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Development of an injection molded ethylene-vinyl acetate copolymer (EVA) intravaginal insert for the delivery of progesterone to cattle

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ABSTRACT

The purpose of this study was to develop a new injection-molded intravaginal insert manufactured from ethylene-vinyl acetate containing progesterone for a 7-day insertion period in cattle. The manufacturing process resulted in a reduction in the residual drug compared to the silicone insert available while still maintaining biological performance.

1. Introduction

In the past 40 years, technologies of drug controlled release have found increasing application in the pharmaceutical, agricultural, veterinary and other fields (Mathiowitz, 1999). The intravaginal devices for the controlled release of progesterone have been successfully used in order to synchronize estrous in cattle (Roche, 1975, 1976; Burggraaf et al., 1997; Rathbone et al., 1998a). Commercially available bovine intravaginal inserts consist of a T-shaped or Y-shaped nylon spine coated with an inert matrix, usually silicone rubber, loaded with progesterone evenly dispersed therein (Roche, 1976; Munro, 1987; Macmillan and Peterson, 1993; Winkler et al., 1997; Rathbone et al., 2002). These devices have several advantages, such as ease of placement and removal from the vagina. In addition, the progesterone administration ends when the device is removed, resulting in a sharp fall in plasma concentration of the hormone. However, they have various drawbacks mainly derived from the use of the silicone rubber. This polymer is expensive, not biodegradable and requires burning or burial for disposal after use. Silicone rubber is a thermoset plastic that, once formed, does not permit reprocessing. In addition, although silicone will cure at low temperatures, the extended times needed to achieve this limit the commercial viability of this approach, and therefore high temperatures (as high as 190 °C) are used commercially. Finally, the initial load of progesterone only decreases between 20 and 40% after the hormonal therapy, which is the main disadvantage because progesterone is the most expensive ingredient in the formulation.

This study was to overcome these problems by designing and developing a new intravaginal device. The key

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requirements for this intravaginal device were: ease of manufacture, low manufacturing temperatures, low cost of manufacture, ease of insertion, high retention rate, ease of removal, little or no damage to the vaginal mucosa and the potential for reprocessing formed inserts. A thermoplastic polymer was used as an alternative to silicone rubber. Ethylene-vinyl acetate (EVA) has interesting properties and is already used for the production of inserts in human medicine (Shastri, 2002). This paper describe the experiments conducted to develop and clinically evaluate an injection molded intravaginal insert manufactured from EVA that delivered progesterone for estrous cycle control and synchronization in cows. The new device was compared to the silicone commercially available DIB® insert which is widely used nowadays (Aller et al., 2010; Prada Torres et al., 2013; Núñez-Olivera et al., 2014).

2. Experimental

2.1. Materials

Ethylene-vinyl acetate (ELVAX® 460, 18% vinyl acetate content) and acetal resin (Delrin 900P) were obtained from DuPont, USA, micronized progesterone (USP 30) was supplied by Farmabase, Brasil, DIB® intravaginal inserts were supplied by Syntex, Argentina, D(+)cloprostenol was supplied by OVER, Argentina, and ethanol PA was from Cicarelli, Argentina. Radio-immunoassay (RIA) kits for quantitative determination of progesterone were Coat-A-Count from Siemens Medical Solutions Diagnostics, USA.

2.2. Compounding of progesterone/EVA and manufacture of intravaginal inserts

ELVAX 460 was selected as an alternative to silicone rubber based on the two progesterone transport properties that have the greatest influence on release: solubility and effective diffusion coefficient in the polymer. EVA pellets were impregnated with progesterone using a solvent impregnation method developed in the laboratory. EVA pellets were placed in a suitable organic solvent containing a specified amount of progesterone. This solvent must cause swelling of the pellets and must have a low boiling point. After reaching the maximum swelling the solvent was evaporated and the pellets were placed in an oven for 24 h.

