The Use of a Vaginal Conductivity Probe to Influence Calf Sex Ratio via Altered Insemination Time

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Summary

One hundred eighty-nine mixed breed beef heifers from 13 consignors enrolled in the MACEP heifer development project were utilized in this study. Heifers were synchronized by feeding 0.5 mg melengestrol acetate (MGA) per head per day for 14 days followed by an injection of prostaglandin $F_2\alpha$ ($PGF_2\alpha$; 25 mg Lutalyse®) 17 days after the last MGA feeding. Each heifer was fitted with a Heatwatch® transmitter on the morning of $PGF_2\alpha$ administration to facilitate detection of estrus. Vaginal conductivity measurements were taken using an Ovatec® probe every 12 hours for 96 hours beginning at the time of $PGF_2\alpha$ injection. Heifers randomly assigned to produce a female calf were inseminated near the onset of estrus (as indicated by probe values of ≤ 55 on the decline). Heifers randomly assigned to produce a male calf were inseminated approximately 24 hours after the onset of estrus (as indicated by probe values of ≥ 60 on the incline). All heifers not inseminated by 96 hours after $PGF_2\alpha$ were mass inseminated in an attempt to impregnate as many heifers as possible. Heifers that were diagnosed as pregnant as a result of the artificial insemination were subjected to ultrasonography for fetal sex determination. Only 70 of the 189 heifers (37.0%) exhibited estrus according to Heatwatch® and incidence of estrus was influenced by heifer average daily gain, reproductive tract score, and disposition score. Heifers receiving a disposition score of 3 (78.7) had a higher (P<.05) probe reading at AI than those receiving a disposition score of 1 or 2 (70.8 and 72.5, respectively). Heifers with probe readings at insemination of 80 - 84 and > 84 had lower (P<.05) pregnancy rates to AI (13.6 and 0.0%, respectively) than heifers with probe readings in the ranges of < 60, 60 - 64, 65 - 69, 70 - 74, and 75 - 79 (35.7, 40.9, 31.4, 35.3, and 26.9% respectively). Heifers that were bred when probe values were increasing had a lower (P<.05) percentage of male fetuses (34.4%) than those bred during a period of decreasing probe values (69.2% male fetuses). These results demonstrate that a vaginal conductivity probe may be a useful tool to determine an insemination time that could potentially alter calf sex ratio.

Introduction

Detection of estrus and timing of insemination are known to be very important contributors to the reproductive efficiency of cattle. For many years, the widely accepted practice has been to artificially inseminate females ~12 hours after the detection of standing estrus, following a protocol known as the am/pm breeding rule. Under this method, any animal observed in estrus in the evening is inseminated the following morning, whereas any animal observed in estrus in the morning is inseminated that evening. By following the am/pm breeding rule, producers have achieved acceptable conception rates in both cows and heifers. By altering insemination time, a decrease in normal pregnancy rate for every ± 12 hour deviation from the am/pm rule can be expected. However, it has been proposed by some that the sex of the resultant offspring may be influenced by altering the insemination time from the am/pm rule may be observed. By inseminating at or before the onset of estrus, one may increase the proportion of female offspring, and by inseminating at or after the end of standing estrus, one may increase the proportion of male offspring. The major problem with applying this theory under field conditions, however, is that it is difficult to predict the onset of estrus.

Since the advent of artificial insemination, researchers have experimented with devices to increase the accuracy and efficiency of estrus detection. One of the tools that can be used to accurately detect estrus is a vaginal conductivity probe, which measures the electrical resistance of the mucus secreted by the vagina. As females near the onset of estrus, the electrical resistance of the vaginal mucus begins to decrease, corresponding to a decrease in observed probe values. The probe readings reach their lowest values near the middle of the estrus period (~12 hours after the onset of estrus), after which they begin to increase again. (The clear mucous discharge commonly observed from females in estrus is usually correlated with the lowest probe values.)

It has recently been reported that the Ovatec® vaginal conductivity probe can be used to determine proper insemination time to significantly alter the sex ratio of the resultant offspring. By inseminating females at probe values of 45 - 55, while probe values are still declining, one can significantly alter the proportion of female offspring (up to 90% female). By inseminating females at probe values of 45 - 80, after probe values have reached a minimum and are increasing, one could significantly alter the proportion of male offspring (up to 90% male). These two breeding times would correlate with approximately 0 hours after the onset of estrus for female offspring and approximately 24 hours after the onset of estrus for male offspring. Normal values obtained with the Ovatec® probe range from 30 (indicating an animal that is in estrus) to ~120 (indicating an animal that is in the luteal phase of the estrous cycle). Variation among animals is commonly observed, with each animal exhibiting unique high and low points and patterns of change.

The purpose of this study was to investigate the feasibility of using the Ovatec® vaginal conductivity probe to determine proper insemination time to significantly alter the sex ratio of offspring.

