

HFA-305
Documents Management Branch

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**FREEDOM OF
INFORMATION SUMMARY**

NADA 141-200

EAZI-BREED™ CIDR®

Intravaginal Progesterone Insert

**For concurrent use with dinoprost tromethamine
(Lutalyse® Sterile Solution)**

For Synchronization of Estrus in Suckled Beef Cows and
Replacement Beef and Dairy Heifers, for Advancement of First
Postpartum Estrus in Suckled Beef Cows and for Advancement of
First Pubertal Estrus in Replacement Beef Heifers

SPONSORED BY:

DEC INTERNATIONAL, Inc.

1919 S. Stoughton Road
P.O. Box 8050
Madison, WI 53708-8050

NADA 141-200

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FREEDOM OF INFORMATION SUMMARY

1 GENERAL INFORMATION

- a. **File Number:** NADA 141-200
- b. **Sponsor:** DEC INTERNATIONAL, INC
1919 S. Stoughton Road
P.O. Box 8050
Madison, WI 53708-8050
- Drug Labeler Code: 67080
- c. **Established Name:** Intravaginal Progesterone Insert
- d. **Proprietary Name:** EAZI-Breed™ CIDR® Cattle Insert
- e. **Dosage Form:** Intravaginal Insert
- f. **How Supplied:** 10 Inserts per Polyethylene Bag
- g. **How Dispensed:** OTC
- h. **Amount of Active Ingredients:** Each Insert contains 1.38 grams of progesterone in molded silastic over a nylon spine.
- i. **Route of Administration:** Intravaginal
- j. **Species/Class:** Bovine/Beef Cows, Beef Heifers and Dairy Heifers
- k. **Recommended Dosage:** One Insert—Remove on Day 7
- l. **Pharmacological Category:** Steroid hormone
- m. **Indications:**
1. Synchronization of estrus in suckled beef cows, and replacement beef and dairy heifers.
 2. Advancement of first postpartum estrus in suckled beef cows.
 3. Advancement of first pubertal estrus in replacement beef heifers.

2 DOSAGE RATIONALE

2.1 Published Literature

Progesterone is an endogenous steroid produced by the corpus luteum of cattle. Progesterone concentration is variable during the estrous cycle and remains relatively high throughout

pregnancy and declines prior to calving. It has been known for 50 years that administration of progesterone inhibits estrus and ovulation in cattle (Ulberg, Christian and Casida, 1951; Trimberger and Hansel, 1955). However, how progesterone inhibits estrus and ovulation emerged gradually from scientific publications in the intervening five decades. Recently the use of ultrasonography to study ovarian follicle growth (Sirois and Fortune, 1988) has filled a major void in knowledge in this area.

In cattle, while early stages of ovarian follicular development occur independently of gonadotropin support, follicle stimulating hormone (FSH) is required for growth of follicles from about 4 to 9 mm diameter, and frequent pulses of luteinizing hormone (LH) are required for final maturation of the follicle (Gong *et al.*, 1996). The physiological bases for the effects of dose and duration of progesterone on LH secretion and the development of follicles in cows were reviewed by Kinder *et al.* (1996). Follicle growth occurs in waves, normally two or three in each estrous cycle in cows. Within each wave of follicle growth, a dominant follicle is selected and can ovulate a fertile oocyte after the endogenous source of progesterone is withdrawn as normally happens during the last 3 days before estrus. During efforts to synchronize estrus and ovulation in cattle, two main factors can affect the viability of the ovulated oocyte; 1) the duration (i.e., persistence) of the dominant follicle, and 2) the amount of progesterone in the blood.

Duration of Inset Treatment: Savio *et al.* (1993) showed that high fertility from estrous synchronization requires treatments leading to an ovulatory follicle that persists for no more than 8 days. This is because an oocyte originating from a follicle that persists for longer periods is likely to be senescent (Mihm *et al.*, 1994). Although such oocytes are fertilizable, they lead to faulty embryonic development and significantly increased embryonic death (Ahmad *et al.*, 1995). The adverse effect of a follicle that persists for periods beyond 8 days has no lasting effect on the reproductive tract, because pregnancy rates were normal from transfer of normal embryos on day 7 after the estrus following a prolonged period of persistent dominant follicle (Wehrman *et al.*, 1996). Furthermore, dinoprost (prostaglandin F_{2α}) will regress corpora lutea of cattle that are on day 6 or greater of the estrous cycle. A 7 day administration of progesterone assures that cattle with a corpus luteum are in the responsive stage of the estrous cycle if dinoprost is administered at or near the end of progesterone administration. Therefore, the cited research reports support a 7-day period for treatment with intravaginal progesterone inserts.

Amount of Progesterone: Based on published reports (Ahmad *et al.*, 1995; Cooperative Regional Research Project, NE-161, 1996; Savio *et al.*, 1993), it was concluded that blood levels of progesterone of 2 ng/mL or more inhibits episodic secretion of LH and thereby minimizes persistent dominant follicles. While the above-cited literature was less than clear as to an absolute concentration of progesterone in the circulation, it did indicate that increased progesterone during the days leading to estrus and breeding improved fertility compared to those animals that had reduced or decreasing progesterone concentrations in the days leading to estrus. Thus, the 2 ng/ml threshold was selected to target an appropriate dose of progesterone to include in the inserts.

2.2 Dose Selection Studies

Studies by the sponsor in ovariectomized heifers showed that the circulating concentrations of progesterone from the intravaginal progesterone insert was dependant principally upon a) the surface area of the intravaginal progesterone insert, and b) the concentration of progesterone in the silastic skin of the intravaginal progesterone insert. Blood progesterone in cattle increased linearly with increasing surface areas of the insert, and surface areas of 120 sq cm or greater resulted in blood progesterone greater than 2 ng/mL (Figure 1). However, increasing surface area (and thus size of insert) beyond 120 sq cm is not practical due to the physical constraints of the vaginal size in the target species and class of animal. Blood progesterone increased in cattle with increasing concentrations of progesterone up to 10% (w/w) in the silastic skin of the intravaginal progesterone insert, but silastic skin progesterone concentrations greater than 10% resulted in no greater concentrations of progesterone in blood (Figure 2). Therefore, the intravaginal progesterone insert selected by the sponsor has 120 sq cm surface area and 10% progesterone in the silicone skin (1.38 g progesterone).

The sponsor conducted two studies to determine blood progesterone in intact cattle administered the selected intravaginal insert, one study in lactating beef cows and another in beef heifers. Blood progesterone in the cows peaked at 3.6 ng/mL and then declined to 1.44 and 1.31 ng/mL on days 6 and 7 after administration of intravaginal progesterone inserts. In intact heifers blood progesterone declined from 5.99 ng/mL on the first day of administration of the progesterone intravaginal insert to 3.79 ng/mL on day 7 of administration. Thus, the insert maintained blood progesterone ≥ 2 ng/mL in beef heifers, but not in beef cows. In beef cows, progesterone concentrations were apparently maintained for sufficient duration (≥ 5 days) to allow for normal follicle turnover and ovulation of a newly-developed follicle with acceptable fertility (see section 2.3 below).

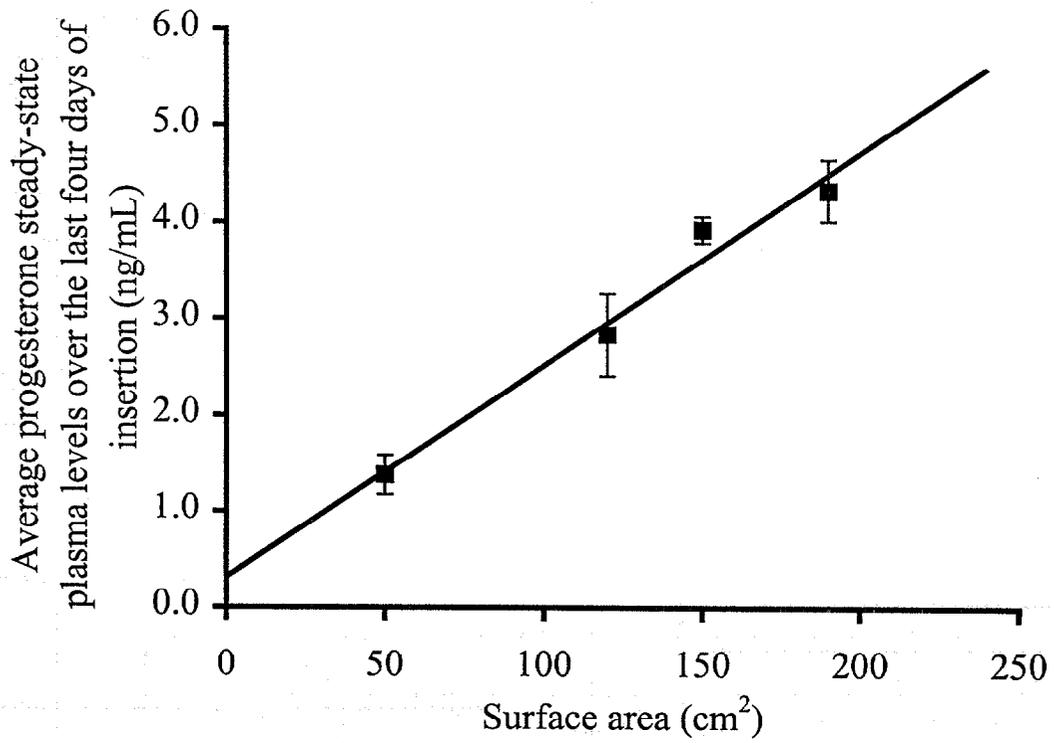


Figure 1. Effect of surface area on average progesterone steady-state plasma levels over the last four days of a 7 day insertion period. Error bars are standard errors of means (n=4 cattle).

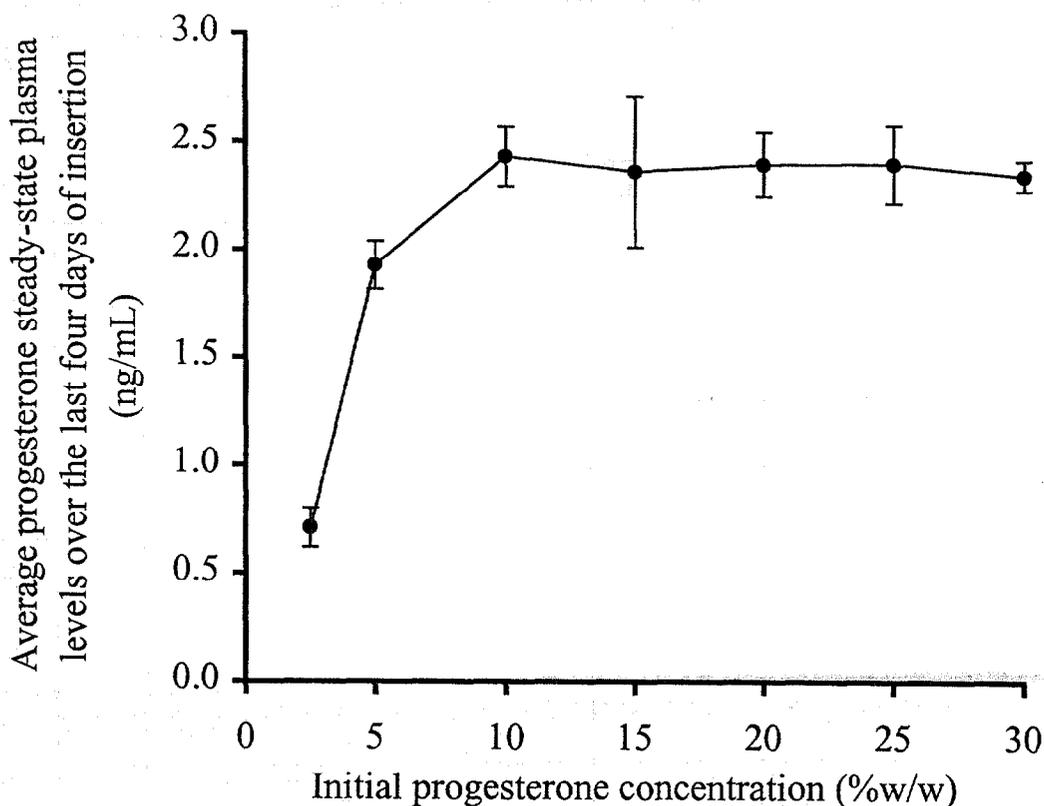


Figure 2. Effect of initial progesterone concentration (%w/w) on the average progesterone steady-state plasma levels over the last four days of a 7 day insertion period. Error bars are standard errors of means (n=4 cattle).

