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Short Communication

Endogenous Progesterone Concentrations Affect Progesterone Release from Intravaginal Devices Used for Oestrous Synchronization in Cattle

HL Neri[1,2], MP Palhao[1], DS Costa[3], JHM Viana[1,4] and CAC Fernandes[1]

[1]*University of Alfenas, Alfenas, MG, Brazil;* [2]*Hertape-Calier, Juatuba, MG, Brazil;* [3]*Federal University of Mato Grosso do Sul, MS, Brazil;* [4]*Embrapa, Juiz de Fora, MG, Brazil*

Contents
Intravaginal progesterone-releasing devices are largely used both as contraceptives in humans and as a component of oestrous synchronization protocols in cattle. To reduce costs in large-scale timed artificial insemination, the reuse of these releasing devices is common. Passive hormone diffusion, however, depends on the concentration gradient, which could affect the amount of residual progesterone present in these devices after a first use. To evaluate the effect of the presence of a corpus luteum in the release of progesterone from intravaginal devices, three synchronization protocols were designed to simulate the effects of inserting the device in the early dioestrus, late dioestrus or anoestrus. Holstein-Zebu cross-bred heifers were randomly allocated into one of these three treatments, and a series of blood samples was taken to evaluate the plasma progesterone concentrations. After 8 days, the intravaginal devices were removed and underwent a previously validated alcoholic extraction technique to measure the residual progesterone. Non-used devices were used as controls. As expected, the simultaneous presence of the intravaginal device and a corpus luteum resulted in increased plasma progesterone concentrations. Conversely, the amount of residual progesterone in the devices after use was inversely proportional to the plasma progesterone concentration. These results demonstrate that the release rate of progesterone from intravaginal devices is affected by the endogenous concentration of this hormone; consequently, the strategy for reuse should account for the category and expected luteal cyclic activity of the animals undergoing synchronization protocols.

Introduction

Oestrous detection in cattle remains a challenge, particularly in high-producing or large herds (Roelofs et al. 2010). Timed artificial insemination (TAI) protocols were initially developed to overcome the low efficiency of visual oestrous detection (Pursley et al. 1995). Subsequently, the inclusion of exogenous progesterone (P4) in the protocols showed that they could also be used to induce ovulation in anoestrous cattle, increasing the range of animals suitable to be included in TAI programmes (Lamb et al. 2010).

The use of intravaginal progesterone-releasing devices (IPRD) avoids multiple P4 injections in the TAI protocols. These devices are usually made of porous silicon polymers impregnated with P4, which is released by passive diffusion when in contact with the vaginal mucosa, similar to the vaginal rings used as contraceptives in humans (Malcolm et al. 2003). To reduce the costs of synchronization protocols, such devices can be reused (van Cleeff et al. 1992; Zuluaga and Williams 2008).

In most TAI programmes, progesterone-based oestrous synchronization protocols are used in cows and heifers at random phases of the oestrous cycle or during anoestrus. This means that IPRD are used under unknown endogenous plasma P4 concentrations. Differences in the gradient of P4 could affect the release rate from the devices. Most studies on the reuse of such devices, however, have focused on the subsequent differences in ovulation and pregnancy rates, not on the pharmacokinetics background. Our hypothesis is that differences in P4 concentration gradient related to the presence of a functional CL would affect P4 release from intravaginal devices. Therefore, the aim of this study was to evaluate the amount of residual progesterone remaining in a new IPRD after it is used in heifers having different endogenous concentrations of progesterone.

Material and Methods

Reproductively sound nulliparous Holstein-Zebu cross-bred heifers (24–30 months and 320–380 kg body weight; N = 30) were used in this experiment. They were selected based on the presence of a corpus luteum (CL) as confirmed by ovarian ultrasonographic evaluation. The animals were randomly allocated into one of three pre-treatment protocols, designed to simulate the effects of inserting the IPRD in early dioestrus (G1, n = 10), late dioestrus (G2, n = 10) or anoestrus (G3, n = 10). To achieve this, in G1 and G2, a pre-treatment was used to synchronize ovulation and determine the presence of a CL: insertion of a 3 mg norgestomet auricular implant (Crestar, MSD Saúde Animal, São Paulo, SP, Brazil) and injection of 2 mg estradiol benzoate (Benzoato HC, Hertape-Calier, Juatuba, MG, Brazil) on day -18 (in reference to the moment of insertion of the IPRD, day 0); removal

of auricular implants and injection of 0.15 mg D-cloprostenol (Veteglan, Hertape-Calier) and 25 IU FSH (Pluset, Hertape-Calier) on day -10; and injection of 1 mg estradiol benzoate (Benzoato HC) on day -9. Ovulations were expected at day -7. Only in G2, an injection of 0.15 mg D-cloprostenol (Veteglan, Hertape-Calier) was given on day 3. In G3, an alternative pre-treatment was used to avoid the presence of CL and consequently ensure low endogenous P4: insertion of a norgestomet auricular implant (Crestar, MSD Saúde Animal) and injection of 2 mg estradiol benzoate (Benzoato HC) on day -9; injection of 0.15 mg D-cloprostenol (Veteglan) on days -4, -2 and 0; and removal of auricular implants on day 0 (Fig. S1).

