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A COMPARISON OF HYDRON AND SILICONE IMPLANTS IN THE BOVINE NORGESTOMET AND ESTRADIOL VALERATE ESTRUS SYNCHRONIZATION PROCEDURE

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ABSTRACT

Hydron and silicone implants impregnated with norgestomet were used in the bovine norgestomet and estradiol valerate estrus synchronization procedure. Total norgestomet secreted from the implants over the 9 day implantation period was similar ($P > .25$) for both implants (both *in vitro* and *in vivo*) and was released *in vitro* in linear declining patterns but decreasing at different rates (slopes of -66.5 [hydron] and -22.1 [silicone] [both $P < .01$]). Furthermore, total *in vitro* secretion over the 9 day period was similar ($P > .25$) to total *in vivo* secretion over the 9 day period for both types of implants. On days 1 and 2, more ($P < .01$) norgestomet was secreted from the hydron implants whereas on days 3 to 9 more ($P < .01$) norgestomet was secreted from the silicone implants. *In vivo* efficacy data demonstrated that synchronized pregnancy rates were greater (44 % *vs* 53 %; $P < .01$) for cattle treated with the procedure utilizing the silicone implant.

INTRODUCTION

Estrus synchronization is the manipulation of the reproductive cycle such that all treated females exhibit estrus at a predefined period with normal fertility. This has been accomplished by using progestins or prostaglandin $F_2\alpha$ (9). One effective progestin procedure utilizes the progestin norgestomet in a nine day implant and an injection of norgestomet and estradiol valerate on the day of implantation (4, 9). Although pregnancy results are sometimes variable (3), cattle responding exhibit estrus at such a uniform time that timed insemination can be utilized. The commercial procedure utilizes a matrix implant composed of norgestomet impregnated in poly(ethylene glycomethacrylate) (hydron) (1). Poly(dimethylsiloxane) (silicone) has also be successfully utilized to delivery steroids in a controlled fashion (5, 6).

The purpose of these studies was to evaluate the alternative silicone implant impregnated with norgestomet in the norgestomet and estradiol valerate estrus synchronization procedure.

MATERIAL AND METHODS

Implants. Hydron implants impregnated with 6.0 mg of norgestomet were purchased from Sanofi Animal Health, Inc.[a]. Silicone implants impregnated with 6.0 mg of norgestomet were donated by Antech Laboratories, Inc.[b]. Both hydron and silicone implants were cylinders of approximately 2.6 mm in diameter and 2.0 cm in length.

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[a] Syncro-Mate B®, Overland Park, KS.

[b] Champaign, IL.

Injectable. The injectable portion of the procedure was the same for animals treated with silicone and hydron implants. The injection consisted of 3.0 mg of norgestomet and 5.0 mg of estradiol valerate in 2 cc of sesame oil (with 10 % benzyl alcohol).

In vitro Secretion. The in vitro conditions used to determine daily secretion rates consisted of 10 ml aliquots of sterile bovine serum[c] placed in 16 X 100 mm culture tubes. Implants were individually placed in the serum, the tubes capped, and held at 37°C (in a water bath) for 24 hours. At the end of 24 hours the implants were removed and placed in new 10 ml aliquots of bovine serum for the next 24 hour period. This was done for nine days. Twelve (12) silicone and 3 hydron implants were included.

In vivo Secretion. Eight heifers were implanted with hydron (n=4) and silicone (n=4) implants. The implants were placed subdermally on the convex surface of the ear. The implants were left in situ for nine days. At the end of the nine day implantation period the implants were removed with a scalpel.

Serum Extraction. Norgestomet was extracted from the cultured serum by placing 200 μl of the serum into a 16 X 150 culture tube. Subsequent to the addition of 2.0 ml of petroleum ether[d] the tubes were vigorously vortexed for 30 seconds and then placed at -20°C until the aqueous portion had frozen. The petroleum ether[d] was then

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[c] Quad 5, Ryegate, MT.

