# Cow/Calf Split Session

*Moderator* - Michael Downey



# Androgenization of Cows as Aids for Estrous Detection and Influence of Biostimulation on Postpartum Reproduction

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## Introduction

Androgenized females have been well established as aids for detection of estrus in beef and dairy herds and offer several advantages in assisting humans in finding cows in estrus. Androgenized females have been observed to cause increased mounting of estrous cows and reduced mounting of non-estrous cows. Further, they have allowed for identification of more cows in estrus than human observation alone.

Biostimulation was a term coined to describe the stimulatory (positive) effects of a male on estrus, ovulation or pregnancy. In cattle, biostimulatory effects were first inferred by producers with well-fed cows on year-round natural mating breeding programs who observed cows returning to estrus earlier post partum than expected. Studies where natural mating showed advantages over artificial insemination also implied a biostimulatory effect for males. Well controlled studies provided evidence that exposure of cows to sterile bulls induced earlier return to estrus post partum than was observed in unexposed cows. Additionally, placement of androgenized females with postpartum cows also shortened postpartum intervals to first estrus, similar to results obtained with exposure to sterile bulls.

## Androgenization of Females

A variety of methods for exogenous delivery of testosterone or synthetic analogs have been reported. Probably the first practical and reliable application was reported by Kiser *et al.*, 1977. Their regimen consisted of an induction treatment with intramuscular (i.m.) injections of 200 mg testosterone propionate every other day for 20 days, followed by subcutaneous (s.c.) injections of 0.5 or 1.0 gm of testosterone enanthate (TE) approximately every 2 weeks. Their conclusions were that testosterone-treated cows (fitted with chin-ball markers) detected cows in estrus not observed by a herdsman. In addition, testosterone-treated cows and surgically altered bulls were equally effective for detection of estrus.

A modification of this treatment, consisting of an induction dosage of 2 gm TE with 0.5 gm injected i.m. and 1.5 gm injected s.c. followed by 0.5 to 0.75 gm administered s.c. every 10 to 14 days, was reported by Heekin, 1983. This treatment required fewer injections, took less time from first injection until cows were active as detector animals, and was equally effective to the testosterone propionate regimen reported by Kiser and coworkers in preparing cows as estrous detectors. This treatment, with the modification of a dosage of 1 gm TE s.c. every 14 days, has evolved into the standard for androgenizing cows for use as detector animals.

Disadvantages with this system, such as frequent handling, short duration of sexual activity without a booster administration every 2 weeks, and relatively high drug costs, led Mortimer *et al.*, 1990, to evaluate use of Synovex-H® implants (Syntex Laboratories, Inc., Palo Alto, CA) to androgenize 4 freemartin heifers. They

reported the likelihood of detecting cows in estrus was increased when heifers androgenized in this manner were added to a cow herd, but cautioned interpretation of results due to lack of non-treated or traditionally-treated controls.

This history led us to a recently completed study at Clemson University. Twenty multiparous, non-lactating, cyclic beef cows were blocked to 5 groups of 4 by descending dominance rank and randomly allotted to 1 of 4 treatment groups: no hormone treatment, control (C); 8 Synovex-H® implant dosages (S); TE treatment (T); and 8 Synovex-H® implant dosages + TE priming (ST).

Treatment for group S consisted of 4 Synovex-H® dosages implanted s.c. on the convex surface of both ears. Each Synovex-H® implant dosage contained 8 pellets with total concentrations of 200 mg testosterone propionate and 20 mg estradiol benzoate per dosage. These 8 dosages of Synovex-H® provided a total dosage of 1600 mg testosterone propionate and 160 mg estradiol benzoate per cow. No injectable hormones were administered to cows in group S. Treatment for group T consisted of 0.5 gm TE administered i.m. and 1.5 gm TE administered s.c. on the first day of treatment followed by 1 gm TE s.c. every 14 days through and including day 84. Females in group ST received 8 Synovex-H® dosages as described previously. Additionally, 0.5 gm and 1.5 gm TE were administered i.m. and s.c., respectively, concurrent with administration of Synovex-H.® No further testosterone preparations were administered to cows in group ST.

Estrous detection trials were conducted on days 35, 56 and 84. During estrous detection trials, 4 females, 1 from each of the 4 treatment groups, were randomly selected and placed with 8 cows known to be in estrus and 4 pregnant cows. This group was monitored for 1 hr and number of mounts and attempted mounts were recorded.

A pool of estrous females was created by synchronization with Syncro-Mate-B® (Sanofi Animal Health, Overland Park, KS) to assure cows in estrus were continuously available throughout each trial. A cow was considered to be in estrus when observed standing to be mounted 2 or more times 30 to 60 min prior to estrous detection trials and considered to be out of estrus if consistently refusing to be mounted 3 times during trials. Pregnant cows utilized in estrous detection trials were in the last trimester of gestation.