Materials and Methods

One hundred eighty-nine mixed breed beef heifers from 13 consignors enrolled in the MACEP heifer development program stationed near Tingley, IA, were used in this study (Table 1). All heifers were taken to the location six months prior to breeding, at which time they were vaccinated, weighed, and scored for body condition, disposition, and frame size. Body weight, body condition score, and disposition score were recorded periodically for each female throughout the six month period preceding breeding. One month prior to the beginning of the experiment, all heifers received their booster vaccinations and were weighed and scored for body condition, disposition, and reproductive tract maturity.

Estrus was synchronized by feeding 0.5 mg melengestrol acetate (MGA) for 14 days followed by an injection of prostaglandin $F_{2}\alpha$ ($PGF_{2}\alpha$; 25 mg Lutalyse®) 17 days after the last MGA feeding. Heifers were randomly assigned within consignor to be bred for either a male or a female calf, and this designation was used to make breeding decisions. Beginning on the morning of $PGF_{2}\alpha$ injection, each heifer was fitted with a Heatwatch® transmitter to aid in the detection of estrus. Probe measurements were also taken beginning on the morning of $PGF_{2}\alpha$ injection and continued every 12 hours for 96 hours. Any heifer assigned to be bred for a female calf was inseminated whenever the first of three criteria was met: 1) probe values reached ≤ 55 on the decline, 2) probe reading was ≤ 65 and ≥ 5 units lower than the previous probe reading, or 3) after the onset of estrus (Figure 1). Any heifer assigned to be bred for a male calf was inseminated whenever the first of two criteria was met: 1) probe value reached ≥ 60 with an increase from the previous reading of ≥ 5 units or 2) the heifer had been in heat for > 18 hours (Figure 2). Any heifer not bred by 96 hours after $PGF_{2}\alpha$ injection underwent a mass insemination in an attempt to maximize the number of heifers pregnant as a result of artificial insemination.

Figure 1. Protocol for breeding for a female calf.
Move down the chart until you come to
BREED or **Wait to Breed**

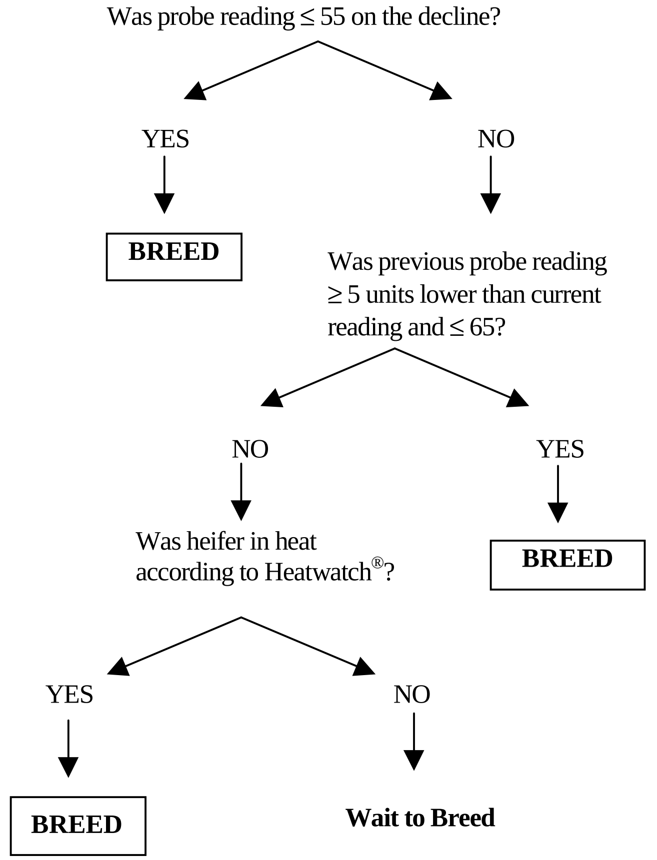
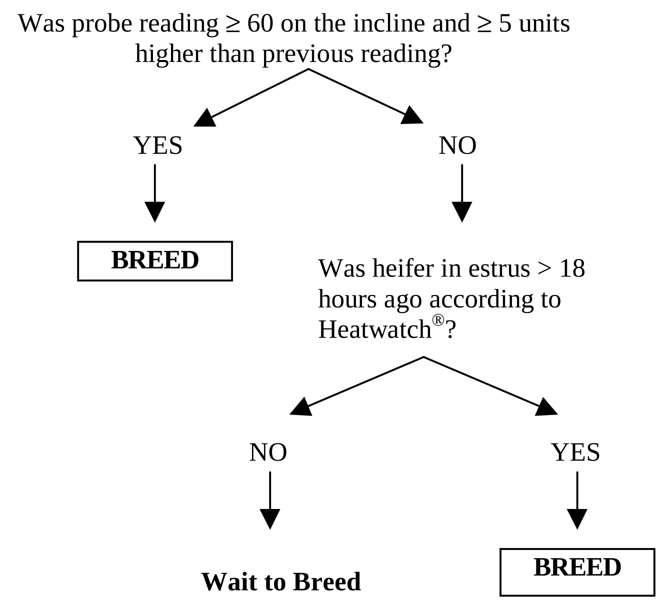


