

## The use of artificial insemination gun protective plastic sheath at the time of artificial insemination did not improve fertility of beef cattle

Ramanathan Kasimanickam

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA

### Abstract

A strict hygienic artificial insemination (AI) technique is critical to maximize reproductive outcomes. The objective of this study was to evaluate the effect of AI gun protective plastic sheaths use on AI pregnancy rates (AI-PR) in beef cattle. Angus cross beef cows (n=321) and Angus cross beef heifers (n=430) were randomly assigned to one of two groups: with (TRT) or without (CON) the use of disposable plastic sheath during the AI procedure. Accounting for estrus expression at or prior to AI ( $P < 0.01$ ), the use of protective sheath neither in cows (sheath, 59.4% [95/160] vs. no sheath, 55.9% [90/161];  $P > 0.1$ ) nor in heifers (sheath, 58.3 [127/218] vs. no sheath, 56.7% [121/212];  $P > 0.1$ ) improved AI-PR. Both cows and heifers that expressed estrus at or prior to AI had greater AI-PR compared to that did not express estrus (cows: estrus, 64.4% [116/180] vs. no estrus, 48.9 [69/141]; heifers: estrus, 64.6 [144/213] vs. no estrus, 50.2 [104/207];  $P < 0.01$ ). In conclusion, carrying out a hygienic AI technique is critical for AI success; however, the use of protective sheath did not improve reproductive outcomes in beef cattle.

**Keywords:** Beef cattle, artificial insemination, AI gun, protective sheath, pregnancy rate

### Introduction

Artificial insemination was the first reproductive biotechnology applied to improve reproduction and genetics of farm animals.<sup>1</sup> The utilization of AI has an enormous impact worldwide in many species, mainly among domestic livestock including cattle, sheep, goats, pigs, horses, poultry (turkeys, ducks, fowl) and rabbits. The worldwide acceptance of AI technology provided a thrust for the development of other assisted reproductive technologies, such as pharmacological regulation of estrous cycle, multiple ovulation and embryo transfer, *in-vitro* embryo production, sexing of sperm and embryo, cryopreservation of gametes and embryo, and somatic cell nuclear transfer and cloning.<sup>1,2</sup> In cattle the safest and best method of insemination is the recto-vaginal method which involves traversing the anatomic barriers of the female reproductive tract, the cervix, and deposition of frozen-thawed semen in the body of the uterus under hygienic conditions. The efficiency of insemination depends on the deposition of proper numbers of normal spermatozoa at the appropriate site in the reproductive tract at the precise time relative to estrus. In addition, an adequate and clean AI technique is recommended to improve reproductive outcome.<sup>3</sup> Improper handling of instruments and unsanitary conditions may lead to lower fertility.

Cattle fertility is influenced by several factors including herd environment and management practices.<sup>4</sup> It should be noted that during the AI procedure, not only semen but also bacteria and debris may be introduced into the lumen of the uterus. Studies revealed that dairy cows that developed post-AI subclinical endometritis had lower conception rates.<sup>5</sup> This could be due to either uterine reaction to sperm, existing infection, or introduction of pathogens from the vagina into the uterus. In beef cows endometrial cytology did not predict final pregnancy status or day of conception when samples were collected >50 d postpartum, indicating that beef cows were able to clear uterine inflammation after resumption of ovarian cyclicity.<sup>6</sup> However, a recent study that followed beef cows for 130 days postpartum concluded that cows with subclinical endometritis had an increase in time to conception and a decrease in the risk of pregnancy.<sup>7</sup>

Use of protective sheaths (double sheath insemination procedure) prevents introduction of vaginal contaminants including pathogenic organisms into the uterus via the AI gun. Previous studies reported no improvement in first service conception rates<sup>3,8</sup> and improved pregnancies per AI for second or greater

services in dairy cattle<sup>9</sup> following the use of AI catheter protective sheaths during AI compared to no protective sheath use.

Use of protective sheaths incurs additional cost to beef producers whereas failure to use protective sheaths may lower pregnancy rate. This study was designed with an intent to provide a comprehensive recommendation on whether or not to use AI gun protective sheaths while performing AI in beef cattle. The objective of this study was to evaluate the effect of AI gun protective plastic sheaths (PS) use on AI pregnancies in beef cattle.