All testosterone treatments raised serum testosterone concentrations above pre-treatment and control levels (P<.05). Mean pre-treatment testosterone concentrations did not differ and were 0.21, 0.23, 0.23 and 0.22 ng/ml for C, S, T and ST cows, respectively. Mean concentrations from 1 to 120 days were 0.19, 0.88, 4.42 and 2.72 ng/ml for C, S, T and ST cows, respectively (P<.05).

Testosterone concentrations for cows in T and ST groups increased immediately to 13 to 14 ng/ml (Figure 1). Treatment T maintained testosterone levels at 2.5 to 6.5 ng/ml until about day 95, while concentrations for ST cows declined by day 30 and were maintained at 0.5 to 2 ng/ml. Treatment S immediately increased testosterone levels to about 1.5 ng/ml and maintained levels at 0.5 to 2 ng/ml. Testosterone levels for cows in group T returned to basal levels 92 days following last injection, while testosterone levels for cows in groups S and ST did not return to basal levels until 225 days after treatment. This finding was disturbing, since one advantage in the use of androgenized females has been freedom to sell these females at the end of a breeding season. This drawback might be overcome by removal of implants; however, physical properties of Synovex-H® implants make this difficult.

Estrous detection scores did not differ over time. Therefore, data from observations on days 35, 56 and 84 were averaged (Table 1). All testosterone-treated groups performed more total mounts and mounts on estrous cows than controls (P<.05). Cows in group ST performed more total mounts and mounts on estrous cows than S and T cows (P<.05), while mounts performed by S and T cows did not differ. Virtually no mounts were performed on pregnant cows and less than 1 mount/h was performed on other testosterone-treated cows. Cows treated with ST received the greatest number of mounts from penmates (P<.05), followed by S and T cows, while C cows received no mounts.

**Table 1.** Mean estrous detection scores[1] obtained during observation of 8 estrous and 4 pregnant cows with a group of 4 treatment cows.[2]

| Treatment[3] | Total Mounts | Mounts on Estrus | Mounts on Pregnant | Mounts on Detectors | Total Times Mounted |
|---|---|---|---|---|---|
| C | 1.5[a] | 1.5[a] | 0.0 | 0.0 | 0.0[a] |
| S | 6.1[bd] | 5.9[b] | 0.0 | 0.2 | 3.3[bc] |
| T | 8.7[be] | 7.8[b] | 0.1 | 0.9 | 2.9[b] |
| ST | 18.2[c] | 18.2[c] | 0.0 | 0.0 | 5.5[c] |

1 Estrous detection scores were averaged from tests conducted on days 35, 56 and 84 after treatment.

2 Each group of 4 included 1 cow from each treatment group.

3 C = Control, no hormone treatment.
S = Synovex-H® with no additional hormones.
T = Multiple TE injections.
ST = Synovex-H® + TE.

[a,b,c] Within variable, values with different letters differ (P< .05).
[d,e] Within variable, values with different letters differ (P< .09).

Mean percent of estrous females detected by cows in groups treated with testosterone was greater than that detected by controls (P<.05; Table 2). Treatment ST cows detected more estrous cows than T or S cows (P<.05). Only cows within treatment T differed over time in percent detected. These cows detected a greater percent on

day 35 than on days 56 or 84 (P<.05; Table 2).

Mounting behavior was not correlated to testosterone concentrations, in agreement with similar studies with androgenized females and bulls. Due to limitations in experimental design, no estimate could be made concerning minimum testosterone concentration required to elicit male behavior. However, once attained, male-like behavior was maintained with blood testosterone concentrations of 0.5 to 2 ng/ml.

**Table 2.** Mean percent of estrous cows detected during a 1 hour observation of 8 estrous and 4 pregnant cows with a group of 4 treatment cows[1] over 3 observation periods.

| | Days After Treatment | | | |
|---|---|---|---|---|
| Treatment[2] | 35 | 56 | 84 | Average |
| C | 21[a] | 5[a] | 2[a] | 9[a] |
| S | 30[abd] | 24[b] | 26[b] | 27[b] |
| T | 42[bf] | 17[abg] | 15[abg] | 25[b] |
| ST | 46[be] | 31[b] | 40[c] | 39[c] |

[1] Include 1 cow from each treatment group.

[2] C = Control, no hormone treatment.
S = Synovex-H® with no additional hormones.
T = Multiple TE injections.
ST = Synovex-H® + TE.