2.3 Dose Confirmation/Efficacy Studies

Estrus was successfully synchronized in each of six herds of suckled beef cows given the insert in the clinical study. Moreover, the fertility of insert-treated cows inseminated during the 31 days after the insert was removed was $50 \pm 4\%$, compared to $49 \pm 4\%$ for cows given $\text{PGF}_{2\alpha}$ and $45 \pm 4\%$ for untreated cows (see Section 3, Table 1, including cows in the herd that were the subject of the dose confirmation study). Comparable averages for heifers in five of the same herds were $47 \pm 7\%$, $36 \pm 7\%$, and $42 \pm 8\%$, respectively (see Section 3, Table 11), including heifers in the herd that were the subject of the dose confirmation study. These data support that sufficient progesterone was administered by the intravaginal progesterone insert to obtain ovulation of an ova with normal fertility.

2.4 Conclusion

The cited published research, coupled with the sponsor's research on surface area and dose titration, provide sufficient scientific justification for the dose and treatment regimen that was tested in their clinical studies.

2.5 Literature Cited

Ahmad, N. R., N. Schrick, R. L. Butcher and E. K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cows. *Biol. Reprod.* 52: 1129-1135.

Cooperative Regional Research Project, NE-161, 1996. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J. Animal Sci.* 1943-1952.

Fike, K. E., M. L. Day, E. K. Inskeep, J. E. Kinder, P. E. Lewis, R. E. Short and H. D. Hafs. 1997. Estrus and luteal function in suckled beef cows that were anestrous when treated with an intravaginal device containing progesterone with or without a subsequent injection of estradiol benzoate. *J. Animal Sci.* 75: 209-215.

Gong, J. G., B. K. Campbell, T. A. Bramley, C. G. Gutierrez, A. R. Peters and R. Webb. 1996. Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicular development in heifers continuously infused with a gonadotropin-releasing hormone agonist. *Biol. Reprod.* 55: 68-74.

Kinder, J. E., F. N. Kojima, E. G. M. Bergfield, M. E. Wehrman and K. E. Fike. 1996. Progesterone and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J. Animal Sci.* 74: 1424-1440.

Mihm, M., N. Curran, P. Hyttel, M. P. Boland and J. F. Roche. 1994. Resumption of meiosis in cattle oocytes from preovulatory follicles with a short and long duration of dominance. *J. Reprod. Fertil. Abstr. Series* 13: 14.

Savio, J. D., W. W. Thatcher, G. R. Morris, K. Entwistle, M. Drost and M. R. Mattiacci. 1993. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J. Reprod. Fertil.* 98: 77-84.

Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol. Reprod.* 39: 308-317.

Trimberger, G. W., and W. Hansel. 1955. Conception rate and ovarian functions following estrus control by progesterone injections in dairy cattle. *J. Animal Sci.* 14: 224-232.

Ulberg, L. C., R. E. Christian and L. E. Casida. 1951. Ovarian response in heifers to progesterone injections. *J. Animal Sci.* 10: 752-759.

Wehrman, M. E., K. E. Fike, E. J. Melvin, E. F. M. Bergfield and J. E. Kinder. 1996. Development of a persistent ovarian follicle during synchronization of estrus does not alter conception rate after embryo transfer in cattle. *Theriogenology* 45: 291 (abstr).

3 EFFECTIVENESS STUDIES

3.1 Clinical study in beef cows

3.1.1 Methods

A study was conducted at the following six sites with a total of 875 suckled beef cows. The objective of this study was to determine the effect of the intravaginal progesterone inserts given concurrently with an injection of dinoprost tromethamine on the interval to first estrus, the synchrony of estrus, and fertility in all of the test cows and in cows that were anestrous when the intravaginal progesterone inserts were administered.

Investigator	Location of Study	Breed
1. Dr. Michael Fields University of Florida Gainesville, FL	Heldon Brangus Ranch Morrison, FL & University of Florida Gainesville, FL	Brangus
2. Dr. Darrel Kesler University of Illinois Urbana, IL	University of Illinois Urbana, IL & Baylis, IL	Angus & Angus/Simmental
3. Dr. James Kinder University of Nebraska Lincoln, NE	University of Nebraska Mead, NE	Angus, Hereford, Pinzgauer, Red Poll Crosses
4. Dr. Matthew Lucy University of Missouri Columbia, MO	University of Missouri Columbia, MO	Angus, Simmental
5. Dr. Robert Short USDA, ARS Miles City, MT	Miles City, MT	Red Angus, Charlois, Tarentaise Crosses or Hereford, Angus, Simmental Crosses
6. Dr. Robert Wettemann Oklahoma State Univ. Stillwater, OK	Oklahoma State University Stillwater, OK	Hereford and Hereford/Angus

The subject cattle were suckled beef cows at least 20 days postpartum, entering the study when the principal investigator estimated that about one-half had begun postpartum estrous cycles. Cattle were determined to be cycling or non-cycling (anestrous) by retrospective determination of plasma progesterone concentration in blood samples collected 7 days before (study day -7) and on the day of administration of intravaginal progesterone inserts (study day 0). Cows were managed in the facilities normally used for cattle at each site, which were

representative of facilities on commercial beef operations in the respective localities. Cows at each site were assigned at random to one of three treatments:

1. Untreated controls,
2. Dinoprost tromethamine (5 mL LUTALYSE® Sterile Solution, equivalent to 5 mg/mL dinoprost),
3. Intravaginal progesterone insert (1.38 g progesterone) administered for 7 days with an injection of dinoprost tromethamine (5 mL Lutalyse® Sterile Solution, equivalent to 5 mg/mL dinoprost) administered on day 6 of the 7 day administration period (hereafter referred to as the insert+dinoprost treatment).

Health status of all cattle was documented at weekly scheduled health observation periods. In addition, health abnormalities were documented at any time when detected during other observation periods (i.e., during estrous detection). Cattle were observed for signs of estrus twice daily for 31 days after the inserts were removed. They were artificially inseminated about half a day after first observed in estrus, and pregnancy was determined by transrectal palpation or real-time ultrasonography.

3.1.2 Statistical Analyses

Survival analyses were used to evaluate the effects of treatment on interval to estrus. Cattle that were not observed in estrus during the first 31 days after removal of the intravaginal progesterone insert were considered censored for the interval to estrus analysis. The Cox proportional hazards model was used for the analysis. The model included terms for site, treatment and site by treatment interaction. The analysis of the binary variables related to synchrony of estrus and pregnancy rate was conducted using a generalized linear mixed model with the logit link and binomial error distribution. The model included the random effect of site, fixed effect of treatment and random interaction of site by treatment. Interestrous interval was analyzed using mixed model analysis of variance. The model included terms for the random effect of site, the fixed effect of treatment and the random effect of treatment by site interaction.

3.1.3 Results

A synopsis of the results is listed in Table 1. Table 2a shows the numbers of cows assigned to each treatment, and the number of cows included in the analyses for estrous synchrony and pregnancy rate for each location. As listed in Table 2b, 13 cows were lost from the estrous synchrony data and an additional 11 cows were lost from the study from the time of AI to pregnancy diagnosis. These losses, principally due to missing data, did not affect the conclusions because they were distributed evenly among treatments. The distribution of estrus after treatments (Table 3) revealed estrous synchrony as normally expected after a single treatment with dinoprost tromethamine, and improved synchrony from the concurrent use of intravaginal progesterone inserts and dinoprost tromethamine.

Table 1. Beef Cows Summary: Median Interval to Estrus in Days, Percent in Estrus During Study Days 8-10 or Study Days 8-9, and Percent Pregnant Following Removal of Intravaginal Progesterone Inserts

Criterion	Untreated Controls	Dinoprost Trom.	Insert + Dino. Trom.
	Median (N)		
Interval to estrus ^a	14.0 (285)	10.0 (283)	2.0 (294)
Interval to estrus ^b	15.0 (151)	13.5 (154)	5.0 (148)
	Average ± SE (N)		
% in estrus d 8-10 ^c	13 ± 4 (285)	32 ± 5 (283)	58 ± 7 (294)
% in estrus d 8-9 ^c	8 ± 2 (285)	26 ± 4 (283)	51 ± 5 (294)
% Pregnant	45 ± 4 (281)	49 ± 4 (280)	50 ± 4 (290)

a All cows

b Anestrous cows

c Inserts were removed on day 7

Tables 4 through 9 list the results for each criterion of response, including data for each site and the statistical analyses. The insert+dinoprost treatment reduced the median interval to first estrus (Table 4) when compared with untreated controls ($P < .001$), and with cows given dinoprost tromethamine alone ($P = .005$). Among cows anestrous at the outset of the study, the insert+dinoprost reduced the interval to first estrus (Table 5) when compared to controls ($P = .024$), but not to cows given dinoprost tromethamine ($P = .179$). The insert also improved the synchrony of estrus as measured by the numbers of cows in estrus on days 8, 9 and 10 (Table 6) and on days 8 and 9 (Table 7), both when compared to untreated controls and to cows given dinoprost tromethamine alone ($P \leq .001$).

Pregnancy rate to first services during the 31 day AI period (Table 8) was not affected by the inserts when compared to untreated controls ($P = .252$) or to cows given dinoprost tromethamine alone ($P = .793$). Among cows not conceiving on the first insemination, the interval to the second estrus (Table 9) averaged 20.9 days for cows given inserts+dinoprost, consistent with the accepted normal length of the bovine estrous cycle.

Investigators reported slight vaginal discharge (one site), some purulent mucus on the inserts when they were removed (two sites), or some cream to yellow mucus on the inserts when they were removed (one site), probably reflecting vaginal irritation. The frequency of these observations ranged from 0 at two sites to about 80% at another. Subsequent fertility was not impaired.

In addition to this clinical study, the sponsor reported a summary of 17 ancillary studies for beef cows (see Section 3.2); 16 studies observed cows for signs of vaginal irritation, with a total of 2,938 cows in Florida, Illinois, Kentucky, Montana, Nebraska, New Jersey, Ohio, and West Virginia. Signs of vaginal irritation were reported in a small proportion of the cows in the ancillary studies.

Among the 298 cows given inserts in the efficacy study, 12 (4.0%) inserts were lost before scheduled removal on day 7 of treatment. The losses ranged from 0 at one site to as high as 8% at another. These cattle were included in the statistical analyses of the data for this study.