All groups received then the same treatment: a 2 mg estradiol benzoate (Benzoato HC, Hertape-Calier, Juatuba, MG, Brazil) injection on day 0, and a new (non-used) IPRD (Primer, Agener União, SP, Brazil) was inserted on day 0 and removed on day 8. At the end of the treatments (day 8), three IPRD from each group were washed in water to remove the residues (mucus, cell debris), disinfected in 10% quaternary ammonia and stored under refrigeration (2–8°C) until P4 extraction.

Blood samples were collected by venipuncture of the coccygeal vessels at days 0, 3, 5 and 8. Samples were immediately stored at 4°C, and in less than 6 h, they were centrifuged at 600 × ***g*** for 10 min. The plasma was then transferred to 1.5-ml tubes and stored at −20°C until P4 analysis. An alcoholic P4 extraction technique was developed and validated for the evaluation of the residual P4 in the IPRD used in groups G1, G2 and G3. Briefly, the silicon was removed from each IPRD, individually weighed (original weight, OW) and mechanically triturated. Two samples of approximately 1 g were then collected from each IPRD and weighed (sample weight, SW). Each silicon sample was transferred to a glass tube, which was filled with methanol alcohol up to a volume of 10 ml (10 × dilution), and locked with a rubber tap. The samples were gently vortexed every 30 min. After 4 h, 1 ml of the supernatant was pipetted and transferred to another glass tube, which were kept opened in incubator at 38°C for 24 h for alcohol evaporation. Progesterone was then washed from the tube wall by vortexing with 1 ml of male serum (previously tested for the absence of measurable amounts of P4) for 1 min. Aliquots of 0.01 ml of serum were serially diluted in saline (NaCl 0.9%) until a final dilution of 10^6 of the initial volume of the silicon samples, which was used for P4 analysis by radioimmunoassay (RIA). The efficiency of the extraction technique was evaluated using new (non-used) IPRD, with a known amount of P4 (1 g), in six replicates. The extraction efficiency was calculated as follows: $Efficiency = ([P4]/\{[(SW/OW)*10^9]/10^6\})*100$, considering: $[P4]$ = progesterone concentration (ng/ml); SW = sample weight (g); and OW = original weight of the silicon from each IPRD (g). The new (non-used) IPRD had 20.6 ± 0.3 g of silicon, from which samples of 1.03 ± 0.02 g (5.0 ± 0.1% of total) were collected. The mean extraction efficiency was 61.7 ± 3.2% (56.5–64.5%), with a variation coefficient of 5.1%. There was no difference ($p > 0.05$) in extraction efficiency among replicates. The average efficiency value of this control was used to determine the P4 concentrations in the samples from the IPRD used in the experiment.

The concentrations of P4 in the plasma and in the IPRD samples were determined using a solid-phase ^{125}I radioimmunoassay (RIA IM 1188, Immunotech Inc., Prague, Czech Republic) at the Genese RIA Laboratory (São Paulo, SP, Brazil). The sensitivity was 0.05 ng/ml, and the interassay and intra-assay CVs were 2.78% and 1.38%, respectively.

The data for the concentrations of P4 in the plasma and in the IPRD samples were examined for normality using the Shapiro–Wilk test and then evaluated by ANOVA. The differences among the groups were assessed by Tukey's *post hoc* test using the GLM procedure of the SAS software (SAS System for Information Delivery, v. 8.02, SAS Institute Inc., Cary, NC, USA) for the IPRD samples and the repeated-measures MIXED procedure for the plasma samples. The results are presented as the means ± SD. Statistical significance was considered at a level of $p < 0.05$.

Results

The plasma P4 concentration during treatment is summarized in Table 1. At day 0, the plasma P4 was similar between G1 and G2 ($p > 0.05$), and both concentrations were higher than that of G3 ($p < 0.05$). After the induction of luteolysis at day 3, the P4 concentration of G2 became lower ($p < 0.05$) than in G1.

The results of the analysis of the residual P4 in the IPRD (corrected for the efficiency of the extraction method, 61.7%) are shown in Table 2. The residual P4 was different ($p < 0.05$) among treatments and between the treatments and the control. The residual P4 in G3 corresponded to only 53.7% of the amount of the residual P4 in G1 and 25.7% of the P4 of a new IPRD.

Table 1. Plasma progesterone concentration (ng/ml) in cross-bred heifers treated with synchronization protocols designed to simulate the effect of inserting an intravaginal progesterone-releasing device (1 g progesterone) during early dioestrus (G1), late dioestrus (G2) or anoestrus (G3). Samples were collected on days 0 through 8 after the insertion of the progesterone devices

Treatment	N	D0	D3	D5	D8
G1	10	5.1 ± 2.3[a]	6.4 ± 2.2[a]	3.9 ± 1.1[a]	4.7 ± 0.9[a]
G2	10	5.3 ± 1.2[a]	6.3 ± 1.4[a]	2.2 ± 0.5[b]	1.6 ± 0.5[b]
G3	10	0.3 ± 0.2[b]	3.3 ± 0.7[b]	1.6 ± 0.4[c]	1.5 ± 0.6[b]

[a,b,c]Within a column, values with a common superscript did not differ ($p > 0.05$).