[a,b,c] Within columns, values with different letters differ (P<.05).
[d,e] Within columns, values with different letters differ (P<.09).
[f,g] Within rows, values with different letters differ (P<.05).

Treatment with multiple injections of TE successfully increased mounting activity, as did treatment with Synovex-H® alone. However, intense mounting behavior was maintained for a shorter period of time with multiple injections of TE. Treatment with Synovex-H® coupled with an initial dose of TE proved most effective in increasing mounting behavior for the purpose of estrous detection.

## Biostimulatory Effects On Reproduction In Postpartum Cows

Average postpartum intervals to estrus range from 46 to 168 days in suckled beef cows (see review of Dunn and Kaltenbach, 1980). With such large variations in reproductive response, many lactating cows may not be cyclic either at the start of, or early in a breeding season. With this in mind, there are many avenues to pursue in improving reproductive performance. Many of these have traditionally utilized management resources to manipulate nutrition and body condition scores to ensure early cyclicity post partum. Biostimulation appears to offer another management option to use in improving beef cow reproduction. To our knowledge, all controlled studies to date have shown biostimulatory effects on reducing postpartum interval to estrus in primiparous as well as multiparous cows.

When multiparous cows were exposed to bulls within 3 days of parturition, onset of estrus was advanced by about 20 days compared to cows isolated from bulls until 53 days post partum. This biostimulatory effect on return to postpartum estrus can also be elicited with testosterone-treated cows. In a study reported from our laboratory (Table 4), it was observed in Exp. 1 that cows exposed to either bulls or testosterone-treated cows had similar postpartum intervals to estrus. However, in Exp. 2, cows exposed to bulls had an 8-day earlier return to estrus than did cows isolated from biostimulation, and in Exp. 3, cows exposed to testosterone-treated cows had a 12-day earlier return to estrus than did cows isolated from biostimulation. It would appear that bulls or androgenized females elicit similar biostimulatory effects in reducing postpartum interval to estrus.

**Table 3.** Days from calving to resumption of estrous cycles (Zalesky *et al.*, 1984).

| | Days from calving to estrus | |
|---|---|---|
| Group | 1981 | 1982 |
| Bull exposure | 43[a] | 39[a] |
| Isolated | 63[b] | 61[b] |

[ab] Values with different superscripts within year differ (P<.01).

**Table 4.** Effects of biostimulation on postpartum intervals to estrus (ITE) and pregnancy (ITP) (Burns and Spitzer, 1992).

| Group | ITE | ITP |
|---|---|---|
| Exp. 1 | | |
| Bull exposure | 43 | 80 |
| TTC[a] exposure | 43 | 85 |
| Exp. 2 | | |
| Bull exposure | 44[b] | 81 |
| Isolated | 52[c] | 85 |
| Exp. 3 | | |
| TTC[a] exposure | 41[b] | 87 |
| Isolated | 52[c] | 91 |

[a] TTC = testosterone-treated cows (Multiple TE injections).
[bc] Values with different superscripts within experiment differ (P<.05).

Note that interval to pregnancy was not different in this series of experiments. This was because biostimulation exerted its stimulatory effects early post partum (Table 5). By 40 days post partum, 29 and 31%

more cows exposed to bulls or testosterone-treated cows, respectively, were observed to be in estrus compared with cows isolated from biostimulation (Exp. 2 and 3, respectively). By 60 days post partum, 23% more cows exposed to testosterone-treated cows were observed to be in estrus compared with cows isolated from biostimulation (Exp. 3). After 60 days post partum, biostimulation had no effect on percentage of cows in estrus. These data correspond to the report of Zalesky *et al.*, 1984, who indicated the biostimulatory effect occurred prior to day 53 post partum.

**Table 5.** Effects of biostimulation on cumulative percentages in estrus by days post partum (Burns and Spitzer, 1992).

| | Cumulative % in estrus | | | | |
|---|---|---|---|---|---|
| Group | 20 d | 40 d | 60 d | 80 d | 100 d |
| Exp. 1 | | | | | |
| Bull exposure | 2 | 52 | 84 | 96 | 100 |
| TTC[a] exposure | 4 | 55 | 85 | 94 | 97 |
| Exp. 2 | | | | | |
| Bull exposure | 4 | 52[b] | 76 | 95 | 96 |
| Isolated | 0 | 26[c] | 62 | 92 | 98 |
| Exp. 3 | | | | | |
| TTC[a] exposure | 3 | 62[b] | 87[b] | 95 | 95 |
| Isolated | 5 | 31[c] | 64[c] | 90 | 100 |

[a] TTC = testosterone-treated cows (multiple TE injections).
[bc] Within experiment, values with different superscripts within day differ (P<.05).