[d] Baker Resi-Analyzed® Reagent, J.T. Baker, Inc., Phillipsburg, NJ.

decanted into 13 X 100 mm culture tubes and placed at approximately 60°C until all the petroleum ether[d] had evaporated.

Implant Extraction. Norgestomet was extracted from new implants and implants after nine days *in situ* by placing them individually along with 10 ml of methanol[e] in 16 X 100 mm capped culture tubes. The intact implants were then placed at 37°C (in a water bath) for 6 days.

Norgestomet Determination. Norgestomet concentrations were determined from the extracted serum samples and the implant methanol[e] solutions spectrophotometrically. The extraction tubes were reconstituted with methanol and absorbance of the methanol[e] solutions was determined at the wavelength of 240 nm.

Efficacy. Efficacy was determined in 260 beef cows and heifers at five locations. Cattle at locations 1 and 3 were beef heifers while cattle at locations 2, 4, and 5 were postpartum beef cows. Day zero was the day that implants were inserted and injections administered. On day nine implants were removed. Approximately 48 hours after implant removal cattle were artificially inseminated with semen from fertile bulls without regard to estrus. Pregnancy was determined nine months later during the calving season (pregnancy rate = number calving to the synchronized insemination ÷ total number treated).

Data Analysis. *In vitro* data were analyzed by linear regression and analysis of variance (10). Efficacy data

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[e] ACS Certified, FisherChemical, Fair Lawn, NJ.

were analyzed by analysis of variance as described by Chinchilli (2).

RESULTS

Validation. The spectrophotometric standard curve (0 to 25 μg) for norgestomet had a linear regression of r = .99998 (P < .01). Extraction of known quantities of norgestomet spiked into bovine serum was 87.5 % (coefficient of variation 1.97 % for 5 spiked samples). All extracted samples were adjusted for recovery loss. One lot of silicone implants was manufactured with 6.3 mg of norgestomet per implant. Norgestomet content extracted from four of these implants was determined to be 6.458 mg (102.51 %; coefficient of variation = 4.57 %).

In vitro and In vivo Secretion. Daily and cumulative norgestomet secretion for the 9 day implantation period are presented in figures 1 and 2. As depicted in figure 1 norgestomet was secreted in a linear declining fashion from both the hydron (slope = -66.5, r = .80, P < .01) and the silicone (slope = -22.1, r = .96, P < .01) implants. The rate of decline, however, was greater for the hydron implants than for the silicone implants (P < .05). The correlation for the daily secretion rate for the hydron implants was improved when day 1 data were removed (r = -.92; P < .01). Even with day 1 removed, the rate of decline (slope = -33.9) for the hydron implants was still greater than for the silicone implants. More (P < .01) norgestomet was secreted from the hydron implants on days 1 and 2 than for the silicone implants. However, more (P < .01) norgestomet was secreted from the silicone implants on days 3 to 9 than for the hydron implants. Combined, the cumulative amount