Figure 2. Protocol for breeding for a male calf.
Follow the chart down until you see
BREED or **Wait to Breed**



Five days after the mass insemination, heifers were penned with clean-up bulls for 63 days. Thirty-eight days after the mass insemination, all heifers were examined for pregnancy by ultrasound to determine those that became pregnant as a result of AI. Heifers that were diagnosed as pregnant as a result of the AI underwent ultrasonographic fetal sex determination 30 days after pregnancy diagnosis (68 days after mass insemination). One hundred twelve days after the mass insemination, heifers underwent a final ultrasound pregnancy diagnosis and were returned to the consignor (if they had not been returned previously).

Data were analyzed by the general linear model (GLM) procedure of SAS. Condition score was on a scale of 1 (very thin) to 9 (very obese). Frame score was calculated using a formula including the animal's hip height and age. Reproductive tract maturity score ranged from 1 (an animal that is pre-puberal [no significant structures present on either ovary and a small infantile reproductive tract]) to 5 (an animal with a palpable corpus luteum [CL], and a large distinct reproductive tract). Pelvic area was adjusted to one year of age using actual pelvic height and width measurements. Target weight at breeding was 65% of mature weight as calculated using frame score tables. Average daily gain prior to breeding was calculated as: (weight at breeding - previous weight)/35 days. Average daily gain after breeding was calculated as: (weight at first pregnancy diagnosis - weight at breeding)/42 days. Disposition score was a subjective evaluation of the behavior of the heifer and ranged from 1 (a calm, easy to handle animal) to 3 (a very flighty, difficult to manage animal). Probe value at AI was the probe value taken at the time artificial insemination was performed. Probe value at estrus was the first probe value recorded after the heifer was determined to be in estrus by Heatwatch®. Probe change before AI was calculated as: (previous probe reading - probe reading at AI). Probe change after AI was calculated as: (probe reading at AI - probe reading 12 hours after AI). Estrus to AI interval was determined as: (time of AI - onset of estrus as determined by Heatwatch®).

Results and Discussion

A high incidence of visible signs of estrus is very important when AI is to be performed and obviously will have a major impact on pregnancy rates. In this study, only 70 of the 189 heifers (37.0%) exhibited estrus according to the Heatwatch® system. This low overall estrus response rate may be explained in part by the conditions of the lots in which the heifers were kept. Due to a large amount of rainfall during the pre-breeding and breeding period, the heifers were kept in very muddy conditions, resulting in very poor footing for the heifers (which is known to decrease mounting activity). In addition, the frequent handling of the animals for probing may have increased the stress level of the animals, thereby decreasing expression of estrus.

The probe readings in this study did not drop as low as indicated in the Ovatec® operator's manual. Figure 3 shows a typical vaginal conductivity probe reading pattern of a heifer in this study bred to produce and pregnant with a female fetus. Figure 4 shows a typical vaginal conductivity probe reading pattern for a heifer bred to produce and pregnant with a male fetus. Figure 5 shows a heifer that did not exhibit any signs of estrus (based on Heatwatch® or vaginal conductivity readings) and was mass inseminated, but did not become pregnant. One explanation for the higher probe values in the present study (compared to those in the operator's manual) could be due to the synchronization protocol. It is possible that a synchronization protocol may interfere with the estrogen:progesterone balance within the female and alter composition of the vaginal mucus. High estrogen levels (normally found in heifers near the time of estrus) are highly correlated with low probe readings. Therefore, it may be necessary to develop a separate breeding protocol based on typical vaginal conductivity measurements specific for the synchronization program.

Average daily gain is an important measure, as it is an indication of the proper growth and development of heifers, as well as the energy balance of the animal. In this study, estrus was affected ($P<.05$) by pre-breeding average daily gain (Table 2). Heifers that gained 1.50 - 1.99 and 2.00 - 2.49 lbs./day had higher (41.4 and 51.8%, respectively) estrus expression rates than heifers that gained > 2.49 lbs./day (18.2%), but none of these three groups differed from heifers gaining < 1.00 lb./day or 1.00 - 1.49 lbs./day (36.0 and 35.1%, respectively). This result was not surprising, as one might expect heifers that are gaining weight very rapidly to have a higher percent body fat, which is negatively correlated with reproductive efficiency.