The 17 ancillary studies also reported incidence of insert loss. Among a total of 2,991 cows given inserts, 125 (4.2%) lost inserts, ranging from 0 (seven sites) to 7% among the seventeen sites. No relationship could be established between these losses and size, age, body condition, management or environment of the cattle.

3.1.4 Conclusions

The concurrent use of intravaginal progesterone inserts and dinoprost tromethamine effectively synchronized estrus by comparison with dinoprost tromethamine treatment alone in suckled beef cows. In suckled anestrous beef cows the intravaginal progesterone insert effectively advanced the first postpartum estrus in comparison to the control group. The fertility of cattle given intravaginal progesterone inserts concurrently with dinoprost tromethamine was not reduced when compared to the other 2 treatment groups. Losses of intravaginal progesterone inserts during the recommended 7-day insert period averaged 4.0%. Mild vaginitis, evident among some of the cattle when the inserts were removed at four of the six study sites, was not evident at the time of inseminations a few days later because abnormal vaginal discharge was not observed.

Table 2a. Numbers of Suckled Beef Cows Initially Assigned, and Numbers Included in Analyses for Estrous Synchrony and Pregnancy Rate at Each Site

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a
	[No. Assigned/No. Synchrony/No. Pregnancy]		
1	49/48/47	47/47/47	50/50/50
2	47/46/46	48/47/47	51/50/49
3	50/50/50	50/50/50	50/50/50
4	45/44/41	45/45/43	45/44/42
5	48/48/48	47/47/46	52/52/51
6	51/49/49	50/47/47	50/48/48
Total	290/285/281	287/283/280	298/294/290

a Includes animals that lost inserts during the study.

Table 2b. Cows Removed from Study, When Removed and the Reason

Site	Trmt ^a	Cow	When Removed ^b	Reason for Removal
1	1	300D	Before	Missing progesterone data ^c
		92Z	After	Died
2	1	9666	Before	Missing data ^c
	2	8404	Before	Missing data ^c
	3	9622	Before	Missing data ^c
		368	After	Died
3		none		
4	1	476X	Before	Poor health
		2102	After	Missing pregnancy data ^c
		468B	After	Missing pregnancy data ^c
		7089	After	Missing pregnancy data ^c
	2	277	After	Missing pregnancy data ^c
		825	After	Missing pregnancy data ^c
	3	2109	Before	Lost calf ^c
		558D	After	Missing pregnancy data ^c
		4C081	After	Missing pregnancy data ^c
5	2	93679	After	Missing pregnancy data ^c
	3	93619	After	Missing pregnancy data ^c
6	1	678	Before	Died
		862	Before	Missing progesterone data ^c
	2	1500	Before	Missing progesterone data ^c
		8632	Before	Missing progesterone data ^c
		453	Before	Missing progesterone data ^c
	3	1400	Before	Missing progesterone data ^c
		783	Before	Calf died ^c

^a Treatment 1 = untreated controls, 2 = dinoprost tromethamine alone and 3 = insert+dinoprost tromethamine.

^b Removed from the study either before or after observation for estrus.

^c Protocol deviation.

Table 3. Distribution of Estrus for Beef Cows

Day^a	Untreated Controls (n=285)	Dinoprost Tromethamine (n=283)	Insert + Dinop. Trom.^b (n=294)
8	8	39	33
9	14	34	117
10	16	17	21
11	4	10	15
12	11	9	8
13	7	13	4
14	13	8	0
15	6	2	0
16	14	3	0
Total (%) in estrus^c	93 (33)	135 (48)	198 (67)

a Inserts removed on day 7.

b Includes animals that lost inserts during the study.

c During the first 9 days after insert removal.

Table 4. Interval to Estrus^a for All Beef Cows

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^b
[median days to estrus (no. in estrus/no. available)]			
1	13.0 (36/48)	9.0 (36/47)	2.0 (44/50)
2	11.5 (42/46)	6.0 (39/47)	2.0 (44/50)
3	10.0 (46/50)	3.0 (48/50)	2.0 (48/50)
4	13.5 (34/44)	17.0 (36/45)	3.0 (38/44)
5	19.0 (26/48)	16.0 (28/47)	32.0 ^c (25/52)
6	31.0 (26/49)	22.0 (31/47)	4.5 (33/48)
Median(Total/Total)	14.0 (210/285)	10.0 (218/283)	2.0 (232/294)
Proportional Hazards Analysis			
Source	Chi-Square	DF	P-Value
DaysPP	3.14	1	0.077
Site	103.83	5	<0.001
Treatment	26.35	2	<0.001
Insert vs Control	26.21	1	<0.001
Insert vs Dinop. Trom.	8.02	1	0.005
Site*Treatment ^d	10.43	10	0.404

a Interval to estrus is the number of days from study day 7 (when inserts were removed) to the day seen in estrus. If cows were not seen in estrus (through day 31), a value of 32 was assigned and these observations were considered censored.

b Includes animals that lost inserts during the study.

c Because over 50% of the cows were not seen in estrus, the median = 32 (the value of the censored observations).

d Site by treatment interaction was dropped from the model before calculation of the testing of the remaining effects.

Table 5. Interval to Estrus^a for Anestrous Beef Cows

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^b
[median days to estrus (no. in estrus/no. available)]			
1	13.0 (18/26)	17.0 (15/25)	2.0 (23/29)
2	7.0 (5/5)	6.0 (13/14)	3.0 (5/5)
3	10.0 (31/34)	4.0 (32/34)	2.0 (31/33)
4	14.0 (16/22)	17.0 (13/19)	22.0 (10/16)
5	25.5 (16/32)	32.0 ^c (13/29)	32.0 ^c (12/34)
6	32.0 ^c (15/32)	30.0 (18/33)	22.0 (17/31)
Median(Total/Total)	15.0 (101/151)	13.5 (104/154)	5.0 (98/148)
Proportional Hazards Analysis			
Source	Chi-Square	DF	P-Value
DaysPP	0.001	1	0.980
Site	95.16	5	<0.001
Treatment	5.15	2	0.076
Insert vs Control	5.11	1	0.024
Insert vs Dinop. Trom.	1.81	1	0.179
Site*Treatment ^d	10.69	10	0.382

- a Interval to estrus is the number of days from study day 7 (when inserts were removed) to the day seen in estrus. If cows were not seen in estrus (through day 31), a value of 32 was assigned and these observations were considered censored.
- b Includes animals that lost inserts during the study.
- c Because over 50% of the cows were not seen in estrus, the median = 32 (the value of the censored observations).
- d Site by treatment interaction was dropped from the model before calculation of the testing of the remaining effects.

Table 6. Beef Cows in Estrus on Days 8, 9, and 10 (Inserts Removed on Day 7)

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a		
[no. in estrus/total available (% in estrus)]					
1	3/48 (6)	14/47 (30)	33/50 (66)		
2	10/46 (22)	17/47 (36)	37/50 (74)		
3	14/50 (28)	27/50 (54)	38/50 (76)		
4	5/44 (11)	7/45 (16)	25/44 (57)		
5	4/48 (8)	13/47 (28)	15/52 (29)		
6	2/49 (4)	12/47 (26)	23/48 (48)		
Total	38/285 (13)	90/283 (32)	171/294 (58)		
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
DaysPP	1		4.74	843	0.030
Site	5	0.3463			
Treatment	2		40.02	10	<0.001
Insert vs Control	1		78.10	10	<0.001
Insert vs Dinop. Trom.	1		25.24	10	0.001
Site*Treatment	10	0.0649			
Extra-Dispersion ^c		0.9823			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value.

c The closer to 1.0 this value, the better the model fit.

Table 7. Beef Cows in Estrus on Days 8 and 9 (Inserts Removed on Day 7)

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a		
[no. in estrus/total available (% in estrus)]					
1	3/48 (6)	12/47 (26)	28/50 (56)		
2	7/46 (15)	14/47 (30)	34/50 (68)		
3	5/50 (10)	19/50 (38)	29/50 (58)		
4	1/44 (2)	5/45 (11)	21/44 (48)		
5	4/48 (8)	13/47 (28)	15/52 (29)		
6	2/49 (4)	10/47 (21)	23/48 (48)		
Total/total (avg)	22/285 (8)	73/283 (26)	150/294 (51)		
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
DaysPP	1		2.05	843	0.152
Site	5	0.1399			
Treatment	2		43.76	10	<0.001
Insert vs Control	1		83.35	10	<0.001
Insert vs Dinop. Trom.	1		26.33	10	<0.001
Site*Treatment	10	0.0463			
Extra-Dispersion ^c		0.9805			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value

c The closer to 1.0 this value, the better the model fit.

Table 8. Beef Cow Pregnancy Rates to the First Service

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a		
[no. pregnant/total available (% pregnant)]					
1	22/47 (47)	24/47 (51)	29/50 (58)		
2	28/46 (61)	29/47 (62)	31/49 (63)		
3	19/50 (38)	23/50 (46)	23/50 (46)		
4	22/41 (54)	25/43 (58)	19/42 (45)		
5	16/48 (33)	15/46 (33)	18/51 (35)		
6	20/49 (41)	22/47 (47)	26/48 (54)		
Total/total (avg)	127/281 (45)	138/280 (49)	146/290 (50)		
Mixed Logistic Regression					
Source	DF	Vari- ance	F-Value	DDF ^b	P-Value
Days PP	1		0.40	832	0.528
Site	5	0.1133			
Treatment	2		0.81	10	0.472
Insert vs Control	1		1.48	10	0.252
Insert vs Dinop. Trom.	1		0.07	10	0.793
Site*Treatment	10	0.0000			
Extra-Dispersion ^c		0.9996			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value

c The closer to 1.0 this value, the better the model fit.

Table 9. Interestrous Interval Analysis for Beef Cows

Site	Untreated Controls			Dinoprost Tromethamine			Insert + Dinop. Trom. ^a		
	N	Avg	Std	N	Avg	Std	N	Avg	Std
1	1	17.0	-	5	16.0	5.7	7	21.3	2.6
2	3	21.0	1.0	5	14.6	8.4	8	21.3	2.5
3	8	15.1	7.4	12	17.2	7.1	12	20.1	2.1
4	6	19.8	5.6	4	17.3	7.5	7	22.3	3.7
5	3	18.0	8.5	3	22.3	2.1	3	19.3	1.5
6	3	19.3	0.6	2	19.0	2.8	4	20.8	1.0
Across Site	24	18.0	5.9	31	17.2	6.5	41	20.9	2.5
Analysis of Variance									
Source	DF	Variance	F-Value	DDF ^b	P-Value				
Site	5	0.1733							
Treatment	2		6.40	49.2	0.003				
Insert vs Control	1		5.29	27.3	0.029				
Insert vs Dinop. Trom.	1		8.73	36.2	0.006				
Site*Treatment	10	0.0000							
Residual ^c	78								
Trt I		6.3028							
Trt N		34.3494							
Trt P		42.9518							

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value

c The residual variance was found to be heterogeneous among treatments and was partitioned by treatment using the group=treatment option on the repeated statement of proc mixed. However the residuals were also found to be highly non-normal with a possible bimodal pattern.

3.2 Ancillary Studies with Beef Cows

Seventeen ancillary studies with beef cows were conducted in the U.S. (Florida, Illinois, Kentucky, Montana, Nebraska, New Jersey, Ohio, Texas and West Virginia) in which observation on insert loss and vaginal irritation were reported. Table 10 summarizes this information for each of the ancillary studies.

