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Response of ewes primed with new CIDRs, previously used CIDRs, or previously used and autoclaved CIDRs to the ram effect during the non-breeding season

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Abstract

The ovulation induced in anestrous ewes by the sudden introduction of males is not accompanied by estrous behavior unless ewes are primed with progestagens. Although CIDR devices (which contain 0.3 g of progesterone; Pfizer, Auckland, New Zealand) may be used for more than one treatment, conception rates decrease when CIDRs previously used for long times are used again. In cows, if previously used CIDRs are autoclaved, the initial rises in plasma progesterone concentrations are not different to those obtained with a new CIDR. Our objective was to compare the estrous and pregnancy rates of ewes induced to ovulate with the ram effect during the non-breeding season to ewes primed with a new CIDR, a previously used CIDR, or a previously used autoclaved CIDR. Ninety-two ewes were randomly allocated to three groups and received a new CIDR (group NC, n = 30), a previously used CIDR (UC, n = 30), or a used-autoclaved CIDR (UAC, n = 32) for 8 days. Used CIDRs were used for 22 days; the CIDRs used in UAC ewes were previously autoclaved. At CIDR withdrawal ewes were joined with adult rams and 20 additional ewes with induced estrous (1 ram:13 ewes). Marked ewes were detected every 24 h for 4 days. Forty days after estrus, pregnancy was determined by transrectal ultrasound. Blood samples were collected from 8 ewes from each group 5 and 6 days after CIDR insertion, and at the moment of CIDR withdrawal, and progesterone was measured by radioimmunoassay. Estrous and pregnancy rates were greater in NC than in UC and UAC ewes (estrous: 56.7 *vs*. 26.7 and 15.6%; pregnancy rate: 50.0 *vs*. 13.3 and 15.6% for NC, UC, and UAC ewes, respectively; $P < 0.05$). Progesterone concentration was greater ($P < 0.001$) in NC than in UC and UAC ewes. No difference was found between UC and UAC groups for any parameter. Overall, we concluded that autoclaving the CIDRs previously used for 22 days had no positive effects on estrous and pregnancy rates when applied as primings for the ram effect during the non-breeding season. This may be explained by the fact that progesterone concentrations in UC and UAC ewes were below luteal levels during the last days of the treatment.

Keywords: estrus, pregnancy rate, progesterone, sheep.

Introduction

In anestrous ewes, the introduction of males triggers an increase in the frequency of luteinizing hormone (LH) pulsatility (see reviews: Martin *et al*., 1986; Delgadillo *et al*., 2009). This increase stimulates follicular development and is followed by a pre-ovulatory surge of LH (Martin *et al*., 1986). Consequently, a high percentage of females exposed to rams ovulate within the first 3-4 days after the stimulus (Martin *et al*., 1986). However, this ovulation is not accompanied by estrous behavior unless ewes are primed with progestagens (Hunter *et al*., 1971). During the non-breeding season, progestagen priming may be used for short periods (6 days), followed by the administration of equine chorionic gonadotrophin (eCG; Ungerfeld and Rubianes, 1999, 2002) or the introduction of rams (Ungerfeld *et al*., 2003). As the response in terms of estrous and pregnancy rates is not affected, shortening the treatments is an important advantage that gives flexibility when working under field conditions.

CIDR devices (controlled internal drug release, Pfizer, Auckland, New Zealand), which contain 0.3 g of progesterone, may be used for more than one treatment (ewes: Ungerfeld, 2009; Vilariño *et al*., 2013; cows: Colazo *et al*., 2004; goats: Oliveira *et al*., 2001; Vilariño *et al*., 2011). Thus, environmental contamination decreases because of the lower amount of hormone remaining in the device and costs decrease as each device is used more than once. However, it has been reported that conception rates decrease according to the duration of previous use (Smith *et al*., 1991; Ungerfeld and Rubianes, 1999). In ewes, we determined that pregnancy rate decreases with the use of CIDRs previously used at least for 11 days compared to new CIDRs (Ungerfeld and Rubianes, 1999). It also decreases when CIDRs were previously used for 18 days compared to those used for 12 days (Ungerfeld, 2009), but only tended to decrease if CIDRs were used for 12 days (Vilariño *et al*., 2013). This decrease in pregnancy rate may relate to the dominant follicle lifespan prolongation,