Reproductive tract scoring is a relatively new method of evaluating the sexual maturity of a group of heifers and can be used to help producers cull females that are sexually immature, thereby reducing costs for synchronization and breeding. In this study, reproductive tract score of the animal had a significant impact on incidence of estrus (Table 2). Heifers with a reproductive tract score of 5 had a higher ($P<.05$) estrus expression rate (46.7%) than heifers with a reproductive tract score of 2 or 3 (20.0 and 21.4%, respectively), with reproductive tract score 4 heifers being intermediate (31.8%). This result follows previous observations that more sexually mature heifers tend to have higher estrus expression rates. It also stresses the fact that reproductive tract scores can be a useful tool for culling those heifers that are reproductively immature prior to breeding or synchronization.

Disposition score is a measure of an animal's behavior and plays a role not only in the ease with which an animal is handled, but also in reproductive efficiency, as it was shown to affect the estrus response rate (Table 2). The estrus response rate of heifers with a disposition score of 1 or 2 did not differ from each other (46.2 and 38.6%, respectively) but was higher (P<.05) than that for disposition score 3 heifers (8.7%). This may be due to the fact that handling these animals two times per day for probing may have caused more stress to the flighty heifers than to calm heifers, thereby negatively impacting reproductive function. This interpretation is further supported by the observation that no differences existed in the overall breeding season pregnancy rate of heifers when compared across disposition scores (Table 2).

Estrus to AI interval refers to the number of hours from the onset of estrus until insemination. This plays an important role not only in pregnancy percentage, as indicated above, but may also have a role in calf sex determination. Age at breeding had an impact on the estrus to AI interval as the 13- and 14-month old heifers had a longer (P<.05) estrus to AI interval (16.6 and 11.3 hours, respectively) than either the 12-month old heifer or the 15-month old heifers (-16.0 and -9.0, respectively; Table 3). This difference can be explained by chance alone, in that the 12-month old heifer (n=1) and the 15-month old heifers (n=2) were all assigned to be bred for a female calf (i.e., they were intended to be bred near or before the onset of estrus).

Probe value at AI is important in determining at what point of the estrus period each heifer was inseminated. Reproductive tract score had an impact on the probe value at AI (Table 3) as heifers with reproductive scores of 2 and 5 had lower (P<.05) probe values at AI (66.2 and 70.8, respectively) than those heifers with a reproductive tract score of 3 (77.1). Heifers with a reproductive tract score of 4 were intermediate (74.2). This result is probably best explained as a sampling phenomenon, as there were very few heifers (n=5) with a reproductive tract score of 2. Those heifers with a reproductive tract score of 2 would have been expected to have a higher probe value at AI than any other group due to a lower incidence of estrus. The lower probe reading at AI for the reproductive tract score 5 heifers compared with those with a reproductive tract score of 3 is expected because the sexually mature heifers exhibited a higher incidence of estrus (resulting in lower probe values at AI) than those heifers with a reproductive tract score of 3, which would be less mature.

Probe reading at AI was also affected by disposition score (Table 3) with disposition score 3 heifers having a higher (P<.05) probe value at AI (78.7) than heifers with a disposition score of either 1 or 2 (70.8 and 72.5, respectively). Because estrus expression and probe reading at AI should be correlated, it is of no surprise that the same group of heifers that had a lower estrus response (disposition score 3) had a higher probe reading at AI. Any heifer that did not exhibit estrus will have a higher probe reading at AI (due to the mass insemination) when compared with heifers that were inseminated due to estrus expression as indicated by Heatwatch® or the vaginal conductivity measurements.

To maximize genetic improvement within a producer's herd, one must maximize the percentage of females that become pregnant by superior AI sires. In this study, the probe reading at AI had an impact on the proportion of heifers that became pregnant as a result of AI (Table 4). Heifers that were inseminated at probe values of > 84 and 80 - 84 had lower (P<.05) pregnancy rates as a result of AI (0.0 and 13.6%, respectively) than heifers that had probe readings of < 60, 60 - 64, 65 - 69, 70 - 74, and 75 - 79 at AI (35.7, 40.9, 31.4, 35.3, and 26.9%, respectively). These results agree with previous reports of the effectiveness of vaginal conductivity probes in detecting estrus and determining proper insemination times to establish pregnancy.

The ability to control the outcome of calf sex or significantly skew the sex ratio of a population of offspring is an exciting and powerful idea. In the beef industry, huge dividends could be reaped in both the seedstock and commercial arenas. Seedstock producers could alter the sex of the offspring to fit more closely with the typical market for their animals (i.e., bulls or replacement heifers). Commercial operations could produce a larger proportion of male calves, resulting in more steer calves for market which would lead to faster gains, better feed efficiency, and a higher carcass value than heifers. In this study, the probe change before AI affected (P<.05) the fetal sex ratio as determined by ultrasound (Table 4). Heifers bred while probe numbers were increasing resulted in a higher proportion of female fetuses (65.6%), whereas heifers bred while probe numbers were decreasing resulted in a higher proportion of male fetuses (69.2%). This alteration of sex ratio was the opposite of what was expected based on a previous report. This difference may be due to the fact that probe values in the present study did not drop as low as indicated by the Ovatec® operators manual (i.e., few heifers were inseminated at probe values of < 65), resulting in insemination of some heifers at the “wrong” time. This conflict also may be due to the variation in probe values within and among heifers, which influenced when insemination was performed. Although the alteration of sex ratio in the present study was not as extreme and was not in the same direction as the previous report, the fact that there was a significant alteration from the expected 50:50 sex ratio can not be ignored.