Table 10. Summary of Loss Rates and Incidence of Vaginal Irritation or Vaginitis Reported in Beef Cows Administered Intravaginal Progesterone Inserts in Ancillary Studies

Study #	Location	# Cows Administered Inserts	# of Inserts Lost*	# Cows with Vaginal Irritation or Vaginitis
1	Ohio	97	3	0
2	Nebraska	23	0	0
3	Nebraska	8	0	0
4	Texas	16	0	5
5	Montana	53	1	Most
6	West Virginia	111	0	0
7	Nebraska	116	0	0
8	Montana	133	5	0
9	Kentucky	524	32	2
10	Ohio	636	32	0
11	West Virginia	126	5	1
12	West Virginia	139	9	0
13	Illinois	412	28	0
14	New Jersey	14	1	0
15	New Jersey	14	0	0
16	New Jersey	88	0	0
17	Florida	481	9	0

* Number of inserts missing on day of removal.

3.2.1 Conclusions for ancillary studies conducted in beef cows.

Across the 17 ancillary studies, approximately 4% of cows administered inserts experienced insert loss, in close agreement with what was noted in the clinical effectiveness study. While vaginal irritation reported in the ancillary studies in beef cows appeared to be minimal, the studies do lend support to a label statement with respect to vaginal irritation.

3.3 Clinical study in beef heifers

3.3.1 Methods

A study was conducted at the following five sites with a total of 763 beef heifers. The objective of this study was to determine the effect of the intravaginal progesterone inserts given concurrently with an injection of dinoprost tromethamine on the interval to first pubertal estrus, the synchrony of estrus, and pregnancy in all of the test heifers and in heifers that were anestrous when the inserts were administered.

Investigator	Location of Study	Breed
1. Dr. Michael Fields University of Florida Gainesville, FL	Heldon Brangus Ranch Morrison, FL & University of Florida Gainesville, FL	Brangus
2. Dr. Darrel Kesler University of Illinois Urbana, IL	University of Illinois Urbana, IL & Baylis, IL	Angus & Angus/Simmental
3. Dr. James Kinder University of Nebraska Lincoln, NE	University of Nebraska Mead, NE	Angus, Hereford, Pinzgauer, Red Poll Crosses
4. Dr. Matthew Lucy University of Missouri Columbia, MO	University of Missouri Columbia, MO	Angus, Simmental
5. Dr. Robert Short USDA, ARS Miles City, MT	Livestock & Range Res. Lab. Miles City, MT	Red Angus, Charlois, Tarentaise Crosses, or Hereford, Angus, Simmental Crosses

Beef heifers were chosen that were of size and age appropriate for breeding, and entered the study when the principal investigator estimated that about one-half had begun postpubertal estrous cycles. Cattle were determined retrospectively to have begun estrous cycles or to remain prepubertal on the basis of plasma progesterone concentration from blood samples collected 7 days prior to and on the day of administration of the intravaginal progesterone inserts. Heifers were managed in the facilities normally used for cattle at each site, and were representative of facilities in the geographical region where each study was conducted. Heifers at each site were assigned at random to one of three treatments:

1. Untreated controls,
2. Dinoprost tromethamine (5 mL LUTALYSE® Sterile Solution, equivalent to 5 mg/mL dinoprost), or
3. Intravaginal progesterone insert (1.38 g progesterone) administered for 7 days with and injection of dinoprost tromethamine (5 mL LUTALYSE® Sterile Solution, equivalent to 5 mg/mL dinoprost) administered on day 6 of the 7-day administration period (here after referred to as the insert+dinoprost treatment).

Health status of all cattle was documented at weekly scheduled health observation periods. In addition, health abnormalities were documented at any time when detected during other observations periods (i.e., during estrous detection). Heifers were observed for signs of estrus twice daily for 31 days after the inserts were removed. They were inseminated artificially about half a day after first observed in estrus, and pregnancy was determined by transrectal palpation or real-time ultrasonography.

3.3.2 Statistical Analyses

Survival analyses were used to evaluate the effects of treatment on interval to estrus. Cattle that were not observed in estrus during the first 31 days after removal of the intravaginal progesterone insert were considered censored for the interval to estrus analysis. The Cox proportional hazards model was used for the analysis. The model included terms for site, treatment and site by treatment interaction. The analysis of the binary variables related to synchrony of estrus and pregnancy rate was conducted using a generalized linear mixed model with the logit link and binomial error distribution. The model included the random effect of site, fixed effect of treatment and random interaction of site by treatment. Interestrous interval was analyzed using mixed model analysis of variance. The model included terms for the random effect of site, the fixed effect of treatment and the random effect of treatment by site interaction.

3.3.3 Results

A synopsis of the results is listed in Table 11. Table 12a shows the numbers of heifers assigned to each treatment at each site, and the number available for estrous, synchrony and pregnancy observations. As listed in Table 12b, 12 heifers were lost from the estrous synchrony data and an additional 14 heifers were lost in the interval from AI to pregnancy diagnosis. These losses were principally associated with investigator errors or inconclusive information on whether the heifers were cycling at the outset. These losses did not influence the conclusions from this study, because they were distributed similarly across treatment groups. The distribution of estrus after treatments (Table 13) reveals estrous synchrony as normally expected after a single treatment with dinoprost tromethamine, and improved synchrony from the concurrent administration of the inserts+dinoprost.

Table 11. Beef Heifers Summary: Median Interval to Estrus, Percent in Estrus During the Days 8-10 or on days 8-9, and Percent Pregnant Following Removal of Intravaginal Progesterone Inserts

Criterion	Untreated Controls	Dinoprost Tromethamine	Insert + Dino. Trom.
	Median (n)		
Interval to estrus ^a	15.0 (251)	16.0 (252)	2.0 (248)
Interval to estrus ^b	27.0 (107)	>31.0 ^c (101)	14.0 (119)
	Average ± SE (n)		
% in estrus d 8-10 ^d	12 ± 3 (251)	25 ± 7 (252)	60 ± 9 (248)
% in estrus d 8-9	8 ± 3 (251)	22 ± 7 (252)	52 ± 8 (248)
% Pregnant	42 ± 8 (246)	36 ± 7 (244)	47 ± 7 (247)

a All heifers

b Anestrus heifers

c More than half the heifers had interval to estrus greater than 31 days.

d Inserts were removed on day 7.

Tables 14 through 19 list the results for each criterion of response including data for each site and the statistical analyses. The insert+dinoprost treatment reduced the median interval in days to first estrus when compared to untreated controls ($P < .001$) and to heifers given dinoprost tromethamine alone ($P < .001$) for all heifers (Table 14), and among heifers deemed prepubertal (Table 15) at the outset of the study. Also, the insert+dinoprost treatment improved the synchrony of estrus when compared to untreated controls ($P < .001$) and to heifers given dinoprost tromethamine alone ($P < .001$) for heifers in estrus on days 8, 9 and 10, as well as for those in estrus on days 8 and 9 ($P < .001$ and $P = .002$, respectively; Tables 16 and 17).

Pregnancy rate to first inseminations during the 31 day AI period (Table 18) of the heifers in the insert+dinoprost treatment was higher than in heifers given dinoprost tromethamine alone ($P = .029$), but not significantly different from that in controls ($P = .315$). Among the heifers that did not conceive on the first insemination, the interval to the second estrus did not differ ($P = .929$) among the three treatments, nor from that normally expected (Table 19).

Investigators reported slight vaginal discharge (one site), some purulent mucus on the inserts when they were removed (one site), or some cream to yellow mucus on the inserts when they were removed (one site), probably reflecting mild vaginal irritation. The frequency of these observations ranged from 0 at two sites to about one-third of the heifers at another. No signs of vaginal irritation were detected when the same cattle were inseminated a few days later,

and fertility was not adversely affected in insert+dinoprost group vs. the other two treatment groups.

In addition to the clinical study, the sponsor summarized data from 20 ancillary studies with beef heifers. These studies were conducted in Australia, Florida, Illinois, Kansas, Kentucky, Montana, Ohio, Oklahoma, Nebraska, New Jersey and Texas. Eighteen studies quantified observations for signs of vaginal irritation, with a total of 1,892 heifers (see Section 3.4 below). Signs of vaginal irritation were reported in a small number of heifers in the ancillary studies.

Among the 251 heifers that were administered inserts in the clinical studies, 27 (10.4%) lost inserts before they were to be removed. The losses ranged from 5% to 21% among the five sites. No relationship was established between these losses and size, age, body condition, management or environment of the cattle, except that losses were greatest at the site with the highest housing density of heifers. These cattle were included in the statistical analyses of the data, just as all of the other cattle. Among the 20 ancillary studies, 2,276 heifers were observed for losses of inserts. A total of 65 (2.9%) of the inserts was lost from these heifers, ranging from 0 (six sites) to 6% among the 20 studies.

3.3.4 Conclusions

The concurrent use of intravaginal progesterone inserts and dinoprost tromethamine effectively synchronized estrus when compared to beef heifers given dinoprost tromethamine treatment alone. In prepubertal heifers administration of intravaginal progesterone insert advanced the pubertal estrus compared to heifers in the control and dinoprost tromethamine alone groups. First service pregnancy rate over 31 days of artificial insemination of heifers given intravaginal progesterone inserts concurrently with dinoprost tromethamine did not differ from heifers in the control group and was higher than that observed in heifers in the dinoprost tromethamine alone group. Losses of intravaginal progesterone inserts during the recommended 7-day insert period averaged 10.4%. Mild vaginitis was evident among some of the heifers when the inserts were removed at four of the five study sites, but was not related to fertility. In conclusion, the intravaginal progesterone inserts are safe and effective to synchronize estrus and advance the date of the pubertal estrus in beef heifers when used as labeled.

Table 12a. Numbers of Beef Heifers Initially Assigned, and Remaining to be Used for Estrous Synchrony and for Pregnancy for Each Treatment at Each Site

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a
	[No. Assigned/No. Synchrony/No. Pregnancy]		
1	47/47/47	49/48/48	48/47/47
2	52/52/52	51/51/51	52/52/52
3	44/42/42	43/42/42	43/42/42
4	52/50/47	53/50/50	52/51/51
5	60/60/58	61/61/53	56/56/55
Total	255/251/246	257/252/244	251/248/247

a Includes animals that lost inserts during the Study.

Table 12b. Beef Heifers Removed from Study, When Removed and the Reason

Site	Trmt ^a	Heifer	When Removed ^b	Reason for Removal
1	2	128E	Before	Pregnant at outset of study ^c
	3	3950913	Before	Failed to remove insert as scheduled ^c
2		none		
3	1	6031	Before	Technical error ^c
		6051	Before	Technical error ^c
	2	6004	Before	Freemartin
	3	3050	Before	Freemartin
4	1	1F218	Before	Inconclusive progesterone data ^c
		2F086	Before	Inconclusive progesterone data ^c
		631	After	Failed to inseminate at estrus ^c
		1F010	After	Pregnancy diagnosis unreliable ^c
		4F121	After	Pregnancy diagnosis unreliable ^c
	2	1F044	Before	Inconclusive progesterone data ^c
		1F167	Before	Inconclusive progesterone data ^c
		2F091	Before	Inconclusive progesterone data ^c
	3	743	Before	Freemartin
5	1	96261	After	Injected Lutalyse by error after AI ^c
		96263	After	Injected Lutalyse by error after AI ^c
	2	96045	After	Injected Lutalyse by error after AI ^c
		96220	After	Injected Lutalyse by error after AI ^c
		96268	After	Injected Lutalyse by error after AI ^c
		96X25	After	Injected Lutalyse by error after AI ^c
		96X53	After	Injected Lutalyse by error after AI ^c
		96X70	After	Injected Lutalyse by error after AI ^c
		96X71	After	Injected Lutalyse by error after AI ^c
	96X87	After	Injected Lutalyse by error after AI ^c	
3	96X22	After	Injected Lutalyse by error after AI ^c	

^a Treatment 1 = untreated controls, 2 = dinoprost tromethamine alone and 3 = insert+dinoprost tromethamine.