With the fixed breeding season used in these experiments, a majority of cows were cyclic before the start of the breeding season, regardless of treatment. In the study of Burns and Spitzer, 1992, after the first 20 days of breeding, 97% of cows exposed to biostimulation and 94% of cows isolated from biostimulation were observed to be in estrus. Therefore, biostimulation had no effect on postpartum interval to pregnancy. However, biostimulation would seem to be beneficial in reducing postpartum interval to estrus in late-calving cows to ensure cyclicity at the start of a breeding season.

## Conclusions

**Long postpartum intervals to estrus in cows is recognized as a major cause of reduced reproductive performance in beef herds. There is overwhelming evidence to support the application of biostimulation in inducing earlier return to postpartum estrus. That this effect may be elicited by testosterone-treated cows may make its application easier.**

Biostimulation appeared to have its effect prior to day 60 post partum, after which no effects were observed. Therefore, biostimulation would be a useful management tool for increasing reproductive performance in late-calving cows by reducing postpartum interval to estrus and having these cows cyclic at either the start of or early in a breeding season to ensure early pregnancy.

## Discussion and Recommendations

Androgenizing cows with the Synovex-H®/TE regimen (4 dosages of Synovex-H® implanted s.c. in both ears concomitant with 0.5 gm TE i.m. and 1.5 gm s.c.) has obvious advantages over previously reported methods in time and labor savings, as well as in reducing drug costs. Additionally, the treatment appears to double or triple mounting activity and increases attempted mounts and investigations for sexual receptivity compared to standard TE regimens or Synovex-H® alone. In our experiences, 90% plus of cows treated will respond with adequate male-like behavior. Mature cows seem to work much better than first-calf-cows and virgin heifers seldom work at all. **PLEASE NOTE**: TE is a controlled substance, prescription drug, and both TE and Synovex-H® are being used in an extra-label manner to androgenize cows. **Withdrawal is 180 days from treatment**. Testosterone enanthate is supplied in 10 ml vials (200 mg/ml) by Geneva Pharmaceutical Inc., Broomfield CO, or Henry Schein, Inc., Port Washington, NY. Certainly be sure to document a valid client-patient-veterinary relationship and incorporate your counsel into the entire system for utilizing testosterone-treated cows in the beef herd management scheme.

Proper cow selection and management are critical to success. We have now used enough lactating cows to feel comfortable in letting our androgenized cow also raise a calf while being a "biostimulator" and/or an "estrous detector." This also helps cover an established withdrawal time, since there is no need to sell a cow until after her calf is weaned at 7 or 8 months of age. Considering herd biosecurity and social interactions among cows in the herd, it would appear wise to select cows to be androgenized **next year** at pregnancy examination this year.

Select pregnant cows which would otherwise be culled on productivity, but will calve early in the next calving season. Choose cows in moderate body condition with no obvious physical problems or undesirable history. Administer testosterone treatments to these cows about 1 month into the calving season. Cows will respond with "male-like" behavior to testosterone treatments administered very early post partum (we have done so on day of calving.) This will maximize early exposure of postpartum cows to provide benefits of biostimulation and should get most herds through an AI breeding period of reasonable length.

A proper ratio of "biostimulators" to postpartum

cows has not been established, and this may differ with whether or not AI will be used. To allow for adequate estrous detection, a ratio of 1:30 to 1:50 would be needed. Without AI, and considering biostimulation appears to affect individual cows fairly early post partum, a ratio of 1:100 may be adequate. More than 1 cow can be used in a pasture successfully, although they will waste a lot of chin-ball marking ink mounting each other when no other cows are in heat.


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# Abstract

## Pulmonary function changes induced by three regimens of bronchodilating agents in calves with acute respiratory distress syndrome


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Two aerosolised bronchodilators, one sympathomimetic and one parasympatholytic, were tested either alone or in combination for their ability to improve the pulmonary function of double-muscled calves suffering from acute respiratory distress syndrome. In control animals treated with 0.9 per cent saline the parameters of pulmonary function and signs of clinical distress did not change significantly within the hour following the first treatment. Among the other animals, both at one hour and seven days after the first treatment, the most clinical improvement was observed in the animals treated with both bronchodilators and the least in the animals treated with clenbuterol hydrochloride. One hour after the first treatment the respiratory system compliance of the animals treated with ipratropium bromide and the arterial oxygen tension of the animals treated with both bronchodilators were significantly enhanced. After seven days the resistive parameters, the rectal temperature and the respiratory rate were also significantly improved in the animals treated with ipratropium bromide or both bronchodilators whereas only the respiratory rate and rectal temperature were significantly reduced in the animals treated with clenbuterol hydrochloride.