Although it is shown here that the proportion of one or the other sex may be increased due to insemination based on probe values, it should be noted that it was difficult to obtain the desired sex in this study, as indicated by the results in Table 4. Of the 47 AI pregnancies, only 46.8% of these had the desired fetal sex. Another problem associated with using vaginal conductivity measurements to determine proper insemination time to alter calf sex is the labor requirement. In this study, all 189 heifers were probed a total of nine times each. Although all of these probings may not be necessary in an applied situation (some of the probings before and after estrus could be omitted), one must have probe measurements prior to the onset of estrus for female breedings and after the end of estrus for male breedings. When the initial cost of the probe (~$2000) is taken into consideration along with the cost of labor associated with taking the measurements, the use of the vaginal conductivity probe may or may not be as economically feasible as other alternatives for controlling the sex of offspring (i.e. embryo sexing and subsequent embryo transfer). This leads to the conclusion that, although this technology may hold promise, much more research needs to be conducted in order to more clearly define a practical breeding protocol to obtain the sex of choice.

Implications

As the cattle industry is being pushed to produce more uniform products, the use of a vaginal conductivity probe to produce offspring of only one sex could be quite advantageous. By producing only male offspring, the commercial producer could market only steer cattle, rather than marketing both steers and heifers. The seedstock producers could more precisely match the sex of the offspring to the demands of the market. Although the breeding protocol tested in this study did not yield the predicted fetal sex, fetal sex was altered when based on changes in probe readings. Further research will undoubtedly uncover the circumstances under which the vaginal conductivity probe can be a highly predictable tool. If a more practical protocol is developed, with estrus synchronization protocols in mind, this technology could have a huge impact on the beef industry.

Acknowledgments

The authors would like to thank Doyle and Connie Richards and Angela Daniels for their help and contributions to this project.

Table 1. Descriptive statistics of the crossbred beef heifers (n=189) utilized in this study.

	Mean	Range
Age at Breeding (mo.)	14	12 - 16
Weight at Breeding (lbs.)	756	545-1005
Condition Score at Breeding (1-9)	5.4	3 - 7
Frame Score (1-9)	5.0	3 - 7
Reproductive Tract Score (1-5)	4.3	2 - 5
Disposition Score (1-3)	1.8	1 - 3
Pelvic Area (cm^2)	151	94 - 217
Target Weight at Breeding (lbs.)	764	678 - 857
Average Daily Gain Prior to Breeding (lbs./day)	1.90	-0.7 - 3.7
Average Daily Gain After Breeding (lbs./day)*	-0.91	-4.2 - 0.8

* 36 heifers were returned to their owner before final pregnancy diagnosis was obtained; therefore, these data are based on only 153 heifers.

Figure 3. Pattern of a heifer that was bred to produce and conceived a female offspring.