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provoked by low progesterone concentrations during treatment (Rubianes *et al*., 1996). Viñoles *et al*. (2001) showed that traditional progestagen treatments (12-14 days) are associated with the ovulation of aged follicles and a decrease in subsequent fertility. To overcome the possible negative effects of low progesterone concentrations, Zuluaga and Williams (2008) introduced the interesting possibility of reusing previously used CIDRs in cows. In effect, they determined that if previously used CIDRs are autoclaved, the rise in plasma progesterone concentrations is not different to that obtained with a new CIDR and greater than those obtained with a non-autoclaved previously used CIDR. On the other hand, progesterone concentration was lower in goats treated with CIDRs previously used for 6 or 12 days and autoclaved than when using new CIDRs during both the breeding (Souza, 2010) and non-breeding (Souza *et al*., 2011) seasons. However, that decrease was not enough to affect reproductive response. Therefore, considering our previous results in ewes (Ungerfeld, 2009) and the lack of a negative effect observed in goats with new CIDRs and CIDRs previously used for 14 days (Alvarez *et al*., 2013), it would be interesting to test if CIDRs previously used for longer times can be reused after autoclaving them to prime ewes exposed to the ram effect. Thus, for this experiment we decided to use CIDRs previously used for 22 days.

Considering all this information, our hypothesis was that the pregnancy rate obtained after the use of used autoclaved CIDRs is greater than that obtained after the application of used non-autoclaved CIDRs in ewes induced to ovulate with the ram effect during the non-breeding season. Therefore, our objective was to compare the estrous and pregnancy rates of ewes induced to ovulate with the ram effect during the non-breeding season primed with a new CIDR, a previously used CIDR, or a previously used autoclaved CIDR.

Materials and Methods

Animal management

The experiment was performed on a commercial farm located in Colonia, Uruguay (35º S) during the non-breeding season (October to December; natural light/dark ratio = 14L:10D). Overall, 92 Corriedale x Milchschaf multiparous ewes with a mean body condition score of 2.9 ± 0.1 (range: 0-5) were used. Ewes grazed together on native and improved pastures, and water was available ad libitum. Ewes were isolated (>1000 m; sight, sound, smell) from rams for 40 days before starting the experiment.

Experimental treatments

The experimental ewes were randomly allocated to three groups according to the CIDR type inserted on day -8 (day 0 = CIDR withdrawal and introduction of the rams). Ewes in group NC (n = 30) received a new CIDR (0.3 g of progesterone; Easy Breed CIDR, Pfizer, Auckland, New Zealand); group UC received a previously used CIDR (n = 30), and ewes in group UAC (n = 32) received a used-autoclaved CIDR. Used CIDRs were previously used two times (10 and 12 days, respectively; total = 22 days) as part of other experiments performed on the same farm. The 32 CIDRs used in UAC ewes were sterilized by autoclaving them at 121°C and 724 mmHg for 20 min (Zuluaga and Williams, 2008) one week before reusing them.

CIDRs remained in place for 8 days in all ewes. At CIDR withdrawal, ewes from all groups were joined in the same paddock with seven sexually experienced adult Corriedale rams fitted with marking harnesses. It has been reported that the maximum reproductive response in anestrous Corriedale ewes submitted to the ram effect is expressed when ewes in estrus are introduced in addition to the rams (Rodríguez Iglesias *et al*., 1991). Therefore, 20 additional ewes were brought into estrus between day 0 and 2 by an im injection of 350 IU eCG (Novormón, Syntex, Buenos Aires, Argentina) after a 6-day CIDR priming period. Marked ewes were detected every 24 h for a period of 4 days. Forty days after estrus, pregnancy was determined by transrectal ultrasound in all ewes.

Progesterone measurement

In order to determine progesterone concentrations during the last days of CIDR treatment, blood samples were collected from the jugular vein of eight ewes from each group. Progesterone was measured the days in which was directly related to the characteristics of the follicle that ovulates: 5 and 6 days after the insertion of the CIDRs and at the time of CIDRs withdrawal (day 0). Samples were allowed to clot for 30 min, centrifuged, and serum was frozen at -20°C. Progesterone concentration was determined in the Laboratorio de Técnicas Nucleares (Facultad de Veterinaria, Montevideo, Uruguay) using a solid-phase RIA (Siemens, Los Angeles, CA, USA). The detection limit of the assay was 0.1 ng/l. The intra and inter-assay coeficients of variation were 9 and 12%, respectively.

Statistical analysis

The percentage of ewes in estrus and pregnancy rates were compared by Chi-square test. Progesterone concentrations were compared using repeated measures ANOVA.

Results

Estrous and pregnancy rates were higher in NC than in UC and UAC groups (Table 1; $P < 0.05$). No

difference was found between UC and UAC groups.

Progesterone concentration (Fig. 1) was greater in NC than in UC and UAC groups ($P < 0.001$). No difference was found between UC and UAC groups.

Table 1. Estrous and pregnancy response of ewes exposed to the ram effect during the non-breeding season. Ewes were primed for 8 days with new CIDRs (NC), 22-day used CIDRs (UC), or 22-day used and autoclaved CIDRs (UAC).