Based on preliminary knowledge of commercially available intravaginal devices, and on advice of veterinarians, the device design was developed. The intravaginal insert comprises an inert Y-shaped acetal resin spine and on its extremes were two EVA flat wings containing progesterone. The inert Y-shaped acetal resin spine (Fig. 1) was designed to ensure vaginal retention, as well as facilitating the insertion and removal of the device. The EVA flat wings (Fig. 2) were designed to ensure a surface area of contact greater than that provided by commercial devices, maintaining a surface area of about 120 cm^2 (Rathbone et al., 1998b). The spine and flat wings were fabricated by injection molding (JM800-C^2, Chen Song Machinery Co., Hong Kong). The injection temperature of EVA flat wings was 170 °C and the injection time was about 1.5 s, allowing progesterone crystals to be uniformly suspended in the EVA matrix.

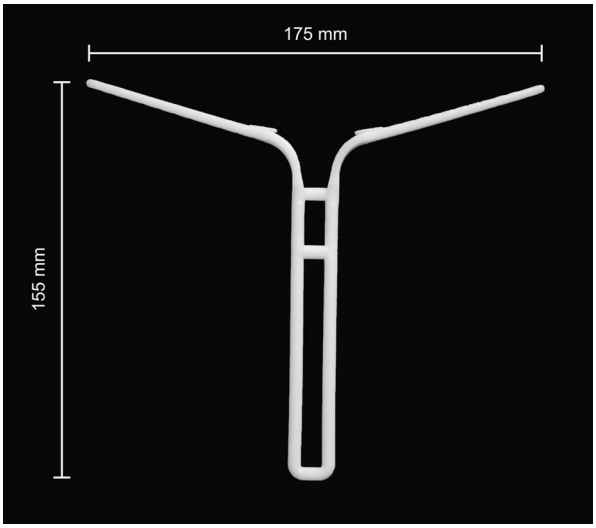

Fig. 1. Inert Y-shaped acetal resin spine.

The EVA insert (Fig. 3) had a surface area of 125.68 cm^2, similar to that of the "traditional" DIB insert (125.65 cm^2). The initial load of progesterone was about 1.0 g.

2.3. Determination of initial and final progesterone load

The initial and final amount of progesterone in an EVA intravaginal insert was determined by cutting it into strips of 5 mm of length, placing the sections into a soxhlet extractor (250 mL) and refluxing for 24 h with ethanol. The ethanolic extract was made up to 250 mL with ethanol. Solutions were then diluted before analysis by UV at 244 nm (UV-2401 PC, Shimadzu).

2.4. In vitro drug release studies (EVA insert vs. DIB insert)

An *in vitro* drug release experiment was performed as described by Bunt et al. (1997) using a dissolution

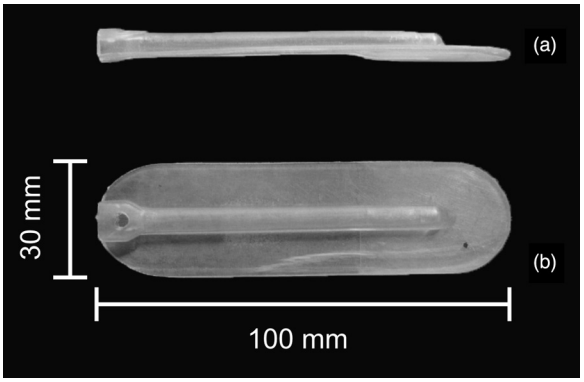

Fig. 2. EVA wing: (a) front view and (b) top view.

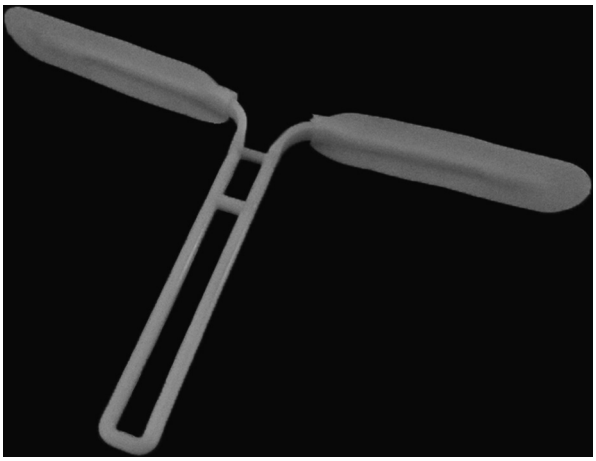

Fig. 3. Designed EVA insert.