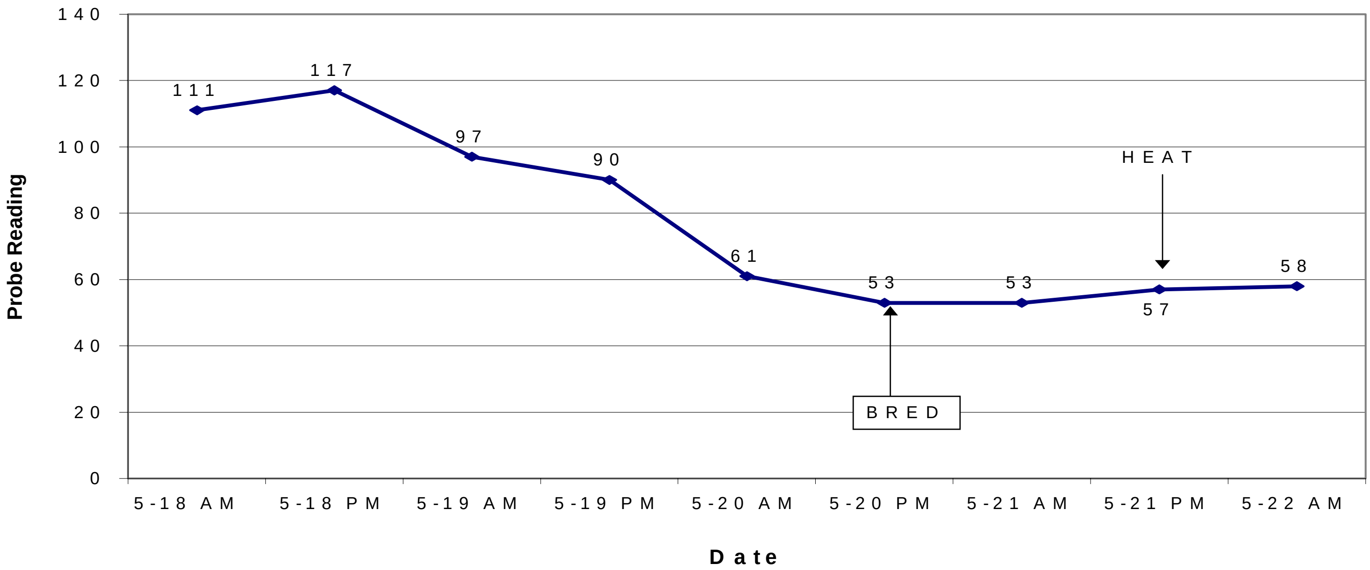


Figure 4. Pattern of a heifer that was bred to produce and conceived a male offspring.

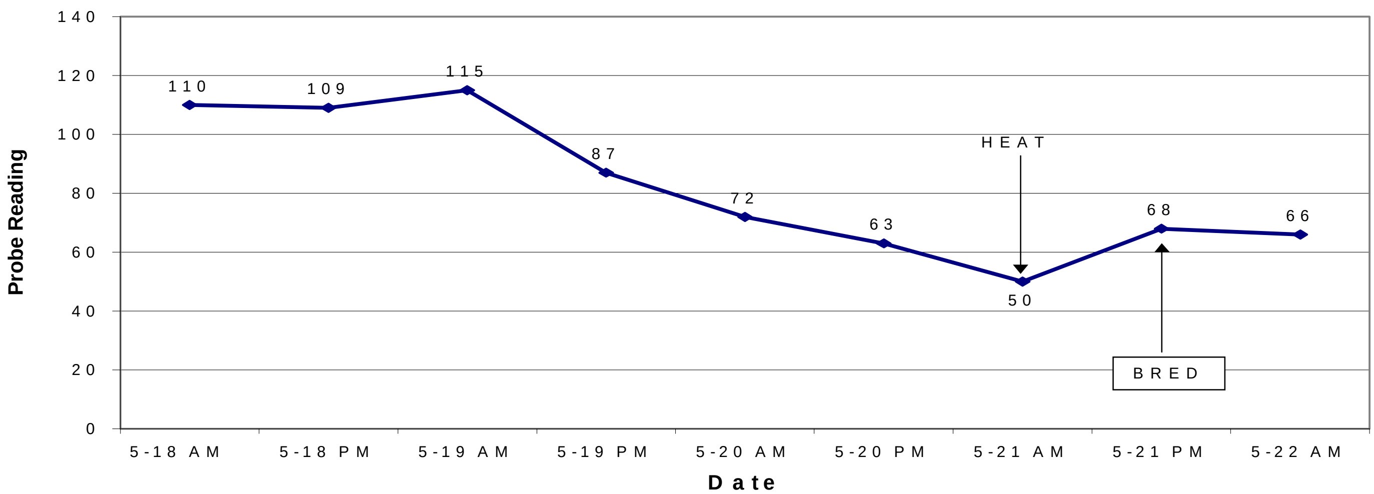


Figure 5. Typical pattern of a heifer that did not exhibit estrus.