	Estrous ewes (%)	Pregnancy rate (%)
NC	17/30 (56.7)[a]	15/30 (50.0)[a]
UC	8/30 (26.7)[b]	4/30 (13.3)[b]
UAC	5/32 (15.6)[b]	5/32 (15.6)[b]

Different letters within the same column indicate statistically significant differences ($P < 0.05$).

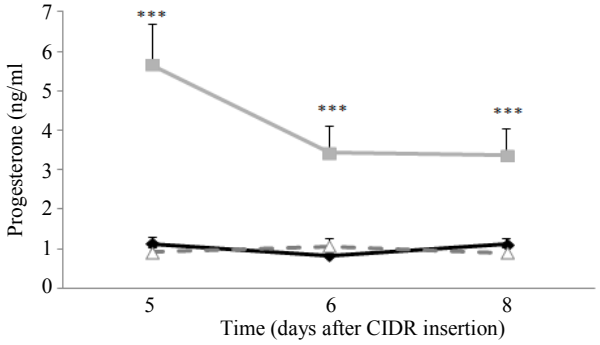

Figure 1. Progesterone concentration (mean ± SEM) in ewes exposed to the ram effect during the non-breeding season. Ewes were primed for 8 days with new (– ◆–), 22-day used CIDRs (--●--), or 22-day used and autoclaved CIDRs (–▲–). ***$P < 0.001$.

Discussion

Under the conditions in which this experiment was performed, our hypothesis was not accepted. The percentage of females that came into estrus and pregnancy rates were significantly greater in NC than in UC and UAC groups, with no difference between UC and UAC groups. Although we expected that autoclaving CIDRs would generate a greater initial rise in progesterone, and thus promote greater pregnancy rates, autoclaving was not enough to redistribute the remaining progesterone content of the CIDRs, and therefore overcome the negative effect of a previous 22 day use. An alternative, complementary explanation is that the progesterone content remaining in the CIDRs was not enough to obtain greater concentrations, and thus, could not be increased by autoclaving the CIDRs. In effect, progesterone concentrations generated by used autoclaved CIDRs were almost the same as those generated by used non-autoclaved CIDRs. In both groups, mean progesterone concentrations remained low, at least for the last days before CIDR withdrawal. Although autoclaving CIDRs is effective in eliminating pathogens before reuse (Souza *et al*., 2011), our results differed from our hypothesis, probably due to the greater depression of progesterone content caused by the previous longer use of the CIDRs. As using the CIDRS for longer periods of time would deplete more progesterone residues, the lack of positive results is a limitation to minimizing the negative problems caused by devices of this type.

While in most previous experiments the main affected parameter by the use of previously used CIDRs was pregnancy rate (Smith *et al*., 1991; Ungerfeld and Rubianes, 1999), in this study, estrous incidence was also affected. This finding agrees with observations from Ungerfeld (2009), who reported that fewer ewes came into estrus after using CIDRs previously used for 18 days compared to CIDRs previously used for 12 days. On the other hand, estrous incidence was not affected when CIDRs previously used for 12 (Vilariño *et al*., 2013), 13 (Uribe *et al*., 2011), or 14 days (Bazzan *et al*., 2011) were applied, suggesting that longer periods of previous use of CIDRs are needed to affect estrous incidence when responding to the ram effect. However, it should be considered that in both experiments (Ungerfeld, 2009, and present), ewes were induced to ovulate with the ram effect, and ovulation was not determined in any of them. Therefore, considering that the first ovulation induced with the ram effect is a silent ovulation unless progesterone primings are applied (Hunter *et al*., 1971), it is possible that more ewes in groups UC and UAC than NC had ovulated without estrous signs.

As it may be expected with the use of previously used CIDRs, low serum progesterone concentrations were observed, and thus, pregnancy was negatively affected. It has been previously reported that the use of previously used CIDRs decreases the pregnancy rates of cyclic ewes (Smith *et al*., 1991), hormonally treated anestrous ewes (Ungerfeld and Rubianes, 1999), and ram-induced ewes (Ungerfeld, 2009). In the last case, treatment with estradiol-17β could not overcome the negative effect of low progesterone levels. The current results expand previous information obtained in anestrous ewes treated with short-term progestagen primings and the ram-effect.

Overall, we conclude that autoclaving CIDRs previously used for 22 days had no positive effects on estrous and pregnancy rates when applied as priming for the ram effect during the non-breeding season. Despite autoclaving, progesterone concentrations were below luteal levels in UC and UAC ewes during the last days of treatment. Considering these results, it remains to be tested if CIDRs previously used for shorter periods of time may be recycled by autoclaving to use them for breeding animals.

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