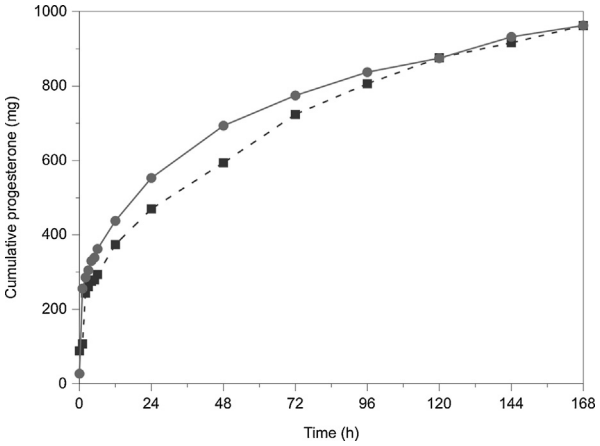

Fig. 4. *In vitro* cumulative release of progesterone obtained from EVA device (●) and DIB insert (■). Values are presented as mean ± SEM of three individual experiments.

instrument (SR8 Plus, Hanson Research). The release media comprised 1000 mL of ethanol:water (60:40) maintained at 37 ± 0.5 °C. The rods were rotated at 100 rpm. Samples (2 mL) were manually collected at predetermined collection times during 7 days. An equal volume of fresh media was added to maintain a constant volume. The concentration of progesterone in the samples was determined by UV spectrophotometry. Data were plotted as a cumulative amount of progesterone released *versus* time.

2.5. In vivo drug release studies (EVA insert vs. DIB insert)

The investigation was conducted in Bradford beef cows ($n = 10$) with a body condition score (BCS) between 2.5 and 3.5, based on a scale from 1 to 5. They were randomly allocated into two groups ($n = 5$): one group received the EVA insert and the other a DIB insert. Before the administration of the insert, ovarian function was monitored using a veterinary ultrasound scanner (AQUILA, Pie Medical, the Netherlands). If a corpus luteum was found, the cows were treated with one dose of 2 mL of prostaglandin (sodic D(+)cloprostenol, 75 μg mL^{-1}), these cows were used in experiments within 24 h after injection. If a follicle were found, the cows were tested in experiments within 24 h after ultrasound monitoring. Cows were specifically excluded from the study if they had a basal progesterone concentration greater than 1 ng mL^{-1} in a pretreatment blood sample. After the insertion of intravaginal device serial blood samples for pharmacokinetic study were collected from each animal at predetermined times (0, 1, 4, 8, 24, 48, 72, 96, 120, 148 and 172 h) until completing 7 days of assay. Blood samples were collected from the coccygeal vessel into tube containing 0.07 mL of EDTA solution (Wiener, 0.342 mol L^{-1}, pH 7.2, Argentina) and processed to yield serum. After the centrifugation of blood at 2000 rpm for 10 min, plasma samples were stored at −20 °C until further analysis.

2.6. Plasma progesterone analysis

Concentrations of progesterone in plasma were determined by radioimmunoassay using a commercial kit (Toribio et al., 1994). Duplicate analysis was performed on each sample. The intra-assay coefficient of variation (CV) was <7% for concentrations between 0.1 and 40.0 ng mL^{-1}, the inter-assay CV was 3.5%. The sensitivity of the assay was 0.01 ng mL^{-1}.

2.7. Statistical analysis

Data analysis was done employing means comparison tests provided by Statgraphics Plus 5.1. Results were considered statistically significant if $p < 0.05$. In order to compare the *in vitro* release profiles the difference factor (f_1) and the similarity factor (f_2) were calculated (Costa and Sousa Lobo, 2001).

3. Results and discussion

3.1. In vitro studies

The average initial progesterone content was 1.034 ± 0.015 g ($n = 3$) for the EVA insert and 1.025 ± 0.022 g for the DIB insert ($n = 3$). The difference was not significant (p-value = 0.2424) and this result indicated that the method developed for the incorporation of progesterone into EVA pellets before manufacturing the device is reliable and reproducible.