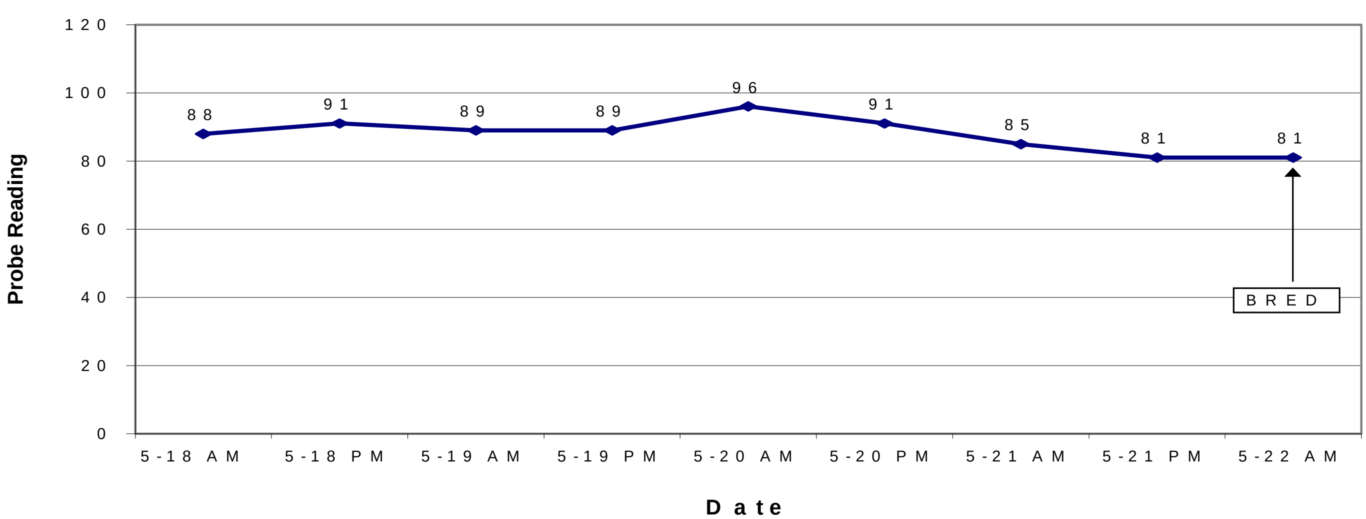


Table 2. Impact of Performance Traits on Reproductive Measures.

	% Estrus			% Pregnant From AI			% Pregnant		
	n	Mean	SEM	n*	Mean	SEM	n†	Mean	SEM
Overall Mean	189	37.0	3.5	174	27.0	3.4	153	85.0	2.9
Pre-Breeding ADG (lbs./day)									
< 1.0	25	36.0[a,b]	9.8	23	30.4	9.8	22	86.4	7.5
1.0 – 1.49	37	35.1[a,b]	8.0	35	28.6	7.7	29	75.9	8.1
1.5 – 1.99	29	41.4[a]	9.3	27	33.3	9.2	24	75.0	9.0
2.0 – 2.49	54	51.8[a]	6.9	47	19.1	5.8	39	89.7	4.9
> 2.49	44	18.2[b]	5.9	42	28.6	7.1	39	92.3	4.3
Breeding Weight (lbs.)									
< 650	13	30.8	13.3	12	16.7	11.2	10	80.0	13.3
650 – 699	27	29.7	9.0	24	25.0	9.0	23	73.9	9.4
700 – 749	47	31.9	6.9	43	23.3	6.5	34	94.1	4.1
750 – 799	47	46.8	7.4	45	26.7	6.7	41	75.6	6.8
800 – 849	35	34.3	8.1	33	39.4	8.6	30	90.0	5.6
> 849	20	45.0	11.4	17	23.5	10.6	15	100.0	0
Condition Score at Breeding									
3	1	0.0	0	1	0.0	0	1	100.0	0
4	12	33.3	14.2	8	25.0	16.4	6	83.3	16.7
5	93	43.0	5.2	85	27.1	4.8	71	81.7	4.6
6	78	32.1	5.3	75	26.7	5.1	70	87.1	4.0
7	5	20.0	20.0	5	40.0	24.5	5	100.0	0
Age (mo.) at Breeding									
12	10	10.0	10.0	9	11.1	11.1	9	77.8	14.7
13	82	36.6	5.4	74	21.6	4.8	64	82.8	4.8
14	91	39.6	5.1	87	33.3	5.1	76	86.8	3.9
15	5	40.0	24.5	3	33.3	33.3	3	100.0	0
16	1	100.0	0	1	0.0	0	1	0.0	0
Reproductive Tract Score (1-5)									
2	5	20.0[a]	20.0	5	20.0	20.0	5	80.0	20.0
3	28	21.4[a]	7.9	27	29.7	9.0	23	78.3	8.8
4	66	31.8[a,b]	5.8	60	23.3	5.5	51	88.2	4.6
5	90	46.7[b]	5.3	82	29.3	5.1	74	85.1	4.2
Disposition Score (1-3)									
1	52	46.2[a]	7.0	45	33.3	6.6	43	76.7	6.5
2	114	38.6[a]	4.6	107	27.1	4.3	89	88.8	3.4
3	23	8.7[b]	6.0	22	13.6	7.5	21	85.7	7.8

[a,b] Values with unlike superscripts differ (P<.05).
* 15 heifers were returned to their owners before the initial pregnancy diagnosis.
† 36 heifers were returned to their owners before the final pregnancy diagnosis.

Table 3. Influence of heifer performance traits on measures used to alter sex ratio.