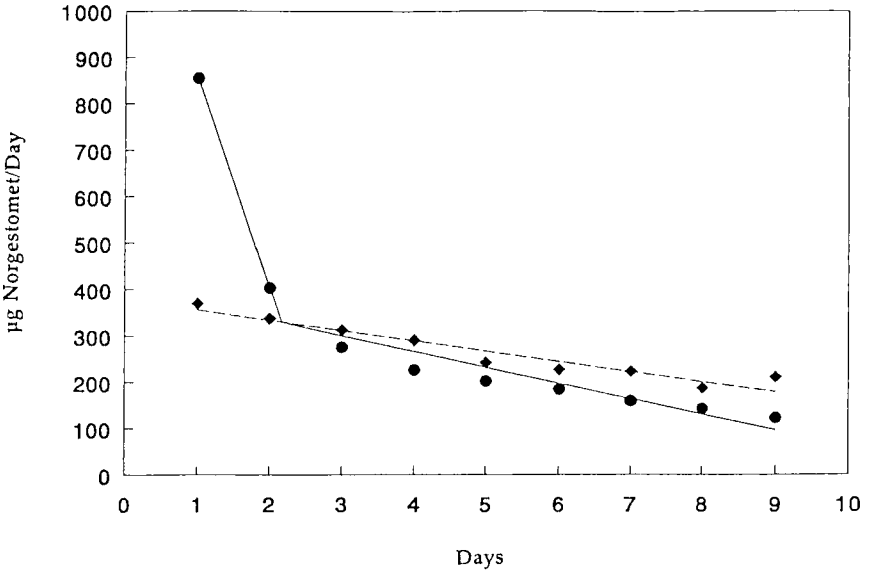

Figure 1. Daily norgestomet secretion (μg per day) from hydron (solid [regression] lines and solid circles [actual data]) and silicone implants (broken [regression] line and diamonds [actual data]) in vitro.

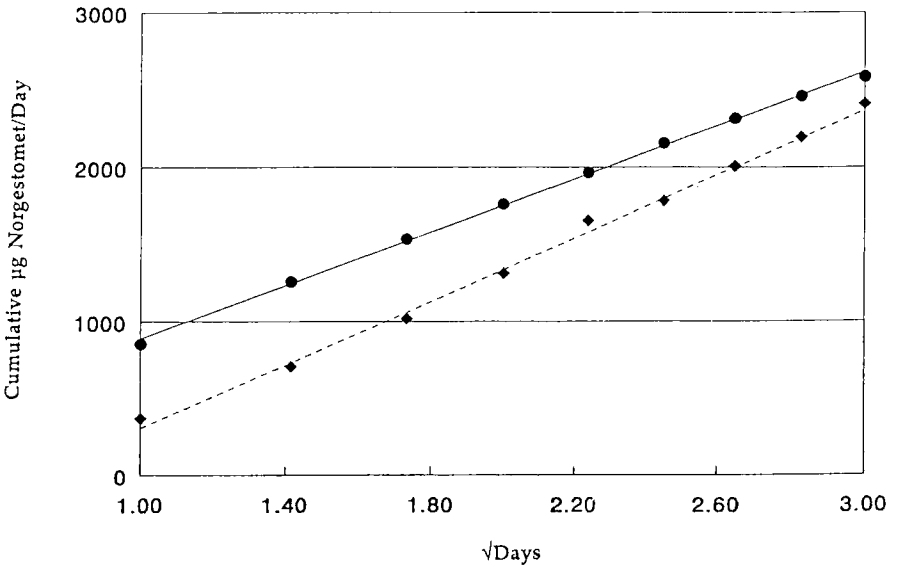

Figure 2. Cumulative norgestomet (μg) secretion over the nine day implantation period from hydron (solid [regression] lines and solid circles [actual data]) and silicone implants (broken [regression] line and diamonds [actual data]) in vitro.

of norgestomet secreted in vitro (figure 2) by the ninth day was similar ($P > .25$) for both types of implants (table 1). The cumulative amount of norgestomet secreted in vitro (figure 2) increased linearly with time for both the hydron ($r = .99$; $P < .01$) and silicone ($r = .99$; $P < .01$) implants but at a greater rate for the silicone (slope = 1030.18) than for the hydron (slope = 861.76) implants.

Also reported in table 1 are the in vivo quantities of norgestomet secreted over the 9 day implantation period which was similar for both types of implants and for in vitro secretions. Variance between the cumulative nine day in vitro and in vivo secretion was 6.35 % and 1.01 % for the hydron and silicone implants, respectively. Therefore, the in vitro secretion over the nine day period appeared to be a good reflection of in vivo secretion for both types of implants. Both types of implants release approximately 2.5 mg of norgestomet over the nine day period. Variance between type of implant for the cumulative nine days was 6.67 % and 1.73 % for in vitro and in vivo secretion, respectively.