The drug release was analyzed by plotting the cumulative release data of progesterone against time. Fig. 4 shows the release behavior of progesterone from the EVA device and DIB insert. The values of the difference factor ($f_1 = 10.82$) and the similarity factor ($f_2 = 60.25$) suggested equivalence between the release profiles (Costa and Sousa Lobo, 2001).

The results obtained suggested that the *in vivo* behavior of EVA device could be similar to that of DIB inserts.

3.2. In vivo studies

Concentrations of progesterone in plasma following administration of EVA or DIB inserts are shown in Fig. 5.

Table 1
Residual progesterone load in spent EVA insert and DIB device and corresponding amount released during a 7-day insertion period ($n = 3$).

Insert type	Initial (g) (Mean ± SEM)	Residual (g) (Mean ± SEM)[a]	Amount released (g) (Mean ± SEM)[a]
EVA intravaginal insert	1.034 ± 0.015	0.601 ± 0.034	0.433 ± 0.034
DIB insert	1.025 ± 0.022	0.819 ± 0.015	0.206 ± 0.015

[a] The difference of average values was significant (p-value < 0.05).

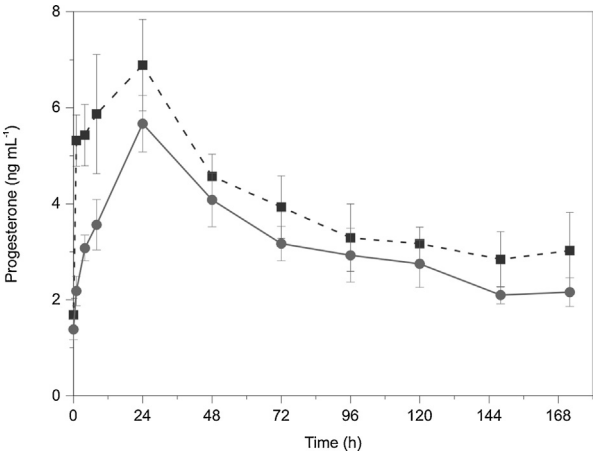

Fig. 5. Blood plasma progesterone levels obtained from EVA device (●) and DIB insert (■). Values are presented as mean ± SEM of five individual experiments.

The comparison between the two *in vivo* profiles showed that both devices maintain plasma concentrations of progesterone greater than 2 ng mL^{-1} over a 7-day treatment period. Devices show no statistically significant differences in concentration of progesterone in plasma at each sampling time (p-value > 0.05).

Table 1 shows residual progesterone content and the corresponding amount released during insertion. The average residual progesterone content was 0.601 ± 0.034 g ($n = 3$) for EVA insert and 0.819 ± 0.015 g for DIB insert ($n = 3$). Residual progesterone content in EVA device was lower than that in the commercial device (p-value < 0.05) but this difference was not reflected in the *in vivo* release profile. This is possibly due to the rate and extent of the progesterone absorption after intravaginal administration may vary depending on formulation and physiological factors such as differences in feed intake and physical activity (Rabiee et al., 1999, 2002). We take into consideration two facts: (a) the cows in each group were of the same species with a similar body condition score and (b) only significant difference is observed in the *in vivo* release profile during the first 8 h of the assay; so that we conclude that the obtained difference was due solely to the formulation factors: release rate and effective contact area (Mariano et al., 2010).

4. Conclusions

The polymer chosen for the release of progesterone and the design adopted seems to have removed the disadvantages of the intravaginal devices based on silicone rubber. The new device appears to have *in vitro* and *in vivo* performance comparable to the commercial insert. After a 7-days insertion the plasma progesterone concentration achieved with the EVA device could remain above 2 ng mL^{-1}, required level for to completely control synchrony and ovulation (Rathbone et al., 1998a). The residual progesterone content is less than 60% of the initial load, and was reduced to more than 20% compared to the commercial one.

Further research is needed to redesign the EVA device to maximize the effective area of release so as to achieve higher levels of plasma concentrations progesterone and to further reduce the residual charge of progesterone in the device. The redesign should be coupled with a pharmacokinetic model (Mariano et al., 2010) in order to predict *in vivo* release profiles.

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