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Efficacy of Norethindrone Acetate and Norgestomet Implants in Suppressing Estrus in Female Beef Cattle

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ABSTRACT

Beef females with corpora lutea on day 12 of the estrus cycle were implanted with 11.5 mg norethindrone acetate implants (7 implanted and 7 nonimplanted controls) at the same time they were administered a luteolytic dose of prostaglandin $F_{2\alpha}$. Although the implants released 340 to 740 μg of norethindrone acetate daily, the implants did not suppress estrus. Norgestomet implants were inserted into beef females 5 days after estrus and implants were left in situ *for 16 days. The numbers of cows in the norgestomet study were 27 nonimplanted, 19 with 6-mg implants, and 21 with 8-mg implants. Control cows were not detected in estrus until day 17 of the estrous cycle (12 days after the time of implantation) and 10 of the 27 cows (37%) were detected in estrus over a 5-day period beginning on day 17 (12 to 16 days after the time of implantation). None of the cows implanted with 8-mg implants were detected in estrus while the implants were* in situ. *However, 3 of the cows with 6-mg norgestomet implants were detected in estrus 13 to 16 days post-implantation. Based on implant secretion data, cows with 6-mg implants began displaying estrus when the implants released less than 138 μg of norgestomet daily. Eight-milligram implants released 137 μg of norgestomet or greater per day during the entire 16-day implantation period.*

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INTRODUCTION

Norethindrone acetate (19-nor-17α-ethynyl-17β-ol-3-one acetate; Fig. 1) is used in combination with ethynylestradiol in the U.S. [with Food and Drug Administration (FDA) approval] as an oral contraceptive in humans. Norethindrone acetate was selected (a) because the acetate provides longer *in vivo* half-life (21), and (b) because esterification enhances steroid secretion from silicone implants (4,9). Norethindrone acetate implants have been used efficaciously as a contraceptive (because it suppresses ovulation) in humans (14).

Norgestomet is approved by FDA for use in cattle for estrus synchronization (5). The procedure, designated Syncro-Mate B®, includes a 9-d implant containing 6 mg of norgestomet and an intramuscular injection that consists of 3 mg of norgestomet and 5 mg of estradiol valerate that is administered at the time of implant insertion (3). The purpose of the implant is to suppress estrus, and when used for estrus synchronization in cattle, subsequent timed (cattle are bred 48 to 52 hr after implant removal) breeding pregnancy rates averaged 40% (12,16).

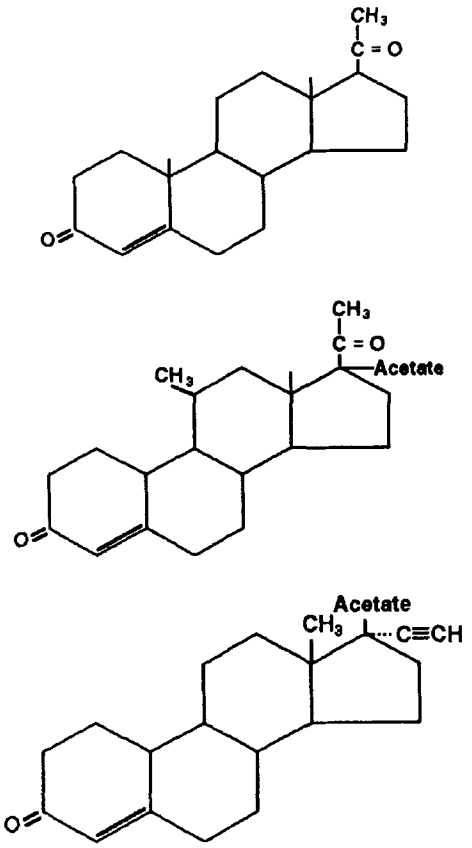

Figure 1. Chemical structures of progesterone (top), norgestomet (middle), and norethindrone acetate (bottom).

Chemically, norgestomet (17α-acetoxy-11β-methyl-19-norpreg-4-ene-20, dione) is a modified 19-norprogesterone (Fig. 1). Norprogesterone is identical to progesterone except that the methyl group at the 19 position is absent. Norgestomet has two other modifications: the presence of a methyl group at the 11 position and the presence of an acetate at the 17 position. Norgestomet is metabolized quickly (15) and is excreted in the urine and feces (20). In both urine and bile, the majority of the excreted metabolites were highly polar materials demonstrated to have only about 4% of the progestational activity of norgestomet in the Clauberg assay (20).