## Materials and methods

### Animals and breeding management

*Cows.* A total of 321 Angus cross beef cows from three similarly managed commercial cow-calf operations inseminated during spring of 2014 were included. Within locations cows were randomly assigned to Ovsynch+ controlled internal drug release (CIDR) or CO-Synch+CIDR groups (Figure 1A) and were assigned a body condition score (BCS; 1-emaciated; 9-obese) at initiation of synchronization protocol (Day 0). Cows in Ovsynch+CIDR group received a CIDR (1.3 g of progesterone; per vagina; Eazi-Breed™ CIDR® Cattle Insert; Zoetis Animal Health, New York, NY) vaginal insert and 100 µg gonadorelin diacetate tetrahydrate (GnRH; 2 mL; im; Cystorelin®, Merial Inc., Duluth, GA) on Day 0, 25 mg prostaglandin F2alpha (PGF; dinoprost tromethamine sterile solution, 5 mL; im; Lutalyse®, Zoetis Animal Health) and CIDR removal on Day 7, 100 µg GnRH, im, 48 h later on Day 9 and insemination on Day 10, 66 hrs after CIDR removal. Cows in CO-Synch+CIDR group received treatment similar to cows in Ovsynch+CIDR group except the second GnRH (100 µg; im) was administered at the time of AI. Sires (n=5) were selected and assigned to cows based on sire traits and to avoid inbreeding. Inseminators (n=6) included in the study were experienced but differed among locations. Cows were maintained in pastoral conditions and during the winter months supplemented with hay. The cows were fed to meet NRC recommendations.<sup>10</sup>

At the time of AI, cows were randomly assigned to one of the two treatment groups, with (TRT, n = 160) or without (CON, n = 161) the use of AI catheter protective sheath. The sheath (30 cm in length × 0.7 cm in diameter) was a rigid polyvinyl chloride tube (King et al., 1984). The sheath was funnel shaped at one end, where the insemination catheter was introduced, whereas the other end was sealed with a prescored soft rubber cap that can be easily punctured when pressure was applied. For all services performed in the TRT group, the AI catheter protected with a sheath was introduced in to the vagina; at the cranial portion of the vagina adjacent to the cervical os, the sheath was punctured by withdrawing it back, exposing the AI catheter that was advanced through the cervix into the uterine body for semen deposition. In the CON group, the AI catheter was introduced to the vagina without the use of sheath and was advanced through the cervix for deposition of the semen in the uterine body.

All cows were fitted with a heat detector aids estrus detection aids (Kamar® Heatmount detector patches; Kamar, Inc., Steamboat Springs, CO) or Estrus Alert patches (Western Point Inc., Apple Valley, MN) or chalk at CIDR removal. After CIDR removal, the cows were observed twice daily until insemination for estrus and estrus detection aid status (estrus, activated aids or lost aids with mount marks vs. no estrus, intact aids) and estrus status (standing to be mounted) was recorded. A cow was determined to be in estrus if she was observed to stand for mounting or if she had an activated, lost (with mount marks) or partially-activated aid. The timing of CIDR insertion, CIDR withdrawal, and interval from CIDR withdrawal to timed-AI was recorded for each animal. Age of the cows was retrieved from the records.

Two weeks later, intact Angus bulls were placed with cows (approximately 1:30 to 1:50), across treatments, for the remainder of the 60 to 70 d breeding season. Cows were examined for pregnancy status approximately 70 days after AI by ultrasonography of the uterus and its contents to differentiate cows bred by AI or natural service.<sup>11,12</sup> The criteria considered were the size of the amniotic vesicle, fetus, and placentomes. The AI pregnancy rate was calculated as the number of cows pregnant to AI divided by the total number of cows inseminated.