	Probe Value at AI			Probe Value at Estrus			Estrus-AI Interval (hrs.)		
	n*	Mean	SEM	n†	Mean	SEM	n	Mean	SEM
Overall Mean	188	72.8	0.8	68	65.9	0.9	70	12.6	1.4
Pre-Breeding ADG (lbs./day)									
< 1.0	25	73.4	3.0	9	68.2	2.0	9	11.4	3.1
1.0 – 1.49	37	74.4	1.6	13	64.2	2.7	13	11.6	2.6
1.5 – 1.99	29	71.1	2.1	12	65.8	1.9	12	14.7	4.0
2.0 – 2.49	53	72.5	1.5	27	66.7	1.3	28	13.4	2.4
> 2.5	44	72.8	1.7	7	62.9	2.4	8	9.4	5.3
Weight at Breeding (lbs.)									
< 650	13	74.6	3.1	4	67.3	5.9	4	25.3	5.4
650 – 699	27	77.4	2.8	8	69.9	1.8	8	10.3	2.2
700 – 749	47	72.6	1.3	15	65.1	1.5	15	13.3	3.1
750 – 799	46	69.8	1.4	22	65.4	1.6	22	13.5	3.0
800 – 849	35	71.9	1.9	10	63.3	2.8	12	12.8	3.1
> 849	20	74.5	3.0	9	66.9	2.1	9	5.3	3.5
Condition Score at Breeding									
3	1	78.0	0	0	-	-	0	-	-
4	12	77.0	3.6	4	69.5	0.65	4	18.0	8.1
5	92	73.6	1.2	38	65.8	1.2	40	14.2	1.6
6	78	71.2	1.2	25	65.1	1.6	25	9.3	2.9
7	5	71.6	7.4	1	71.0	0	1	8.0	0
Age (mo.) at Breeding									
12	10	69.6	4.5	1	57.0	0	1	-16.0[a]	0
13	81	74.2	1.2	28	66.1	1.3	30	16.6[b]	2.2
14	91	71.8	1.2	36	65.9	1.3	36	11.3[b]	1.7
15	5	76.0	7.6	2	63.0	5.0	2	-9.0[a]	1.0
16	1	71.0	0	1	71.0	0	1	8.0[a,b]	0
Reproductive Tract Score (1-5)									
2	5	66.2[a]	4.9	1	66.0	0	1	9.0	0
3	28	77.1[b]	2.0	6	70.7	1.4	6	15.7	3.9
4	66	74.2[a,b]	1.6	20	64.3	1.8	21	13.3	2.9
5	89	70.8[a]	1.0	41	65.9	1.1	42	11.9	1.8
Disposition Score (1-3)									
1	52	70.8[a]	1.5	23	64.5	1.5	24	14.1	2.1
2	113	72.5[a]	1.0	43	66.6	1.1	44	11.9	2.0
3	23	78.7[b]	3.0	2	65.5	1.5	2	9.0	2.0

* Probe value at AI was not collected for one heifer.

† Includes only those heifers that exhibited estrus according to Heatwatch®, with missing values for two heifers.

a,b Values with unlike superscripts differ (P<.05).

Table 4. Effect of probe readings and estrus to AI interval on sex ratio and pregnancy.

	% Pregnant from AI			Sex Ratio (% Male)			% Fetuses of the Desired Sex		
	n*	Mean	SEM	n†	Mean	SEM	n†	Mean	SEM
Overall Mean	174	27.0	3.4	47	45.7	7.3	47	46.8	7.4
Probe Reading at AI									
< 60	14	35.7[a]	13.3	5	60.0	24.5	5	40.0	24.5
60 - 64	22	40.9[a]	10.7	9	44.4	17.6	9	44.4	17.6
65 - 69	35	31.4[a]	8.0	11	54.5	15.7	11	45.5	15.7
70 - 74	34	35.3[a]	8.3	12	33.3	14.2	12	41.7	14.9
75 - 79	26	26.9[a]	8.9	7	42.9	20.2	7	57.1	20.2
80 - 84	22	13.6[b]	7.5	3	33.3	33.3	3	66.7	33.3
> 84	20	0.0[b]	0	0	-	-	0	-	-
Probe Change Before AI									
Increasing Values	105	30.5	4.5	32	34.4[a]	8.5	32	50.0	9.0
Decreasing Values	66	19.7	4.9	13	69.2[b]	13.3	13	38.5	14.0
Probe Change After AI									
Increasing Values	36	36.1	8.1	13	38.5	14.0	13	38.5	14.0
Decreasing Values	81	29.6	5.1	24	50.0	10.4	24	50.0	10.4
Estrus to AI Interval (hrs.)									
< 0	8	37.5	18.3	3	33.3	33.3	3	33.3	33.3
0 - 8	14	42.9	13.7	6	33.3	21.1	6	50.0	22.4
9 - 15	12	58.3	14.9	7	42.9	20.2	7	42.9	20.2
16 - 24	17	47.1	12.5	8	75.0	16.4	8	62.5	18.3
> 24	9	33.3	16.7	3	66.7	33.3	3	66.7	33.3

[a,b] Values with unlike superscripts differ (P<.05).
* 15 heifers were returned to their owners before the initial pregnancy diagnosis.
† Includes only those heifers that were pregnant as a result of artificial insemination.