Norgestomet has been demonstrated to be a highly biologically active progestin. Gilbert et al. (7) reported that norgestomet was 15 times more biologically active than progesterone when orally administered to rabbits. Gilbert et al. (7) further demonstrated that norgestomet was 216 times more biologically active than progesterone when subcutaneously administered to estradiol-17β-treated mice. Wishart (24) demonstrated that 140 μg of norgestomet and 45 mg of progesterone via daily intramuscular injections were required to suppress estrus in all treated heifers (which suggests that norgestomet is 321 times more potent than progesterone in this model).

The study of Wishart (24) is the only published report demonstrating the dosage of norgestomet required to suppress estrus. In that study, with a limited number of animals, norgestomet was administered via injection whereas in practice norgestomet is delivery via an ear implant to suppress estrus. Also, claims are often made that females with implants *in situ* are detected in estrus. Norethindrone acetate has not been evaluated in cattle. This study was conducted to determine (*a*) if norethindrone acetate would suppress estrus in cattle, and (*b*) the minimal dosage of norgestomet via implant delivery that efficaciously suppresses estrus in cattle.

MATERIALS AND METHODS

Experiment 1

Fourteen beef heifers were selected for the study. Heifers were divided into two groups. All heifers had been previously synchronized with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse® (Pharmacia and Upjohn, Inc., Kalamazoo, MI) (16) and observed for estrus. Twelve days after detected estrus all heifers were bled and

plasma was assayed by a validated ELISA (10) for progesterone concentrations. All 14 heifers had progesterone concentrations greater than 1.5 ng/ml, which suggests that they had corpora lutea that developed after the previously detected estrus. One-half (7) of the heifers were subcutaneously implanted with a norethindrone acetate matrix silicone implants. The cylindrical implants were 3.5 mm in diameter and 2.5 cm in length, and were implanted subdermally on the convex surface of the ear. Each treated heifer received one implant that contained 11.5 mg of norethindrone acetate (Sigma Chemical Company, St. Louis, MO; equivalent to 8.35 mg of norethindrone). At the time of implant insertion, all heifers were injected with a luteolytic dose (25 mg) of $PGF_{2\alpha}$ (Lutalyse) (16). Implants were left *in situ* for 4 days and after removal, total remaining norethindrone acetate was determined (13). *In vitro* implant secretion over 4 days was also determined and corrected for *in vivo* secretion by a procedure reported by Kesler et al. (13).

Experiment 2

Cylindrical silicone implants impregnated with approximately 6 mg or approximately 8 mg of norgestomet (Antech Laboratories, Inc., Savoy, IL) were used for these studies. Implants were 2.67 mm in diameter and were 18.5 mm or 25 mm long. Quantity of norgestomet per milligram of silicone was the same for both the 6-mg and the 8-mg norgestomet implants.

Four implants from each implant dose were used to determine *in vitro* secretion using the method described by Kesler et al. (13). Daily *in vitro* secretion was determined for 16 days. *In vivo* secretion and total content of norgestomet from the implants (four implants of each dosage) were also determined as described by Kesler et al. (13).

Sixty-nine postpartum beef cows were synchronized with Syncro-Mate B® (Rhone Merieux, Inc., Athens, GA). This synchronization protocol consisted of an intramuscular injection of 5 mg estradiol valerate and 3 mg norgestomet in sesame oil (and 10% benzyl alcohol) administered on the same day that cows were administered a 6-mg norgestomet/hydron implant on the convex surface of the ear. Implants were left *in situ* for 9 days. On the fifth day after estrus and insemination, the cows were randomly assigned to three groups. Two groups were implanted with silicone implants impregnated with either 6 or 8 mg of norgestomet. These implants were subdermally implanted on the convex surface of the ear and were left *in situ* for 16 days. Twenty cows received 6-mg norgestomet/silicone implants and 22 cows were implanted with 8-mg norgestomet/silicone implant. Two cows lost their implants, one 6-mg implant and one 8-mg implant, and were excluded from the analysis. The other 27 postpartum beef cows were not administered norgestomet/silicone implants. Beginning the day of implantation of the norgestomet/silicone implants, cows were observed twice daily for estrus for 31 days. Cows were considered in estrus only when they stood to be mounted by other females.