*Heifers.* A total of 430 Angus cross beef heifers from four similarly managed commercial cow-calf operations inseminated during spring of 2013 were included. The goal of the participating farms was to have heifers calve at two year of age. The heifers were fed to meet NRC recommendations.<sup>10</sup> At enrollment, heifers were assigned a BCS (1 to 9; 1, emaciated; 9, obese) and a reproductive tract score (RTS, 1 to 5; 1, immature, anestrus; 5, mature, cycling). Within the herd, heifers were randomly assigned to long term (LT)-72 (14-d CIDR-PGF-GnRH; n=203) or short term (ST)-72 (5-d CO-Synch+CIDR; n=227) estrous synchronization protocol groups (Figure 1B). Briefly, heifers in the LT-72 group received a CIDR from Days 0 to 14, followed by 25 mg of PGF 16 days later (Day 30). In order to inseminate heifers in both LT and ST groups at the same time, the synchronization of heifers in ST-72 group was initiated on Day 25. Heifers in the ST-72 group received a CIDR and 100 µg of GnRH on Day 25 followed by 25 mg of PGF at CIDR removal on Day 30 and a second dose of PGF six hours later (Day 30). Artificial insemination was performed at 72 h (Day 33) after CIDR removal. All heifers were given GnRH (100 µg, im) at the time of insemination. The sires (n=7) were selected and assigned to heifers based on sire traits and to avoid inbreeding. Inseminators (n=7) were experienced but differed among locations.

At the time of AI, heifers were randomly assigned to one of the two treatment groups, with (TRT, n=218) or without (CON, n=212) the use of protective sheath as described previously. All heifers were fitted with a heat detector aids estrus detection aids (Kamar® Heatmount detector patches; Kamar, Inc., Steamboat Springs, CO or Estrus Alert patches; Western Point Inc., Apple Valley, MN) or chalk at CIDR removal. After CIDR removal, the heifers were observed twice daily until insemination for estrus and estrus detection aid status (estrus, activated or lost aids with mount marks vs. no estrus, intact aids) and estrus status (standing to be mounted) was recorded as described previously. The timing of CIDR insertion, CIDR withdrawal, interval to the second PGF injection and timed-AI was recorded for each animal. Age of the heifers was retrieved from the records.

Two weeks later, intact Angus bulls were placed with heifers (approximately 1:30 to 1:50), across treatments, for the remainder of the 60 to 70 d breeding season. Heifers were examined for pregnancy status approximately 70 days after AI by ultrasonography of the uterus and its contents to differentiate heifers bred by AI or natural service sires.<sup>11,12</sup> The criteria considered were the size of the amniotic vesicle, fetus, and placentomes. The AI pregnancy rate was calculated as the number of heifers pregnant to AI divided by the total number of heifers inseminated.

#### Statistical analyses

Data were analyzed with a statistical software program (SAS Version 9.4 for Windows, SAS Institute, Cary, NC). Mean BCS, age, days postpartum, the mean interval (h) CIDR insertion to CIDR withdrawal, and mean interval (h) from CIDR withdrawal to timed-AI in cows between TRT and CON groups were analyzed using one-way ANOVA (PROC GLM of SAS). Similarly differences in the mean BCS, RTS, age, the mean interval (h) from CIDR insertion to CIDR withdrawal, the mean interval (h) from first to second PGF injection and the mean interval from CIDR withdrawal to timed-AI in heifers between TRT and CON groups were analyzed using one-way ANOVA (PROC GLM of SAS).

PROC GLIMMIX of SAS was used to examine the differences in AI pregnancy rate between TRT and CON groups. Variables included in the model for cows were treatments (sheath vs. no sheath), synchronization treatments, BCS categories ( $\leq 5$  vs  $> 5$ ), days postpartum categories ( $\leq 60$  vs.  $> 60$  days), age categories (2, 3 to 6 and  $> 6$  yrs), estrus (expression at or prior to AI vs. no expression) and appropriate interaction. Variables included in the model for heifers were treatments (sheath vs. no sheath), synchronization treatments, BCS categories ( $\leq 5$  vs  $> 5$ ), age categories ( $\leq 15$  vs.  $> 15$  mo), RTS (2 to 5), estrus (expression at or prior to AI vs. no expression) and appropriate interactions. In the model, AI sires, inseminators and locations were used as random effects. All main effects were retained during model reduction in the model. The 'P' value at 0.05 was considered significant.