^b Before or after observation for estrus.

^c Protocol deviation.

Table 13. Distribution of Estrus for Beef Heifers

Day^a	Untreated Controls (n=251)	Dinoprost Tromethamine (n=252)	Insert + Dinop. Trom.^b (n=248)
8	9	36	30
9	10	20	98
10	11	8	21
11	12	9	5
12	8	5	2
13	4	5	2
14	14	5	1
15	3	2	0
16	17	1	2
Total (%) in estrus^c	88 (35)	91 (36)	161 (65)

a Inserts removed on day 7.

b Includes animals that lost inserts during the study.

c During the first 9 days after insert removal.

Table 14. Interval to Estrus^a for All Beef Heifers

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^b
[median days to estrus (no. in estrus/no. available)]			
1	11.0 (41/47)	13.5 (43/48)	2.0 (44/47)
2	14.0 (43/52)	13.0 (40/51)	2.0 (45/52)
3	23.0 (26/42)	29.0 (22/42)	7.5 (34/42)
4	9.0 (47/50)	9.0 (43/50)	2.0 (50/51)
5	32.0 ^c (26/60)	32.0 ^c (30/61)	23.0 (30/56)
Median(Total/Total)	15.0 (183/251)	16.0 (178/252)	2.0 (203/248)
Proportional Hazards Analysis			
Source	Chi-Square	DF	P-Value
Site	114.80	4	<0.001
Treatment	35.83	2	<0.001
Insert vs Control	27.99	1	<0.001
Insert vs Dinop. Trom.	25.05	1	<0.001
Site*Treatment ^d	5.56	8	0.697

- a Interval to estrus is the number of days from study day 7 (when the inserts were removed) to the day seen in estrus. If heifers were not seen in estrus (through day 31), a value of 32 was assigned and these observations were considered censored.
- b Includes animals that lost inserts during the study.
- c Because more than half of the heifers were not seen in estrus the median takes on the value of the censored observations equal to 32.
- d Site by treatment interaction was dropped from the model before testing of the remaining effects.

Table 15. Interval to Estrus^a for Anestrus Beef Heifers

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^b
[median days to estrus (no. in estrus/no. available)]			
1	9.5 (7/8)	9.5 (7/10)	3.5 (7/10)
2	26.0 (2/4)	32.0 ^c (1/3)	26.0 (2/4)
3	22.0 (22/35)	32.0 ^c (14/33)	14.5 (27/34)
4	14.0 (18/21)	9.0 (14/18)	2.0 (28/28)
5	32.0 ^c (9/39)	32.0 ^c (9/37)	32.0 ^c (20/43)
Median(Total/Total)	27.0 (58/107)	32.0 ^c (45/101)	14.0 (84/119)
Proportional Hazards Analysis			
Source	Chi-Square	DF	P-Value
Site	74.06	4	<0.001
Treatment	27.07	2	<0.001
Insert vs Control	15.86	1	<0.001
Insert vs Dinop. Trom.	21.52	1	<0.001
Site*Treatment ^d	7.46	8	0.488

- a Interval to estrus is the number of days from study day 7 (when inserts were removed) to the day seen in estrus. If heifers were not seen in estrus (through day 31), a value of 32 was assigned and these observations were considered censored.
- b Includes animals that lost inserts during the study.
- c Because over 50% of the heifers were not seen in estrus, the median = 32 (the value of the censored observations).
- d Site by treatment interaction was dropped from the model before calculation of the testing of the remaining effects.

Table 16. Beef Heifers in Estrus on Days 8, 9, and 10 (Inserts Removed on Day 7)

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a		
[no. in estrus/total available (% in estrus)]					
1	5/47 (11)	15/48 (31)	34/47 (72)		
2	6/52 (12)	19/51 (37)	36/52 (69)		
3	1/42 (2)	2/42 (5)	20/42 (48)		
4	11/50 (22)	21/50 (42)	41/51 (80)		
5	7/60 (12)	7/61 (11)	18/56 (32)		
Total	30/251 (12)	64/252 (25)	149/248 (60)		
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
Site	4	0.5881			
Treatment	2		43.45	8	<0.001
Insert vs Control	1		78.72	8	<0.001
Insert vs Dinop. Trom.	1		40.55	8	<0.001
Site*Treatment	8	0.0590			
Extra-Dispersion ^c		0.9896			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value

c The closer to 1.0 this value the better the model fit.

Table 17. Beef Heifers in Estrus on Days 8 and 9 (Inserts Removed on Day 7)

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a		
[no. in estrus/total available (% in estrus)]					
1	3/47 (6)	13/48 (27)	31/47 (66)		
2	3/52 (6)	17/51 (33)	28/52 (54)		
3	0/42 (0)	1/42 (2)	19/42 (45)		
4	9/50 (18)	20/50 (40)	36/51 (71)		
5	4/60 (7)	5/61 (8)	14/56 (25)		
Total	19/251 (8)	56/252 (22)	128/248 (52)		
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
Site	4	0.5973			
Treatment	2		29.68	8	<0.001
Insert vs Control	1		55.69	8	<0.001
Insert vs Dinop. Trom.	1		22.28	8	0.002
Site*Treatment	8	0.1403			
Extra-Dispersion ^c		0.9497			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value.

c The closer to 1.0 this value the better the model fit.

Table 18. Beef Heifer Pregnancy Rates to the First Service

Site	Untreated Controls	Dinoprost Tromethamine	Insert + Dinop. Trom. ^a		
[no. pregnant/total available (% pregnant)]					
1	24/47 (51)	26/48 (54)	28/47 (60)		
2	25/52 (48)	16/51 (31)	21/52 (40)		
3	10/42 (24)	7/42 (17)	19/42 (45)		
4	30/47 (64)	26/50 (52)	33/51 (65)		
5	15/58 (26)	13/53 (25)	16/55 (29)		
Total	104/246 (42)	88/244 (36)	117/247 (47)		
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
Site	4	0.3913			
Treatment	2		3.54	8	0.079
Insert vs Control	1		1.15	8	0.315
Insert vs Dinop. Trom.	1		7.01	8	0.029
Site*Treatment	8	0.0000			
Extra-Dispersion ^c		0.9968			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value.

c The closer to 1.0 this value the better the model fit.

Table 19. Interestrous Interval Analysis for Beef Heifers

Site	Untreated Controls			Dinoprost Tromethamine			Insert + Dinop. Trom. ^a		
	N	Ave	Std	N	Ave	Std	N	Ave	Std
1	3	13.0	10.0	6	19.8	1.5	9	20.1	5.0
2	10	16.7	5.4	14	18.3	3.3	20	18.9	5.1
3	3	19.3	3.5	6	15.5	6.6	5	14.0	5.5
4	10	17.4	4.8	9	16.4	7.4	17	15.2	6.5
5	2	20.0	1.4	6	17.2	5.2	8	19.1	1.8
Across Site	28	17.1	5.4	41	17.5	5.1	59	17.6	5.5
Analysis of Variance									
Source	DF	Variance	F-Value	DDF ^b	P-Value				
Site	4	0.1782							
Treatment	2		0.07	8	0.929				
Insert vs Control	1		0.15	8	0.713				
Insert vs Dinop. Trom.	1		0.01	8	0.927				
Site*Treatment	8	0.7973							
Residual ^c	113	27.8500							

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value

c The residuals were found to be highly non-normal with a possible bimodal pattern.

3.4 Ancillary Studies with Beef Heifers

Twenty ancillary studies with beef heifers were conducted (Australia, Florida, Illinois, Kansas, Kentucky, Montana, Ohio, Oklahoma, Nebraska, New Jersey and Texas) in which observations on insert loss and vaginal irritation were reported. Table 20 summarizes this information for each of the ancillary studies.

Table 20. Summary of Loss Rates and Incidence of Vaginal Irritation or Vaginitis Reported in Beef Heifers Administered Intravaginal Progesterone Inserts in Ancillary Studies

Study #	Location	# Heifers Administered Inserts	# of Inserts Lost*	# Heifers with Vaginal Irritation or Vaginitis
1	Nebraska	8	1	8
2	Montana	62	5	Most
3	Florida	8	0	0
4	Nebraska	88	5	5
5	Kansas	40	0	15%
6	Ohio	60	1	1
7	Kansas	43	0	4
8	Nebraska	85	1	0
9	Montana	76	4	0
10	Oklahoma	68	1	0
11	Kentucky	212	15	1
12	Ohio	184	6	0
13	Illinois	287	10	0
14	Florida	60	0	0
15	U. of Florida	313	9	0
16	New Jersey	12	0	0
17	New Jersey	9	1	2
18	New Jersey	51	3	6
19	Florida	288	3	1
20	Australia	322	0	Not Reported

* Number of inserts missing on day of removal.

Conclusions from Ancillary Studies with Beef Heifers

Across the 20 ancillary studies, approximately 3% of heifers administered inserts experienced insert loss, less than what was seen in the clinical study. These ancillary data indicate with a larger number of heifers than in the clinical study, insert loss is a manageable concern for the producer. While vaginal irritation reported in the ancillary studies in beef

heifers appears to be minimal, the studies do lend support to a label statement with respect to vaginal irritation.

3.5 Clinical study in dairy heifers

3.5.1 Methods

A study was conducted at the following four sites with a total of 275 cycling Holstein heifers. The objective of this study was to determine the effect of the intravaginal progesterone insert given concurrently with an injection of dinoprost tromethamine on the synchrony of estrus and pregnancy.

Investigator	Location of Study	Breed
1. Dr. Ron Butler Cornell University Ithaca, NY	Cornell University Ithaca, NY	Holstein
2. Dr. Darrel Kesler University of Illinois Urbana, IL	University of Illinois Urbana, IL	Holstein
3. Dr. Matthew Lucy University of Missouri Columbia, MO	University of Missouri Columbia, MO	Holstein
4. Dr. William Thatcher University of Florida Gainesville, FL	Alliance Dairy Trenton, FL	Holstein

The heifers were chosen that were of age and size adequate for breeding. Whether or not the heifers were cycling at the outset of the study was determined retrospectively based on plasma progesterone concentration in blood samples collected 7 days before and on the day of administration of intravaginal progesterone inserts. Heifers were managed in the facilities normally used for cattle at each site which were representative of facilities in use for cattle in the geographical region the study was conducted. After they were determined fit for the study, heifers at each site were assigned at random to one of two treatments:

1. Dinoprost tromethamine (5 mL LUTALYSE® Sterile Solution, equivalent to 5 mg/mL dinoprost) or
2. Intravaginal progesterone inserts (1.38 g progesterone) administered for 7 days with an injection of dinoprost tromethamine (5 mL LUTALYSE® Sterile Solution, equivalent to 5 mg/mL dinoprost) administered on day 6 of the 7 day administration period (here after referred to as the insert+dinoprost treatment).

Health status of all heifers was documented at weekly scheduled health observation periods. In addition, health abnormalities were documented at any time when detected during other

observation periods (i.e., during estrous detection). Heifers were observed for signs of estrus twice daily for 31 days after the inserts were removed. They were inseminated artificially about half a day after first observed in estrus, and pregnancy was determined by transrectal palpation or real-time ultrasonography.