The rationale for the experimental design was as follows. First, norgestomet secretion from silicone implants (13) and the general amount of norgestomet needed to suppress estrus (24) were known. Second, norgestomet implants have been used for resynchronization of nonpregnant beef females previously inseminated (6). Therefore, the design was to place implants in cows during the luteal phase so that secretion from the implants was around the minimal level needed to suppress estrus when the nonpregnant cows would be returning in estrus.

Qualitative data were analyzed by chi-square analysis (22).

RESULTS

Experiment 1

Norethindrone acetate was released from the silicone implants in a linearly declining fashion [$r = -.997$; $y = x\ (-.21) + 1.15$] (11,13,18). Over the 4-day period, a total of 2.53 mg (22% of the total) was delivered *in vivo*. Three of the 7 control heifers (43%) were detected in estrus whereas all 7 (100%) of the treated heifers were detected in estrus (Table 1). Estrus was

Table 1

Norethindrone Acetate Implant Secretion and Estrus Suppression Efficacy in Beef Heifers

Item	Control	Treated
Number	7	7
Number in estrus	3 (43%)	7 (100%)[a]
Mean interval to estrus (hr)	61	59
Norethinidrone acetate secreted (μg)		
Day 1	0	947
Day 2	0	738
Day 3	0	501
Day 4	0	341

[a]Differed from the control group at the 0.02 level of significance.

detected at similar times after $PGF_{2\alpha}$ treatment for both groups. To verify $PGF_{2\alpha}$-induced luteolysis, all heifers were bled 2 days after $PGF_{2\alpha}$ treatment and plasma was assayed for progesterone concentrations (10). All heifers had progesterone concentrations suggestive that luteolysis was ensuing or had ensued.

Experiment 2

Content of the implant was only 3.8% higher than anticipated (Table 2) and both methods of determining total *in vitro* secretion over the 16-day period produced similar results. *In vivo* secretion, however, was approximately 0.73 of *in vitro* secretion and therefore *in vitro* secretion was adjusted to more truly reflect *in vivo* secretion.

The incidence of estrus behavior during the implantation period is reported in Table 3. Cows implanted with 8-mg implants did not display estrus during the 16-day period. Thirty-seven percent of the nonimplanted cows, however, were detected in estrus during the implantation period. More ($p < 0.05$) nonimplanted cows were detected in estrus than 8-mg-implanted cows. Cows implanted with 6-mg implants were intermediate. There tended ($p = 0.13$) to be fewer 6-mg-implanted cows in estrus than nonimplanted cows, and there tended ($p = 0.07$) to be more 6-mg-implanted cows in estrus than 8-mg-implanted cows.

As reported in Tables 2 and 4, more ($p < 0.05$) norgestomet diffused into the serum *in vitro* for the 8-mg implants than for the 6-mg implants. The release was in a linear declining fashion as predicted for matrix-type silicone implants (Table 4) (11,13,18). Daily quantity released *in vitro* was correlated ($r = -.96$ and $r = -.94$ for 6-mg and 8-mg implants, respectively; both $p < 0.01$) with day *in vitro*, and slopes were similar as depicted in the regression equations (Table 4). Overall, approximately 49 μg more norgestomet diffused from the 8-mg implants daily than from the 6-mg implants.

Based on cows implanted with 6-mg norgestomet implants, cows began to display estrus when daily norgestomet secretion fell below 138 μg per day. Daily secretion of norgestomet from the 8-mg implants, implants that completely suppressed estrus for the 16-day implantation period, was lowest on day 16 at 137 μg per day. Therefore, it was concluded that 137 to 138 μg of norgestomet per day completely suppressed estrus in cattle.

Table 2

Content (mg), and In Vivo *and* In Vitro *Secretion (mg) of Norgestomet from 6-mg and 8-mg Silicone Implants*

	Norgestomet Implants	
	6 mg	8 mg
Content (mg)	6.21	8.33
In vivo secretion[a]	3.04	3.76
In vitro secretion		
Method 1[a]	4.19	5.18
Method 2[b]	4.01	5.46
In vivo/in vitro ratio	0.73	0.73

[a]Content minus content remaining after 16 days *in situ* or *in vitro*.
[b]Cumlative *in vitro* secretion observed during the 16 days *in vitro*.