#### Results

The mean ( $\pm$  SEM) age, mean BCS and mean days postpartum for cows are given in Table 1. The mean age, mean body condition scores and mean days postpartum in cows did not differ between TRT and CON groups (Table 1;  $P>0.1$ ). Similarly, the mean ( $\pm$  SEM) age, mean BCS and mean RTS for heifers are given in Table 1. The mean age, mean BCS and mean RTS in heifers did not differ between TRT and CON groups (Table 1;  $P>0.1$ ). The proportion of RTS 5, 4, 3, and 2 were 51.9 (223), 21.6 (93), 17.2 (74) and 9.3% (40), respectively. The mean time (h) from CIDR insertion to CIDR withdrawal, and interval from CIDR withdrawal to timed-AI in cows did not differ between TRT and CON groups ( $P>0.1$ ). Similarly, the mean time (h) from CIDR insertion to CIDR withdrawal, interval from first to second PGF injection and time from CIDR withdrawal to timed-AI in heifers did not differ between TRT and CON groups ( $P>0.1$ ).

Accounting for estrus expression at or prior to AI ( $P<0.01$ ), the use of protective sheath did not improve AI-PR in cows (sheath, 59.4 [95/160] vs. no sheath, 55.9% [90/161];  $P>0.1$ ; Figure 2). The AI-PR for synchronization treatments (Ovsynch+CIDR, 58.8% [100/170] vs. CO-Synch+CIDR, 56.3% [85/151]), BCS categories ( $\leq 5$ , 57.4% [58/101] vs.  $> 5$ , 57.7% [127/220]), days postpartum categories ( $\leq 60$ , 56.7% [148/261] vs.  $> 60$  days, 61.2% [27/60]) and age categories (2, 52.0% [13/25]; 3 to 6, 59.2% [115/192] and  $> 6$  yrs, 54.8% [57/104]), were not significantly different ( $P>0.1$ ; Table 2). Cows that expressed estrus at or prior to AI had greater AI-PR compared cows that did not express estrus (estrus, 64.4% [116/180] vs. no estrus, 48.9% [69/141];  $P<0.01$ ).

Accounting for estrus expression at or prior to AI ( $P<0.01$ ), the use of protective sheath did not improve AI-PR in heifers (sheath, 58.3% [127/218] vs. no sheath, 56.7% [121/212];  $P>0.1$ ; Figure 2). The AI-PR for synchronization treatments (ST-72, 58.6% [133/227] vs. LT-72, 56.3% [115/203]), BCS categories ( $\leq 5$ , 57.1% [93/163] vs.  $> 5$ , 58.1% [155/267]), and RTS categories (5, 60.9% [136/223], 4, 56.5% [52/93], 3, 55.4% [41/74], 2, 47.5% [19/40]) were not significantly different ( $P>0.1$ ; Table 2). Age categories had a trend for differences in AI-PR ( $\leq 15$ , 55.4% [56/101];  $> 15$  mo, 58.4% [192/329];  $P<0.1$ ). Heifers that expressed estrus at or prior to AI had greater AI-PR compared heifers that did not express estrus (estrus, 64.6% [144/213] vs. no estrus, 50.2% [104/207];  $P<0.01$ ).

## Discussion

The results of the present study revealed that the use of protective sheath did not improve AI-PR in beef heifers and in beef cows. Overall AI-PR was comparable to studies that used similar reproductive protocols.<sup>13,14</sup>

The purpose of using protective sheath is to minimize contamination of the AI catheter at the time of AI in order to prevent introduction of pathogens into the uterus and to improve reproductive outcome. Although bacterial isolation was not performed in this study, a study in dairy cattle showed a greater proportion of recognized potential and opportunistic uterine pathogens isolated from samples taken from the AI gun in cows inseminated without the protective sheath compared with cows inseminated with the protective sheath.<sup>9</sup> The recognized uterine pathogens isolated were *Trueperella pyogenes*, *Prevotella melaninogenica*, *Escherichia coli*, *Fusobacterium necrophorum*, and *Proteus* spp., potential uterine pathogens isolated were *Bacillus* spp. and *Pasteurella* spp., and opportunistic uterine contaminants isolated were *Streptococcus* spp., *Providencia* spp., *Klebsiella* spp., and *Corynebacterium* spp. These pathogens have been associated with postpartum uterine diseases and reduced fertility in dairy cattle. Even though, the prevalence and severity of uterine disease in beef cattle is not as great as those in dairy cattle (17% vs. 51% prevalence at 7 wk. postpartum, respectively),<sup>6,15</sup> it is possible that these pathogens could reside in the vagina of beef cattle.