3.5.2 Statistical Analyses

The analysis of the binary variables related to synchrony of estrus and pregnancy rate was conducted using a generalized linear mixed model with the logit link and binomial error distribution. The model included the random effect of site, fixed effect of treatment and random interaction of site by treatment. Interestrous interval was analyzed using mixed model analysis of variance. The model included terms for the random effect of site, the fixed effect of treatment and the random effect of treatment by site interaction.

3.5.3 Results

A synopsis of the results is listed in Table 21. Table 22a shows the numbers of heifers assigned to each treatment, and number of heifers included in analyses for estrous synchrony and pregnancy rate for each location. As listed in Table 22b, 13 heifers were lost from the estrous synchrony data and 6 heifers were lost in the interval from the time of inseminations until pregnancy diagnosis. The principal reasons for these losses were a) the heifers were not cycling at the outset of the study as required in the study protocol, and b) failure to inseminate the heifers as scheduled. These losses did not likely affect the conclusions from this study, because they were distributed similarly between treatments.

Table 21. Dairy Heifers Summary: Percent in Estrus During Days 8-10 or days 8-9, and Percent Pregnant Following Removal of Intravaginal Progesterone Inserts

Criterion	Dinoprost Tromethamine	Insert + Dino. Trom.
	Average \pm SE (n)	
% in estrus d 8-10 ^a	59 \pm 4 (132)	83 \pm 3 (130)
% in estrus d 8-9	54 \pm 4 (132)	68 \pm 8 (130)
% Pregnant	51 \pm 10 (127)	54 \pm 8 (129)

a Inserts were removed on day 7

The distribution of estrus after treatments (Table 23) reveals estrous synchrony as normally expected after a single treatment with dinoprost tromethamine, and increased synchrony with concurrent administration of intravaginal progesterone inserts and dinoprost tromethamine.

Tables 24 through 27 list the results for each criterion of response including data for each site and the statistical analyses. The insert+dinoprost treatment significantly improved the synchrony of estrus compared with heifers given dinoprost tromethamine alone for heifers in estrus on days 8, 9 and 10 ($P = .025$; Table 24). However, this difference was not significant ($P = .084$; Table 25) for heifers in estrus on days 8 and 9.

Pregnancy rate to first services during the 31 day AI period of the heifers given inserts+dinoprost was not different from that in heifers given dinoprost tromethamine ($P = .648$; Table 26). Among the heifers that did not conceive on the first insemination, the interval to the second estrus did not differ ($P = .196$; Table 27) between the two treatments, nor from that normally expected. Among the 136 heifers given intravaginal progesterone inserts, 14 (10.3%) inserts were lost before they were scheduled to be removed. Eight of these were among 10 treated heifers in one pen at the Ithaca, New York site.

Nearly all the heifers at one site had purulent mucus on the inserts at insert removal, suggesting vaginal irritation. Fertility was not lower in the dinoprost+insert vs. dinoprost alone treatment groups.

The sponsor summarized information from ancillary studies conducted in Delaware and New Jersey that observed heifers for signs of vaginal irritation, with a total of 119 Holstein heifers (see Section 3.6 below). Signs of vaginal irritation were reported in a small proportion of heifers in the ancillary studies.

Among the 136 heifers that were administered inserts in the clinical study, 14 (10.3%) lost inserts before they were to be removed. The losses ranged from 0 (two sites) to 33% at one site, and no relationship was established between these losses and size, age, body condition, or management of the cattle, except that the density of the heifers was highest (in a free stall barn) at the site with the highest losses. The cattle that lost inserts were included in the statistical analyses of the data just as all of the other cattle. The sponsor summarized information from six ancillary studies in New Jersey, New Zealand, and Australia reporting losses of inserts (see Section 3.6 below). Among a total of 1,603 dairy heifers given inserts at these six sites, 20 (1.2%) of the inserts were lost, ranging from 0 (two sites) to 1.3%.

3.5.4 Conclusions

The concurrent use of intravaginal progesterone inserts and dinoprost tromethamine more effectively synchronized estrus than dinoprost tromethamine treatment alone in dairy heifers. Further, the intravaginal progesterone inserts are safe for dairy heifers, as there was only transient vaginal irritation and no reduction in fertility compared to cattle given dinoprost tromethamine alone. Losses of intravaginal progesterone inserts during the recommended 7-day insert period averaged 10.3%. Vaginal irritation was not evident at the time of inseminations a few days later and did not impair fertility when comparing pregnancy rates of the insert+dinoprost vs. dinoprost treatment groups. In conclusion, the intravaginal progesterone inserts when used as labeled are safe and effective to synchronize estrus in dairy heifers.

Table 22a. Numbers of Dairy Heifers Initially Assigned, and Remaining to be Used for Estrus Synchrony and for Pregnancy for Each Treatment at Each Site

Site	Dinoprost Tromethamine	Insert + Dinop. Trom.^a
	[No. Assigned/No. Synchrony/No. Pregnancy]	
1	30/30/27	30/30/30
2	16/16/16	16/16/16
3	30/29/27	30/30/30
4	63/57/57	60/54/53
Total	139/132/127	136/130/129

a Includes animals that lost inserts during the study.

Table 22b. Dairy Heifers Removed from Study, When Removed and the Reason

Site	Trmt ^a	Heifer	When Removed ^b	Reason for Removal
1	1	6290	After	Failed to inseminate at estrus ^c
		6301	After	Failed to inseminate at estrus ^c
		6313	After	Failed to inseminate at estrus ^c
2		none		
3	1	825	Before	Freemartin
		161	After	Failed to inseminate at estrus ^c
		190	After	Failed to inseminate at estrus ^c
4	1	3377	Before	Not cycling at the outset of study ^c
		3380	Before	Not cycling at the outset of study ^c
		3442	Before	Not cycling at the outset of study ^c
		3457	Before	Not cycling at the outset of study ^c
		3478	Before	Not cycling at the outset of study ^c
		6427	Before	Not cycling at the outset of study ^c
	2	3324	Before	Not cycling at the outset of study ^c
		3462	Before	Not cycling at the outset of study ^c
		3485	Before	Not cycling at the outset of study ^c
		3509	Before	Not cycling at the outset of study ^c
		4450	Before	Not cycling at the outset of study ^c
		6473	Before	Not cycling at the outset of study ^c
		4334	After	No pregnancy determination ^c

^a Treatment 1 = dinoprost tromethamine alone and 2 = insert+dinoprost tromethamine.

^b Removed from study either before or after observation for estrus.

^c Protocol deviation.

Table 23. Distribution of Estrus for Dairy Heifers

Day ^a	Dinoprost Tromethamine (n=132)	Insert + Dinop. Trom. ^b (n=130)
8	52	23
9	19	67
10	7	18
11	3	5
12	1	0
13	1	0
14	1	0
15	0	1
16	1	1
Total (%) in estrus ^c	85 (64)	115 (88)

- a Inserts removed on day 7.
- b Includes animals that lost inserts during the study.
- c During the first 9 days after insert removal.

Table 24. Dairy Heifers in Estrus on Days 8, 9, and 10 (Inserts Removed on Day 7)

Site	Dinoprost Tromethamine		Insert + Dinop. Trom. ^a		
	[no. in estrus/total available (% in estrus)]				
1	16/30 (53)		25/30 (83)		
2	9/16 (56)		11/16 (69)		
3	20/29 (69)		26/30 (87)		
4	33/57 (58)		46/54 (85)		
Total	78/132 (59)		108/130 (83)		
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
Site	3	0.0000			
Treatment (Insert vs Dinop. Trom.)	1		17.26	3	0.025
Site*Treatment	3	0.0000			
Extra-Dispersion ^c		1.0077			

- a Includes animals that lost inserts during the study.
- b DDF is the denominator degrees of freedom associated with the F-Value
- c The closer to 1.0 this value the better the model fit.

Table 25. Dairy Heifers in Estrus on Days 8 and 9 (Inserts Removed on Day 7)

Site	Dinoprost Tromethamine		Insert + Dinop. Trom. ^a		
	[no. in estrus/total available (% in estrus)]				
1	15/30 (50)				24/30 (80)
2	9/16 (56)				7/16 (44)
3	19/29 (66)				24/30 (80)
4	28/57 (49)				35/54 (65)
Total	71/132 (54)				90/130 (69)
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
Site	3	0.0817			
Treatment (Insert vs Dinop. Trom.)	1		6.48	3	0.084
Site*Treatment	3	0.0000			
Extra-Dispersion ^c		0.9978			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value.

c The closer to 1.0 this value the better the model fit.

Table 26. Dairy Heifer Pregnancy Rates to the First Service

Site	Dinoprost Tromethamine		Insert + Dinop. Trom. ^a		
	[no. pregnant/total available (% pregnant)]				
1	17/27 (63)				22/30 (73)
2	4/16 (25)				5/16 (31)
3	19/27 (70)				18/30 (60)
4	24/57 (42)				25/53 (47)
Total	64/127 (50)				70/129 (54)
Mixed Logistic Regression					
Source	DF	Variance	F-Value	DDF ^b	P-Value
Site	3	0.4877			
Treatment (Insert vs Dinop. Trom.)	1		0.26	3	0.648
Site*Treatment	3	0.0000			
Extra-Dispersion ^c		0.9962			

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value.

c The closer to 1.0 this value the better the model fit.

Table 27. Interestrous Interval Analysis for Dairy Heifers

Site	Dinoprost Tromethamine			Insert + Dinop. Trom. ^a		
	N	Ave	Std	N	Ave	Std
1	2	15.5	7.8	5	19.4	9.7
2	3	20.7	1.5	4	14.3	7.3
3	3	12.0	10.1	8	21.6	3.2
4	14	15.9	7.1	14	18.6	4.9
Across Site	22	16.0	7.1	31	18.9	6.0
Analysis of Variance						
Source	DF	Variance	F-Value	DDF ^b	P-Value	
Site	3	0.0000				
Treatment (Insert vs Dinop. Trom.)	1		2.75	3	0.196	
Site*Treatment	3	0.0000				
Residual ^c	45	41.6240				

a Includes animals that lost inserts during the study.

b DDF is the denominator degrees of freedom associated with the F-Value.

c The residuals were found to be highly non-normal with a possible bimodal pattern.

3.6 Ancillary studies with dairy heifers

Six ancillary studies with dairy heifers were conducted (Delaware, New Jersey, New Zealand and Australia) in which observation on insert loss and vaginal irritation were reported. Table 28 summarizes this information for each of the ancillary studies.

Table 28. Summary of Loss Rates and Incidence of Vaginal Irritation or Vaginitis Reported in Dairy Heifers Administered Intravaginal Progesterone Inserts in Ancillary Studies

Study #	Location	# Heifers Administered Inserts	# of Inserts Lost*	# Heifers with Vaginal Irritation or Vaginitis
1	Delaware	64	1	12
2	New Jersey	34	0	4
3	New Jersey	21	0	4
4 & 5	New Zealand	1,344	17	Not Reported
6	Australia	140	2	Not Reported

* Number of inserts missing on day of removal.

Insert loss prior to scheduled removal occurred in approximately 1% of heifers administered inserts. While the reported incidence of vaginal irritation was limited, occurrence of vaginal irritation in these ancillary reports supports a label statement with respect to this possibility.

