Ovulation was detected before the end of oestrus in 33% of goats (6/18) whereas 67% (12/18) of goats ovulated after oestrus, i.e. in metaestrus.

There was no difference ($P > 0.05$) between nulliparous and lactating goats on the following end points, respectively: interval to oestrus (41.6 ± 3.9; 35.7 ± 2.8 h), duration of oestrus (30.9 ± 3.7; 32.0 ± 2.1 h), percentage of ovulating goats [83% (10/12); 53% (8/15)], ovulation rate (1.4 ± 0.2; 1.6 ± 0.3 units of corpora lutea), interval from device removal to ovulation (76.8 ± 4.5; 78.0 ± 5.1 h), interval from oestrus to ovulation (37.2 ± 4.2; 36.0 ± 3.2 h), largest follicle diameter (6.7 ± 0.2; 6.9 ± 0.2 mm) and pregnancy rate [53% (9/17); 50% (25/50)].

Plasma progesterone concentration

From the 27 goats, 23 (85.2%) had progesterone ≥1 ng/mL on Day –7 and/or Day 0, indicating that goats were cyclic. The other four goats had an increase in progesterone after devices insertion. No significant differences were observed among treatments (Fig. 1). Concentrations superior to 1 ng/mL at the moment of device removal were detected in all females. On the following days (Day 7 to Day 9), progesterone decreased to subluteal concentrations in 17 goats whereas 10 goats remained with high concentrations (Fig. 2). The average progesterone value considering the period that devices were inserted (Day 0.25 to Day 6) was 8.5 ± 0.7 (CONTROL), 6.0 ± 0.4 (USED6-treated), 7.2 ± 0.5 ng/mL (USED12-treated) and regardless treatment was 7.2 ± 0.3 ng/mL. There was no category effect ($P > 0.05$) between nulliparous or lactating does for progesterone concentrations (ng/mL), respectively:

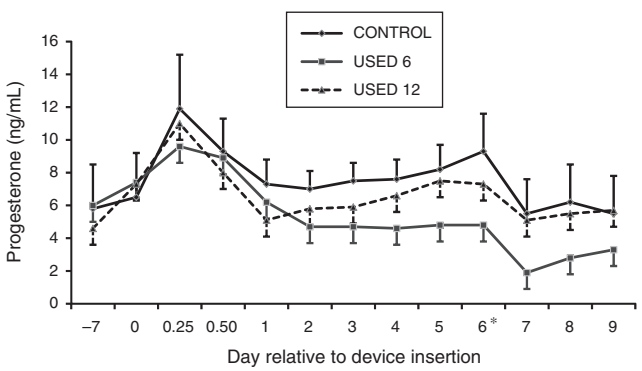

Fig. 1. Plasma progesterone concentrations in Toggenburg goats submitted to oestrus synchronisation (breeding season) receiving new CIDR (CONTROL), autoclaved CIDR previously used for 6 days (USED6) or 12 days (USED12) ($P > 0.05$). *Moment of device removal.

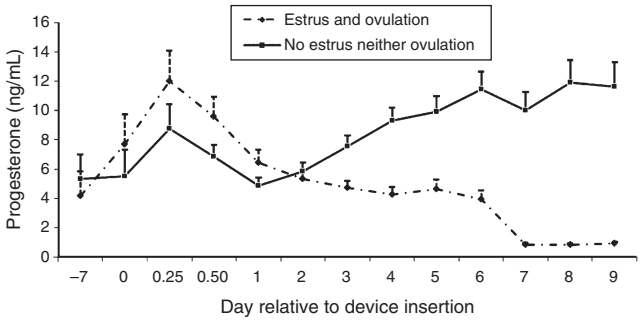

Fig. 2. Plasma progesterone concentrations in Toggenburg goats submitted to oestrus synchronisation (breeding season). The dotted line represents 17 does that demonstrated oestrus and ovulated. The continuous line characterises the group of 10 goats that remained with progesterone elevated after Day 7 and did not show oestrus or ovulation.

Day 0 (6.8 ± 2.5 and 7.6 ± 1.8 ng/mL), Day 0.25 (13.1 ± 3.0 and 9.3 ± 1.7), Day 0.5 (10.8 ± 1.8 and 7.0 ± 0.8), Day 1 (7.4 ± 1.2 and 5.1 ± 0.5), Day 2 (6.2 ± 0.7 and 5.3 ± 0.6), Day 3 (5.3 ± 0.4 and 6.1 ± 0.7), Day 4 (5.1 ± 0.7 and 6.7 ± 0.9), Day 5 (5.4 ± 0.8 and 7.2 ± 1.0) or Day 6 (5.0 ± 1.1 and 7.6 ± 1.2).

Discussion

All sexual behavioural end points reported in the present study are in agreement with other reports in literature (Oliveira *et al*. 2001; Romano 2004; Guido *et al*. 2008; Zhao *et al*. 2010; Souza *et al*. 2011; Fonseca *et al*. 2012). The pregnancy rate achieved in the present study was not different between all treatments, varying from 48% to 54%. This is similar in comparison to other reports when does received new (53%) or reused CIDR (47%) (Guido *et al*. 2008). Our pregnancy rate was lower than 65–100% reported in Saanen goats receiving reused intravaginal devices (Oliveira *et al*. 2001). Considering the studies which have autoclaved the devices during the non-breeding season, our pregnancy rate was slightly lower than 69% (Alvarez *et al*. 2013) and 58–67% (Souza *et al*. 2011); but higher than 16% for sheep receiving CIDR previously used for 22 days (Ungerfeld *et al*. 2013). Thus, our data suggest that autoclaved devices are able to maintain progesterone above concentrations necessary to synchronise cyclic does. According to the literature, the present study is the first report in cyclic goats treated with reused CIDR after undergoing sterilisation by autoclaving. The autoclaving process may be the best option when reusing CIDR devices for oestrus synchronisation since it considerably reduces the risk of disease transmission (Gefrides *et al*. 2010) and also results in greater blood progesterone concentrations when compared with a disinfectant solution (Zuluaga and Williams 2008).