Beef females included in this study were synchronized by CIDR protocols that required vaginal placement of inserts from 5 to 14 days. The placement of an intravaginal device provides the opportunity for bacterial vaginitis. Bacterial culture of swabs of the vagina after a 7-d treatment period revealed moderate growth of coliforms, environmental *Streptococcus* spp. and *Staphylococcus* spp., and other gram-positive, rod-shaped organisms.<sup>16</sup> Bulman et al sampled close to the cervical os after 14 d of progesterone-releasing intravaginal device (PRID) treatment and isolated *Enterococcus* spp., *Escherichia coli*, and *Proteus* spp. from 90% of the treated animals and 40% of animals examined with a speculum.<sup>17</sup>

However, from repeated sampling, the authors reported no bacterial growth in 90% of the cows 7 d after device removal and in 100% of the cows 14 d after device removal.<sup>17</sup> It should be noted that application of vaginal insert did not reduce reproductive outcome in beef cattle.

In general, beef females are exposed to bulls during the rest of the breeding season after the first AI. This poses a greater risk of beef females acquiring pathogens from beef bulls. Female reproductive tract contamination by *Ureaplasma* spp., *Mycoplasma* spp.<sup>18</sup> and venereal pathogens such as *Campylobacter fetus*<sup>19,20</sup> and *Tritrichomonas fetus*<sup>21</sup> poses a greater risk for reduced reproductive outcomes.

In lactating dairy cows, a higher proportion of polymorphonuclear neutrophils (PMN) (>15%) immediately before<sup>22</sup> and 4 h after AI<sup>5</sup> were associated with poor reproductive performance. This indicates that the dairy cows with lowered reproductive performance possibly suffered from poor uterine clearance and poor immunological response due to reduced estradiol concentration around the time of AI.<sup>23,24</sup> It should be noted that beef cows are able to clear uterine inflammation after resumption of ovarian cyclicity.<sup>6</sup> Beef cows that exhibited estrus had significantly greater preovulatory peak estradiol concentrations than cows not exhibiting estrus.<sup>20</sup> Similarly, preovulatory plasma estradiol concentrations on day of AI for dairy cows diagnosed pregnant was greater compared to cows that are not pregnant.<sup>25</sup> Results from these studies indicated that preovulatory estradiol concentrations were greater in beef cows compared to dairy cows. This elevated estradiol concentrations in beef females could possibly contributed to the required uterine clearance and immunological response around the time of AI resulted in similar AI pregnancy between TRT and CON groups.

Beef females that expressed estrus had greater AI pregnancy compared to those that did not express estrus. Preovulatory estradiol is essential for preovulatory luteinizing hormone secretion,<sup>26</sup> and preparation of ideal oviduct and uterine environments for proper embryo development.<sup>27,28</sup> Therefore it is possible that elevated concentrations of estrogen in cows that expressed estrus had effects on follicle development and ovulation, and effects on uterine environment to provide a more conducive environment for fertilization and subsequent embryo development.

## Conclusions

In conclusion, a hygienic AI technique is critical for AI success; however, the use of protective sheath did not improve reproductive outcomes in beef cattle. Beef females that expressed estrus had greater AI pregnancies likely as a result of differences follicle development and favorable uterine environment for fertilization and subsequent embryo development.

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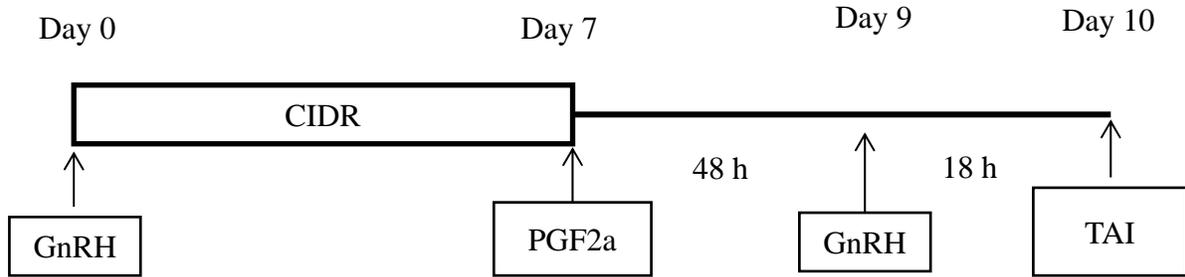
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Figure 1: Schematic presentation of synchronization protocol  
Figure 1A