3.6.1 Conclusions from Ancillary Studies with Dairy Heifers

Data from these ancillary studies, indicate that with a larger sample size than in the clinical study, that insert loss likely is manageable. In addition, these data support inclusion of a label statement on vaginal irritation.

4 TARGET ANIMAL SAFETY

4.1.1 Target Animal Safety Study

4.1.1.1 Investigator

Steven P. Washburn, PhD
 North Carolina State University
 Raleigh, North Carolina

Study No. B-90-14
 November 1990 to January 1991

4.1.1.2 Methods

This study was performed in accordance with Good Laboratory Practice regulation 21 CFR Part 58 established by the Federal Food, Drug and Cosmetic Act.

Holstein heifers selected for this study were within normal ranges for breeding age (14 and 18 months) and body weight (350-434 kg). Based on visual observation of estrus, all heifers were cycling at the beginning of the study. The heifers were contained in two outdoor pens and were fed alfalfa hay for ad libitum intake plus ground corn (plus mineral, vitamin supplement) to achieve body weight gains of approximately 0.8 kg/day (National Research Council, Nutrient Requirements for Dairy Cattle, 1989). Heifers were observed at least twice daily for estrous behavior, eating behavior and signs of illness or toxicity.

Heifers were assigned at random, to one of six treatments (N = 8 per treatment) as follows:

1. Placebo intravaginal inserts with no progesterone for 15 days, replaced twice for a total of 45 days,
2. Simultaneous administration of three placebo intravaginal inserts with no progesterone for 15 days,
3. Intravaginal progesterone inserts with 1.9 gm progesterone for 15 days,
4. Intravaginal progesterone inserts with 1.9 gm progesterone for 15 days, replaced twice for a total of 45 days,
5. Simultaneous administration of three intravaginal progesterone inserts each containing 1.9 gm progesterone for 15 days, and
6. Simultaneous administration of three intravaginal progesterone inserts each containing 2.5 gm progesterone for 15 days.

The heifers were observed for estrous behavior for 72 days after removal of intravaginal inserts. Heifers were inseminated about a half day after they were observed in estrus. Pregnancy to first inseminations was determined by transrectal palpation between days 34 and 77 after inseminations.

A complete veterinary physical examination was conducted on each heifer at the outset of the study, on the final day of treatment and 7 days after removal of the intravaginal inserts. On these latter 2 dates, vaginal examinations were performed using a speculum, with erosions and ulcerations scored with the following system: 0 = normal or none detected, 1 = healing erosion(s), 2 = one erosion or ulcer, and 3 = two or more erosions or ulcers. On the day of insert removal, amount and cloudiness of mucus were scored on a scale of 0 to 5: amount – 0 = none to 5 = copious amount; cloudiness – 0 = clear to 5 = heavy, thick, pus-filled.

Blood samples were collected at weekly intervals from three weeks prior to treatment, during treatment and for 1 week after treatment. These blood samples were used to measure concentration of progesterone and determine blood chemistry and hematology values.

Blood Chemistry Variables:

Sodium	Serum Glutamic-Oxaloacetic Transaminase
Potassium	Creatinine
Carbon Dioxide	Lactate Dehydrogenase
Chloride Ion	Serum Glutamic-Pyruvic Transaminase
Glucose	Gamma Glutamyl Transpeptidase
Blood Urea Nitrogen (BUN)	Uric Acid
Calcium	Phosphate
Total Protein	BUN/Creatinine Ratio
Albumin	Globulin
Bilirubin	Cholesterol
Alkaline Phosphatase	Triglyceride

Hematology Variables:

White Blood Cell Count	Platelets
Red Blood Cell Count	Mean Platelet Volume
Hemoglobin	Segmented Neutrophils
Hematocrit	Eosinophils
Mean Corpuscular Volume	Basophils
Mean Corpuscular Hemoglobin	Lymphocytes
Mean Corpuscular Hemoglobin Concentration	Monocytes
Red Cell Distribution Width	Reactive Lymphocytes
	Clumped Platelets

4.1.1.3 Results

Average daily gain during the experimental period did not differ among treatment groups. Among treatment groups, average daily gain was 0.6 to 0.7 kg/day, thereby achieving gains close to those targeted at the outset of the study.

The 48 heifers were given a total of 128 inserts during the 45-day treatment period. Five heifers lost a total 7 inserts (5.5% of 128). All of the heifers with placebo inserts showed estrus during the treatment period, while none of the heifers showed estrus during the administration of intravaginal progesterone inserts.

Heifers with one 1.9 gm intravaginal progesterone insert had blood progesterone concentrations typical of the luteal phase of the estrous cycle for the 15-day (e.g., 8.6 ng/mL on 7th day of 15-day treatment period, treatment 3) or 45-day (e.g., 3.7 ng/mL on day 37 of the 45-day treatment period, treatment 4) treatment periods. As expected, heifers with three 1.9 or 2.5 gm intravaginal progesterone inserts for 15 days had increased progesterone concentrations when compared to heifers administered one insert. Progesterone

concentrations on the 7th day of the 15 day treatment period were 13.1 and 12.3 ng/mL for heifers in treatment groups 5 and 6, respectively.

Isolated statistical differences among treatments for blood chemistry and hematology variables were observed. However, these differences were very small and all values fell within normal physiological ranges, and thus were not deemed to be of biological significance.

Physical presence of the inserts, whether placebo or progesterone-impregnated, resulted in detectable vaginal irritation as indicated by the erosion/ulceration scores recorded from vaginal observations with a speculum at insert removal (Table 29). No statistical differences were noted among the six treatment groups. Vaginal observations via speculum were also made 7 days after insert removal, and scores were reduced dramatically in all treatment groups. Again, no statistical differences were noted among the six treatment groups. No differences among the six treatments were seen in the amount or cloudiness of mucus on the inserts at scheduled time of removal (data not shown). The study veterinarian noted that inserts were malodorous at the time of removal, but no offensive odors were noted during the vaginal observations taken at 7 days after insert removal. In total, these findings indicate that vaginal irritation caused by the inserts was transient in nature. When used according to label directions, insert use did not impair fertility in the clinical studies with beef cows, beef heifers and dairy heifers (Section 3).

Table 29. Vaginal scores via speculum taken at the time of insert removal and again at 7 days following removal.

Vaginal Score	Treatment ^a						P-Value
	1	2	3	4	5	6	
At time of insert removal ^b	1.75	2.25	1.88	1.25	2.13	2.38	N.S. ^c
7 days after insert removal ^b	0.13	0.88	0.50	0.75	0.63	0.63	N.S.

^aN = 8 per treatment group

^bCategorical model (CATMOD)

^cN.S. = non-significant (P > 0.10)

Reproductive performance is presented in Table 30. As anticipated, animals given the intravaginal progesterone-releasing insert (treatments 3 through 6) had a higher degree of estrous synchrony and a reduced interval to post-treatment estrus when compared to placebo controls (treatments 1 and 2). First-service conception rate was greater in placebo-treated vs. progesterone-treated heifers, though overall conception rate did not differ among treatment groups. Given the extended exposure to exogenous progesterone for heifers in this study (15-45 days) compared to the intended use of the product (7 day treatment period), it is likely that ovulation of persistent follicles resulted in poorer fertility (see scientific rationale in Section 2). Conclusions should be guarded however, because too few animals were used

evaluate the effects of treatment on fertility. Rather, the clinical studies in beef cows, beef heifers and dairy heifers were performed with greater animal numbers, and there were no differences detected between heifers treated with inserts+dinoprost vs. untreated controls or those given dinoprost tromethamine alone (see Section 3). Thus, use of this product in the manner proposed (insert for 7 days with dinoprost tromethamine treatment on the 6th day) did not impair fertility vs. animals not administered the inserts.

Table 30. Reproductive performance in heifers treated with the intravaginal progesterone-releasing insert.

Variable	Treatment ^a						Significance
	1	2	3	4	5	6	
% synchronized ^b	25	37.5	100	87.5	100	100	P < 0.01
Days to estrus ^b	10.1	9.4	2.2	2.2	3.2	2.4	P < 0.01
1 st Service conception rate (%) ^c	87.5	100	62.5	71.4	37.5	50	P < 0.05
Overall conception rate (%) ^c	100	100	87.5	100	100	87.5	N.S. ^d

^aN = 8 per treatment group

^bGeneral Linear Model (GLM)

^cCategorical model (CATMOD)

^dN.S. = non-significant (P > 0.10)

4.1.1.4 Conclusions

Use of the inserts caused transient vaginal irritation that resolved within 7 days of insert removal. The number of animals per treatment was not sufficient to make reliable conclusions on fertility in this study, though there was a trend for a reduction in 1st service conception rate in progesterone-treated vs. placebo animals.

4.1.2 Clinical Studies

Intensive animal safety observations were made on animals involved in the clinical studies described in Section 3. Three studies were conducted, one each with beef cows, beef heifers and dairy heifers. Observations were made relative to fertility, animal health, adverse events, vaginal irritation and estrus. Please reference Section 3 for detailed information on these observations.

Use of the inserts in the clinical studies was not associated with general health problems or adverse reactions other than vaginal irritation. Fertility was not impaired in insert+dinoprost treated animals when compared to untreated controls (beef cows and heifers) or those given

dinoprost alone (beef cows, beef and dairy heifers). Results on vaginal irritation in these studies, along with the results from the target animal safety study indicate that the product be labeled accordingly.

4.2 Conclusions for target animal safety

In conclusion, intravaginal progesterone inserts cause localized vaginal irritation in some animals. After the insert was removed, the irritation resolved by the time of insemination. The product label carries the following statement relative to vaginal irritation:

You may notice: clear, cloudy or bloody mucus on the outside of the EAZI-BREED CIDR Cattle Insert when removed from animals. The mucus may have an offensive odor. This is a result of mild irritation to the vaginal lining by the presence of the EAZI-BREED CIDR Cattle Insert, and generally clears between the time of removal and insemination. This irritation does not affect fertility.

No other effects detrimental to animal safety were observed. The data support the conclusion that the intravaginal progesterone insert is safe for cattle when used as directed on the label.

5 HUMAN FOOD SAFETY

Toxicity Tests:

The allowable increments of progesterone concentrations in edible tissues are codified under 21 CFR 556.540: 3 ppb for muscle, 6 ppb for liver, 9 ppb for kidney and 12 ppb for fat.

5.1 Residue Depletion Study

5.1.1 Investigators

Dr. Donald M. Henricks, *et al.*
Animal, Dairy and Veterinary Sciences Department
Clemson University
Clemson, SC 29634
Study No. B-90-16
October 9, 1990 to August 4, 1992

Beef cows 4 to 9 years of age, weighing from 560 kg to 748 kg, were blocked into groups based on body weight and body condition scores. Three cows served as controls and six were each given an intravaginal progesterone insert containing 1.9 gm progesterone. The intravaginal progesterone inserts were removed after 16 days, and the cows were slaughtered at 24 hours later. An additional 3 control cows and 6 cows given intravaginal progesterone

inserts for 36 days were slaughtered at 24 hours after the intravaginal progesterone inserts were removed.

Levels of progesterone were measured in plasma and edible tissues by a radioimmunoassay (RIA) specific for progesterone. The antibody recognizes 100% progesterone. It does not cross react with estradiol and cross reacts less than 1% with cholesterol and other steroids such as testosterone and pregnenolone. [¹²⁵I]-progesterone (for plasmatic samples) and [³H]-progesterone (for edible tissues) were used as tracers.