Table 3

Incidence of Estrus During Implantation with Norgestomet Implants

	Norgestomet		
Days After Estrus	6 mg	8 mg	Nonimplanted
16	0	0	0
17	0	0	1
18	1	0	4
19	0	0	2
20	1	0	1
21	1	0	2
Combined	3/19[a]	0/21[b]	10/27
Combined %	16%	0%	37%

[a]Tends to differ ($p = 0.13$) from nonimplanted cows and tends to differ ($p = 0.07$) from 8-mg-implanted cows.
[b]Differs ($p < 0.01$) from nonimplanted cows and tends to differ ($p = 0.07$) from 6-mg-implanted cows.

DISCUSSION

This first evaluation of the ability of norethindrone acetate implants to suppress estrus in cattle demonstrated that it was ineffective at the 340 to 740 μg per day dosage Levenorgesterel, a compound similar to norethindrone acetate, implants were ineffective in white-tail deer (another ruminant species) (23). Therefore, it may be that synthetic progestins that inhibit ovulation in primates are ineffective at similar dosages in ruminants. Norgestomet, which suppresses estrus in cattle, is also effective in white-tail deer (8).

The minimal required dose of norgestomet to suppress estrus in cattle was confirmed with silicone implant delivery of norgestomet in this study. Our study

Table 4

Daily Norgestomet Secretion[a] by 6- and 8-mg Implants as Related to Estrus Suppression

6-mg Implants		8-mg Implants	
Day	μg Norgestomet[b]	μg Norgestomet[c]	Day
		329	1
		316	2
		303	3
		290	4
1	281	278	5
2	267	264	6
3	255	252	7
4	242	239	8
5	229	227	9
6	216	214	10
7	203	201	11
8	190	188	12
9	177	175	13
10	164	162	14
11	151	150	15
12	138	137	16
	Estrus Suppression[d]		
13	125		
14	112		
15	99		
16	86		

[a]Adjusted to reflect daily *in vivo* secretion.
[b]Daily corrected secretion (Y) based on the following regression equation ($r = -0.96$). $Y = -17.8059X + 402.2250$.
[c]Daily corrected secretion (Y) based on the following regression equation ($r = -0.94$). $Y = -17.5603X + 468.3250$.
[d]No cows were detected in estrus for the days above this point. For the 6-mg implant, one cow was detected in estrus on day 13, one on day 15, and one on day 16. Implants were removed on day 16 for both 6-mg and 8-mg implants.

and the data of Wishart (24) would demonstrate that 137 to 140 μg of norgestomet per day will suppress estrus in cattle.

Experiments utilizing the commercial hydron (polyethylene glycomethacrylate) norgestomet implant (6 mg) have demonstrated that when it was implanted during proestrus, the dominant follicle present was maintained for the duration of the treatment and there was no growth of medium or small follicles (17). Systemic estradiol concentrations were also elevated, and there was insufficient progestin activity to maintain a strong negative feedback on luteinizing hormone (LH) pulse frequency in a manner comparable to that of the luteal phase of a normal estrous cycle (19). Rajamahendran and Taylor (17) suggested that this implied that the norgestomet treatment given during proestrus mimics the actions of low concentrations of progesterone. This time period is, in fact, a time of low norgestomet secretion by the hydron implant (13) and, therefore, obtaining a low progestin effect would be expected. In fact, when implants were changed during the persistence of the dominant follicle, LH pulse frequency decreased, estradiol concentrations decreased, and follicular atresia occurred (15). Therefore, when given in appropriate amounts, norgestomet was effective in provoking the progestin-like negative feedback on LH pulse frequency and on follicular atresia.

This was supported by Butcher et al. (2). These authors reported that daily injections of 100 mg/day was required to elevate systemic progesterone concentrations to levels of the luteal phase (5 to 7 ng/ml), whereas daily injections of only 45 mg/day were required to suppress estrus in all treated animals (24). The dosage selected for the norgestomet implant was based on the minimal quantity required to suppress estrus.

Fertility of ovulated persistent follicles is low (1). Therefore, the dosage of norgestomet for bovine estrus synchronization should be further evaluated and consideration given to regression of follicles rather than just on estrus suppression in order to have higher fertility rates in females treated with norgestomet for the synchronization of estrus. Kesler et al. (13), for example, used an implant with more norgestomet release and had improved synchronized fertility rates.

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