Table 2. Residual progesterone extracted from intravaginal progesterone-releasing devices used in synchronization protocols designed to simulate the effect of inserting the devices during early dioestrus (G1), late dioestrus (G2), or anoestrus (G3). Relative (concentration in the extract in ng/ml) and absolute (mg) values are compared with the results of new (non-used) devices used as control

Treatment	Residual Progesterone Relative (ng/ml)	Residual Progesterone Absolute (mg)
Control	30.8 ± 1.1[a]	1000 ± 102[a]
G1	14.7 ± 1.7[b]	478 ± 78[b]
G2	11.0 ± 1.2[c]	349 ± 61[c]
G3	7.9 ± 1.2[d]	257 ± 46[d]

[a,b,c,d]Within a column, values with a common superscript did not differ ($p > 0.05$).

Discussion

This study was designed to evaluate the residual P4 present in the IPRD that are used in heifers and were previously treated to simulate early dioestrus, late dioestrus or anoestrus. The plasma P4 concentrations during the experiment demonstrate that these pre-treatment and synchronization protocols were effective in achieving the expected reproductive status. In G1, the simultaneous presence of a CL and the IPRD resulted in high P4 concentrations throughout the experiment, as expected for early dioestrus, whereas in G3, the IPRD was the only source of P4, as expected for anoestrus. In G2, the P4 concentrations were similar to G1 until the induction of luteolysis at day 3, and they subsequently become similar to those observed in G3 at day 8.

The intravaginal devices from G1, which were used concomitant with the presence of a functional CL during days 0 through 8 of the treatment period, had higher amounts of residual P4 than did the IPRD from treatments in which a functional CL was present for only half of the period (G2) or was not present at all (G3). The IPRD from G1, which was designed to simulate the synchronization protocol beginning in early dioestrus, had almost twice the residual P4 than did those from G3, which were designed to simulate anoestrus. Thus, the P4 release from the IPRD was affected by the endogenous P4 concentrations. In such devices, hormones reach the blood stream by passive transport through a concentration gradient, and diffusion is facilitated by the high permeability of the vaginal mucosa to steroids (Rothen-Weinhold et al. 2000). Therefore, a lower endogenous concentration of P4 indicates that the concentration gradient is higher and, consequently, that the diffusion of the hormone from the device to the blood stream is greater, as observed in the current study.

Previous studies have evaluated the possibility of the reuse of IPRD, with controversial results (Colazo et al. 2004; Meneghetti et al. 2009). The plasma P4 concentrations were shown to be lower in ovariectomized cows that received a previously used IPRD compared with those receiving a new IPRD (van Cleeff et al. 1992). However, to our knowledge, no other study has addressed the effects of ovarian luteal cyclic activity on the P4 withdrawn from the IPRD during its first use. The present results demonstrate that the residual P4 in the IPRD after a single use is inversely proportional to the concentrations of endogenous P4, which has clear implications for synchronization protocols based on the reuse of the IPRD. Moreover, when the IPRD were used in heifers not bearing a CL (G3), residual P4 after the first use represented only approximately 25% of the amount present in the new IPRD. This low residual P4 may be insufficient to ensure the positive effects of this hormone on pre-ovulatory follicle development and post-ovulatory fertility, as previously reported (Pursley and Martins 2012), particularly if the IPRD is reused in animals in anoestrus or with high hepatic P4 metabolism, such as high-producing dairy cows. Moreover, the procedures used to clean (disinfect or sterilize) may also affect the dynamics of progesterone release from intravaginal devices (Zuluaga and Williams 2008). Thus, the interpretation of results of the IPRD reuse in TAI synchronization protocols and the comparison of these results within studies using different animal categories should consider possible differences for the residual P4 in the used IPRD, as demonstrated in the current study.

Conclusion

Intravaginal devices release more progesterone when used in animals with low endogenous concentrations, resulting in less residual progesterone after the first use.

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Conflict of interest

None of the authors has any conflict of interest to declare.

Author contribution

CACF designed and coordinated the study. CACF, HLN, DSC and MPP carried out the experiment. MPP performed the hormonal assay. MPP and JHMV contributed to the data and statistical analysis. JHMV and HLN drafted the manuscript, which was revised by all authors. All authors read and approved the final manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Pre-treatment protocols designed to simulate the effect of inserting the devices during early dioestrus (G1), late dioestrus (G2) or anoestrus (G3).

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Author's address (for correspondence): Carlos AC Fernandes, Department of Veterinary, University of Alfenas. Campus Universitario. Rod. MG 179, Km 0, Alfenas, MG 37130-000, Brazil. E-mail: carlos@biotran.com.br