5.1.2 Tissue Residue Studies

Progesterone concentrations were measured in muscle, liver, kidney and fat. Progesterone was extracted from tissues by solvent partitioning, and purified by reverse phase chromatography.

Table 31. Tissue Concentrations (ng/gm) of Progesterone in Cattle Given Intravaginal Progesterone Inserts for 16 or 36 Days

Tissue	Cattle*	16-Day Treatment	36-Day Treatment
Muscle	Controls	1.45±0.87	4.00±1.45
	Treated	2.62±3.22	6.72±6.55
Liver**	Controls	0.41±0.00	0.41±0.00
	Treated	0.41±0.00	0.41±0.00
Kidney	Controls	0.60±0.63	2.27±0.12
	Treated	2.18±4.86	2.89±2.51
Fat	Controls	33.1±24.6	142.6±87.7
	Treated	79.0±37.2	99.8±75.8

* Averages ± standard deviations for 3 controls and 6 treated animals.

** Liver concentrations were all below the sensitivity of the assay (0.42 ng/mL).

5.1.3 Plasma Studies

Plasma progesterone profile: Concentrations of progesterone were determined in blood plasma collected from each animal during the 16- or 36-day CIDR treatment periods. Plasma levels of progesterone for animals administered an intravaginal progesterone insert resembled that of the controls, during the 16 days as well as during the 36 days of treatment.

Blood Clearance of Progesterone: In two ancillary studies (RA 870/02 & RA 896 and IDC #022), blood progesterone in treated animals returned to pretreatment levels within 6 to 24 hours after the intravaginal progesterone insert was removed. Additionally, the half-life of progesterone depletion was approximately 15 hours.

5.2 Conclusions

The results of the progesterone tissue residues at 16 and 36 days of treatment revealed that the levels of progesterone do not exceed the permitted increments of progesterone (21 CFR 556.540). Blood studies indicate that plasma profiles in treated and control animals are similar, and that plasma levels of progesterone return to pretreatment levels 6 to 24 hrs following removal of intravaginal progesterone insert. A residue study involving concurrent treatment of animals with CIDR and dinoprost tromethamine would not alter the conclusion that edible products from animals treated with CIDR are safe for human consumption. Most animals treated concurrently with a CIDR and dinoprost tromethamine are intended for reproduction and will not be slaughtered for human food use for at least 21 days post-treatment. Given these facts, we conclude that edible products treated with CIDR and dinoprost tromethamine in the manner indicated in the labeling, are safe for human consumption.

6 AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that the concurrent use of the Intravaginal Progesterone Insert and dinoprost tromethamine, when administered according to label is safe and effective for synchronizing estrus in suckled beef cows, and replacement beef and dairy heifers, for advancing the first postpartum estrus in suckled beef cows and for advancing first pubertal estrus in replacement beef heifers.

The Center for Veterinary Medicine has concluded that, for this product, adequate directions of use by the layman have been provided and the product will have over-the-counter (OTC) status. Label directions are accompanied by pictorial diagrams and detailed instruction in plain language. The drug is not a controlled substance. Thus, the product is assigned OTC status, and the labeling is adequate for the intended use.

Under section 512(c)(2)(F)(ii) of the Federal Food, Drug and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval.

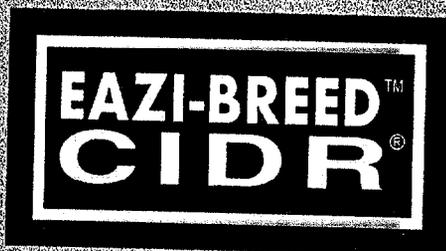
7 ATTACHMENTS

Facsimile Labeling is attached as indicated below:

Primary Package Label (Front Panel)

Primary Package Label (Back Panel)

Carton Label



Cattle Insert

NDC 0009-5207-01

NET CONTENTS

10 EAZI-BREED CIDR Cattle Inserts per bag
 Each EAZI-BREED CIDR Cattle Insert contains 1.38 grams of progesterone in molded silicone over a flexible nylon spine. Attached to each EAZI-BREED CIDR Cattle Insert is a polyester tail.

Caution: Federal law prohibits extra-label use of this drug to enhance food and/or fiber production in animals.

DRUG FACTS

Active ingredient: Progesterone, 1.38 grams per EAZI-BREED CIDR Cattle Insert

Uses:

- Synchronization of estrus in suckled beef cows and replacement beef and dairy heifers
- Advancement of first postpartum estrus in suckled beef cows
- Advancement of first pubertal estrus in replacement beef heifers

The EAZI-BREED CIDR Cattle Insert provides an exogenous source of the hormone progesterone during the 7 day administration period. Removal of the EAZI-BREED CIDR Cattle Insert on treatment day 7 results in a rapid fall in plasma progesterone levels which results in synchronization of estrus in those animals responding to treatment.

WARNINGS:

Human Warning: Wear latex gloves when handling the inserts. Keep this and all medications out of the reach of children.

Environmental Warning: Store removed EAZI-BREED CIDR Cattle Inserts in a plastic bag or other sealable container until they can be properly disposed in accordance with applicable local, state and Federal regulations.

Do Not Use:

- In animals with abnormal, immature or infected genital tracts
- In beef cows that are less than 20 days postpartum
- In beef or dairy heifers of insufficient size or age for breeding
- In lactating dairy cows
- An insert more than once. To prevent the potential transmission of venereal and blood borne diseases the EAZI-BREED CIDR Cattle Insert should be disposed after a single use.

When Using This Product:

- You must use the EAZI-BREED CIDR Cattle Insert concurrently with an injection of 5 mL of LUTALYSE® Sterile Solution (equivalent to 5 mg/mL dinoprost) administered on day 6 of the 7 day administration period to assure maximum effectiveness.
- In animals that respond to treatment the onset of estrus generally occurs within 1 to 3 days after removal of the EAZI-BREED CIDR Cattle Insert.
- Intravaginal administration of EAZI-BREED CIDR Cattle Insert for periods greater than 7 days may result in reduced fertility.

You May Notice:

- Increased loss of EAZI-BREED CIDR Cattle Inserts in animals housed under crowded conditions, especially in heifers. Avoid crowded conditions during treatment as other cattle, particularly heifers, may remove EAZI-BREED CIDR Cattle Inserts by pulling on the tail of the EAZI-BREED CIDR Cattle Insert. If loss rates are high re-evaluate insertion technique and cattle handling facilities.
- Clear, cloudy or bloody mucus on the outside of EAZI-BREED CIDR Cattle Insert when removed from animals. The mucus may have an offensive odor. This is a result of mild irritation to the vaginal lining by the presence of the EAZI-BREED CIDR Cattle Insert and generally clears between the time of removal and insemination. Such irritation does not affect fertility.

Directions:

Wear latex gloves when handling inserts.
 Only use the specially designed EAZI-BREED CIDR Cattle Insert Applicator for administration.
 Administer one EAZI-BREED CIDR Cattle Insert per animal for 7 days.
 Inject 5 mL of LUTALYSE® Sterile Solution (equivalent to 5 mg/mL dinoprost) 1 day prior to EAZI-BREED CIDR Cattle Insert removal, on day 6 of the 7 day administration period.
 Observe animals for signs of estrus on days 1 to 3 after removal of EAZI-BREED CIDR Cattle Inserts and inseminate animals about 12 hours after onset of estrus.

Insertion:

1. Restrain cattle appropriately (head catch, squeeze chute, gate, etc.) prior to administration.
2. Wash the EAZI-BREED CIDR Cattle Insert Applicator in a non-irritating antiseptic solution, and then lubricate the front portion of the EAZI-BREED CIDR Cattle Insert Applicator with a veterinary obstetrical lubricant.
3. Push the flexible tail end of the EAZI-BREED CIDR Cattle Insert into the EAZI-BREED CIDR Cattle Insert Applicator taking care to assure the tail is extending upward through the slot of the EAZI-BREED CIDR Cattle Insert Applicator and is pointed toward the handle.
4. Fold the wings of the EAZI-BREED CIDR Cattle Insert to make it longer and continue to advance the EAZI-BREED CIDR Cattle Insert into the applicator until it is fully seated. When fully seated only the tips of the wings should protrude (one half inch) from the end of the EAZI-BREED CIDR Cattle Insert Applicator. (see Figure 1 below).
5. Lubricate the protruding tips of the wings of the EAZI-BREED CIDR Cattle Insert with veterinary obstetrical lubricant.
6. Lift the tail of the animal and clean the exterior of the vulva.
7. Open the lips of the vulva and gently place the loaded EAZI-BREED CIDR Cattle Insert Applicator through the vulva. The slot in the EAZI-BREED CIDR Cattle Insert Applicator should face upwards (see Figure 2 below).
8. Once the loaded EAZI-BREED CIDR Cattle Insert Applicator is past the vulva slope the EAZI-BREED CIDR Cattle Insert Applicator slightly upwards (35-45° angle) by lowering the handle, and then forward, without forcing, until the EAZI-BREED CIDR Cattle Insert Applicator is fully inserted or resistance is felt (see Figure 3 below).
9. Squeeze the finger grips within the handle of the EAZI-BREED CIDR Cattle Insert Applicator to deposit the EAZI-BREED CIDR Cattle Insert in the anterior vagina (see Figure 4 below) and then pull the EAZI-BREED CIDR Cattle Insert Applicator backwards to remove it from the vagina.
10. With the EAZI-BREED CIDR Cattle Insert correctly placed, with the wings open in the anterior portion of the vagina, the tail of the EAZI-BREED CIDR Cattle Insert should be visible, pointing downward from the vulva of the animal. Tails of EAZI-BREED CIDR Cattle Inserts that protrude more than 2.5 inches from the vulva may be clipped to minimize removal by other animals.

Removal:

1. Remove EAZI-BREED CIDR Cattle Inserts by pulling gently but firmly on the protruding polyester tail.
2. EAZI-BREED CIDR Cattle Inserts have been reported to reverse direction within the vagina; therefore, if the polyester tail of the insert is not visible on the day of removal, check the vagina to determine if an insert is present.

Figure 1

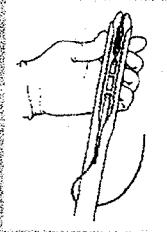


Figure 2



Figure 3

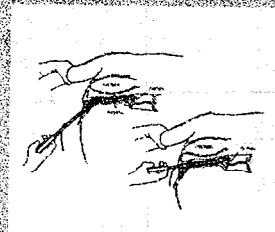


Figure 4

**Other Information:**

Store below 86°F (30°C)

Manufactured by:

DEC International, NZ, LTD
 558 Te Rapa Road
 Hamilton, New Zealand
 DAW 103B

Lot Number: XXXXX

Expiration Date: XXXXX

Inactive ingredients: silicone rubber, nylon and polyester.

Questions/Comments: 1-866-493-1954

NADA XX-XXXX, Approved by FDA

EAZI-BREED and CIDR are registered trademarks of DEC International, NZ, LTD
 LUTALYSE is a registered trademark of Pharmacia & Upjohn Company

EAZI-BREED™
CIDR®

NDC 67080-5207-1

Cattle Insert

For Use in Animals Only

Caution: Federal law prohibits extra-label use of this drug to enhance food and/or fiber production in animals.

Net Contents: Contains 5 bags each containing 10 inserts (1.38 grams progesterone per insert).

Read package (bag) label before using this drug.

NADA #141-200, Approved by FDA

Store below 86°F (30°C)

Manufactured by:
DEC International, NZ, Ltd.
558 Te Rapa Road, Hamilton, New Zealand

LOT

EXP

DEC00A