Ovsynch + CIDR



CO-Synch + CIDR

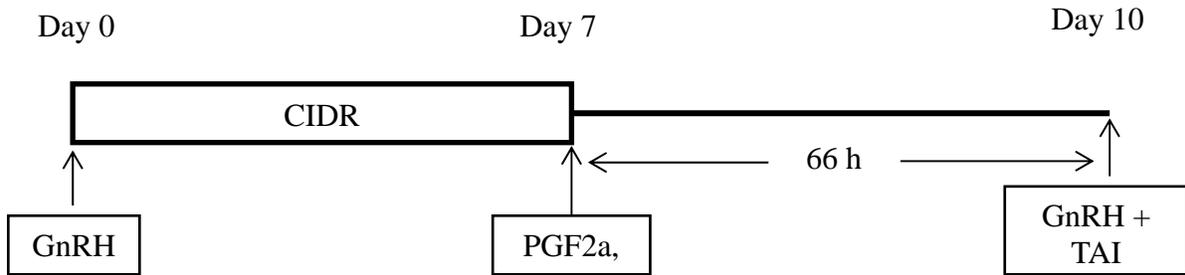




Table 1. Mean  $\pm$  SEM of age, body condition scores, and days postpartum of beef cows belonging to treatment groups in different locations

Cattle groups	Variables	Treatment	n	Mean $\pm$ SEM
Cow	Age (yr)	CON	161	5.73 $\pm$ 0.21
		TRT	160	5.58 $\pm$ 0.21
	BCS	CON	161	6.07 $\pm$ 0.08
		TRT	160	6.43 $\pm$ 0.45
	DPP	CON	161	52.8 $\pm$ 0.67
		TRT	160	52.9 $\pm$ 0.68
Heifers	Age (mo)	CON	212	15.3 $\pm$ 0.43
		TRT	218	15.6 $\pm$ 0.37
	BCS	CON	212	5.93 $\pm$ 0.97
		TRT	218	6.12 $\pm$ 1.49
	RTS	CON	212	4.09 $\pm$ 1.08
		TRT	218	4.14 $\pm$ 1.07

SEM, Standard error of the mean; CON, AI gun with no protective sheath; TRT, AI gun with protective sheath; BCS, Body condition score (1 to 9; 1, emaciated; 9, obese); DPP, days postpartum; RTS, Reproductive tract score (1 to 5; 1, immature, anestrus; 5, mature, cycling);

Table 2. Effect of explanatory variables on AI pregnancy rates from beef cows and beef heifers that were inseminated with (TRT) and without (CON) the use of a protective plastic sheath (PS)

Beef cattle	Explanatory variables	Degrees of Freedom	F value	P value
Cow	Protective sheath	1	0.08	0.78
	Synchronization treatment	1	0.41	0.52
	Estrus	1	8.07	0.005
	Age	2	0.71	0.49
	Body condition score	1	0.10	0.79
	Days postpartum	1	0.43	0.52
	Heifer	Protective sheath	1	0.09
Synchronization treatment		1	0.42	0.52
Estrus		1	9.72	0.002
Age		1	2.86	0.09
Body condition score		1	0.10	0.92
Reproductive tract score		3	0.53	0.66

Refer to Figure 1 for cow and heifer synchronization treatment;

Estrus - expression of estrus at or prior to AI vs no expression;

Cow age categories (yrs)- 2, 3 to 6, > 6 years;

Body condition score (1 to 9; 1, emaciated; 9, obese) categories,  $\leq 5$  and  $> 5$ ;

DPP, Days postpartum categories  $\leq 60$  and  $> 60$ ;

Reproductive tract score (1 to 5; 1, immature, anestrus; 5, mature, cycling), categories 2 to 4;

Heifer age categories (mo),  $\leq 15$  and  $> 15$ ;

Cow - Covariance parameter estimates: Location, 0.07831; AI sire, 0.06429; Inseminators, 0.03344; Residual 0.28937; Fit statistics - BIC =1276.06; -2 Res log likelihood =1254.51;

Heifer - Covariance parameter estimates: Location, 0.10586; AI sire, 0.08713; Inseminators, 0.08237; Residual, 0.22820; Fit statistics, BIC = 1386.8; -2 Res log likelihood = 1371.8;