Oestrus response rate was on average 75% (50/67), considerably lower than the 95% we reported earlier with the same breed, treatments and location, but during the non-breeding season (Souza *et al*. 2011). Conversely, it was previously shown that the oestrus synchronisation treatment was more effective during the breeding than in non-breeding season in indigenous does (Zhao *et al*. 2010). It is important to highlight that these authors did not use gonadotropin such as eCG during the breeding season (Zhao *et al*. 2010), contrarily to the present study. In sheep, researchers concluded that the treatment with eCG in the breeding season had no advantage in association with long-term treatment and had a deleterious effect in combination with short-term treatment (6 days like in the present study) (Viñoles *et al*. 2001). The protocol without eCG synchronised a greater number of females up to 96 h after sponge removal and the number of ewes in oestrus after receiving or not eCG was similar just after 144 h (Viñoles *et al*. 2001). Likewise, Oliveira *et al*. (2001) concluded that was not always necessary to include eCG in oestrus induction and synchronisation protocols for cyclic dairy goats.

Corpora lutea can be responsive on goats from Day 3 of oestrous cycle to the day of natural luteolysis (Fonseca *et al*. 2012) and an active corpus luteum at the time of prostaglandin 2α (PGF2α) administration is the key point to determine the success of oestrus synchronisation (reviewed by Abecia *et al*. 2012). Animals with progesterone lower than 1 ng/mL at PGF2α administration could have been in oestrus 2–3 days earlier, or even entering in oestrus at that moment. Higher number of goats ovulating is expected when treating does in early luteal phase (Days 3–7 of oestrous cycle), but animals in very early luteal phase (Days 0–3 of oestrous cycle) would fail responding to PGF2α (Fernandez-Moro *et al*. 2008; Abecia *et al*. 2012; Fonseca *et al*. 2012). We administered PGF2α on Day 0, however, perhaps this moment was too early and if PGF2α was administered few days after CIDR insertion, it could have promoted higher luteolysis and a higher synchronisation rate would be achieved during the breeding season. This could be the reason of the relatively low pregnancy rate obtained in the present study.

In the present study 85% of goats were cyclic, as indicated by the progesterone analysis in at least one of two samples collected within a 7-day interval. After the insertion, similar concentrations were detected between the CONTROL treatment when compared with the others – USED6-treated and USED12-treated does. Conversely, in cattle, the insertion of reused autoclaved devices appears to markedly increase serum concentrations of progesterone during the first 8 h after insertion compared with a non-autoclaved, new CIDR (Zuluaga and Williams 2008). Recently, Alvarez *et al*. (2013) reported no differences in progesterone concentrations between new and autoclaved CIDR, also in goats.

No significant difference was identified in the progesterone profile in nulliparous compared with lactating goats, different from what we observed before in the non-breeding season (Souza *et al*. 2011). It is noteworthy that supraluteal concentrations were maintained up to device removal in all goats. Therefore, we infer that the autoclaving process did not adversely affect progesterone availability.

After reaching maximum plasma levels at 24 h after device insertion, progesterone concentration gradually decreases (Souza *et al*. 2011; Vilariño *et al*. 2011; Alvarez *et al*. 2013). However, perhaps due the influence of endogenous corpora lutea, we observe in Fig. 1 that this typical pattern did not occur due to the 10 goats that remained with elevated progesterone (Fig. 2). The average progesterone value considering the period that devices were inserted (Day 0.25 to Day 6) was 8.5 (CONTROL), 6.0 (USED6-treated) and 7.2 ng/mL (USED12-treated). It is interesting to note that this total is considerable greater than that reported during the non-breeding season for CONTROL (4.3), USED6-treated (2.9) or USED12-treated

(3.0 ng/mL) (Souza *et al*. 2011). Similarly, mean progesterone concentrations were 3.3 (new CIDR) and 2.6 (used CIDR) in females in deep anoestrus (Güngör *et al*. 2009). These authors mentioned that higher concentrations (4.1 ng/mL) were obtained when the animals were in mid-anoestrus. Therefore, it is reasonable to assume that the season plays an important role and the possible interactions with endogenous progesterone strong influence its concentration in plasma.

Conclusions

Autoclaved, intravaginal progesterone devices that were previously used for either 6 or 12 days were able to maintain progesterone above concentrations necessary to synchronise oestrus and ovulation in cyclic does. It seems evident that CIDR devices are able to release concentrations of progesterone for a longer time than that recommended for oestrus synchronisation. A new approach, which claims the use of 'biostimulation' in place of exogenous hormones and drugs to control and improve the productivity of goats, might be useful to consider in the future. In order to begin to move towards ethical practices, the reuse of autoclaved progesterone devices up to three times instead of the regular one could be a reality providing minimal health risk. This approach also decreases the cost of oestrus synchronisation programs and makes such programs more economically feasible for producers.

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