In vivo Efficacy. Pregnancy rates determined in 260 beef heifers and cows demonstrated that utilization of the silicone implant with the norgestomet and estradiol valerate estrus synchronization was more efficacious ($P < .01$) than utilization of the hydron implant (table 2). Pregnancy rates were 19 % higher when silicone implants were utilized in the estrus synchronization procedure. Results were consistently higher (ranging from 10 % to 64 % improvement) for the silicone implanted animals at all locations. The location effect and the location by treatment interaction were non-significant ($P > .25$).

Table 1. Norgestomet secreted *in vivo* and *in vitro* during the nine day implantation period.

Item	Implant: Hydron	Implant: Silicone
In vitro secretion	2.578 mg	2.406 mg
In vivo secretion	2.424 mg	2.382 mg
In vitro/*in vivo*:		
Difference[a]	0.154 mg	0.024 mg
% Variance[b]	6.35 %	1.01 %
Implant:		
Difference *in vitro*		
mg	0.172 mg[c]	
%	6.67 %[d]	
Difference *in vivo*		
mg	0.042 mg[c]	
%	1.73 %[d]	

[a] *In vitro* secretion minus *in vivo* secretion.
[b] Difference divided by *in vivo* secretion.
[c] Difference = hydron implant secretion - silicone implant secretion.
[d] Difference divided by hydron implant secretion.

Table 2. Pregnancy rates subsequent to a timed insemination after a hydron implant and silicone implant norgestomet and estradiol valerate estrus synchronization procedure[a].

Location	Hydron	Silicone
1. Heifers	10/ 28 (36 %)	13/ 30 (43 %)
2. Postpartum Cows	15/ 34 (44 %)	17/ 35 (49 %)
3. Heifers	4/ 12 (33 %)	6/ 11 (55 %)
4. Postpartum Cows	6/ 14 (43 %)	6/ 11 (55 %)
5. Postpartum Cows	23/ 43 (53 %)	26/ 42 (62 %)
Combined	58/131 (44 %)	68/129 (53 %)

[a] The norgestomet and estradiol valerate estrus synchronization procedure consists of the implantation of a 6 mg norgestomet impregnated implant and the injection of 3 mg of norgestomet and 5 mg of estradiol valerate on day 0. On day 9 implants are removed and cattle are bred either by estrus detection or by timed breeding 48 to 52 hours after implant removal.

DISCUSSION

Data from this study demonstrated that the delivery of the same quantity of norgestomet in two fashions and by two different polymers changed *in vivo* efficacy. The approximately 2.5 mg of norgestomet delivered by the hydron implants (over the nine day period) was delivered in greater quantities early during the implantation period. The approximately 2.5 mg of norgestomet delivered by the silicone implants (over the nine day period) was delivered more constantly (although in a declining fashion) during the nine day implantation period.

It is unknown as to which aspect of the delivery pattern increased *in vivo* efficacy. It could have been one of following three reasons.

1. Reduced norgestomet secretion early in the implantation period.
2. Increased norgestomet secretion during day 3 to 9 of the implantation period.
3. More constant delivery of norgestomet during the entire implantation period or a combination of both numbers 1 and 2.

The other factors such as total loading rate for the implant, implant size, and total amount of norgestomet released over the nine day implantation period were held constant.

The *in vitro* system used in this study appeared to produce similar results to *in vivo* secretions. In an earlier publication (7) we reported *in vivo* secretion from silicone and hydron implants. Another *in vitro* system was utilized in that publication and although correlations could be made between *in vivo* and *in vitro*

secretions, differences were reported (7). Similar differences were not detected with the in vitro system utilized in this study.

In summary, it is recommended that the 6 mg norgestomet/silicone implant be used to deliver norgestomet in vivo in the norgestomet and estradiol valerate estrus synchronization program in cattle. Subsequent fertility, the goal of the program, was improved when the silicone implant was utilized. The silicone/norgestomet implant has also been successfully used for estrus synchronization in sheep (8).

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