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## Laboratory Diagnosis of Bovine Respiratory Disease

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*(Excerpted from 1996 Proceedings of the AABP)*

Bovine respiratory disease remains a significant cause of economic loss to the cattle industry and source of frustration to the cow/calf sector, the feedlot industry, veterinary practitioners, and laboratory diagnosticians.

While numerous infectious agents have been reported to be involved in the Bovine Respiratory Disease complex (BRD) only a handful appear to be of major etiologic significance (Table 1).

Identifying the specific risk factor(s) and agent(s) involved in a particular group of cattle best involves a team approach and this includes feedlot management and personnel, on-site and consulting veterinarians and diagnostic laboratory personnel. Good communication between field veterinarian and the diagnostic laboratory are particularly critical as is an understanding by field veterinarians of the limitations of the various laboratory tests.

Early identification and treatment of sick animals is considered to be the key to success in outbreaks of BRD and early, accurate diagnosis is prerequisite to successful treatment. The inherent time lag in many laboratory tests necessitates that the practitioner often has to act on their initial clinical impression but laboratory confirmation and monitoring can be valuable tools for adjusting treatment and designing preventative protocols.

### Sample Collection and Submission: Upper Respiratory Tract

Nasal swabs have been used to monitor potential pathogens in a group of cattle or as diagnostic procedures in early clinical respiratory disease.

Collection technique is very important when using nasal swabs and different types of swabs are necessary for bacterial cultures versus virus isolation.

Dry cotton swabs are adequate for bacterial cultures. Such swabs need to be inserted

*continued on page 2*

**Table 1.** Infectious agents involved in BRD complex in feedlot cattle.

<b>Major Importance</b>	<b>Minor or Unknown Importance</b>
Viruses	Viruses
Bovine Herpes 1 (IBR)	Bovine Respiratory Syncytial Virus (BRSV)
Bovine Virus Diarrhea	Parainfluenza-3 virus (PI <sub>3</sub> )
Bacteria	Coronavirus
<i>Pasteurella hemolytica</i>	Adenoviruses
<i>Pasteurella multocida</i>	Other herpes viruses
<i>Hemophilus somnus</i>	Bacteria
	<i>Actinomyces pyogenes</i>
	<i>Streptococcus</i> sp.
	<i>E. coli</i>
	<i>Salmonella</i> sp.
	<i>Staphylococcus</i> sp.
	<i>Mycoplasma</i> sp.
	<i>Chlamydia</i> sp.

**BRD continued from page 1**

well into the nasal cavity taking care not to contaminate the swab with the many non-pathogens in the nostrils. The swab should then be placed in prepared transport medium or at least saline or lactated ringers solution for transport to a laboratory.

*Pasteurella hemolytica* and *Pasteurella multocida* are known to inhabit the nasal cavities and upper respiratory tract of normal cattle. The rationale for culturing the upper respiratory tract of incoming or resident cattle, or cattle in early stages of respiratory disease, is to obtain antibiotic sensitivity data on suspected or potential pathogens. Unfortunately, strains that predominate in the upper respiratory tract may not be the same as those that under appropriate circumstances are capable of colonizing the lower respiratory tract and causing pneumonia. Therefore, the antibiotic sensitivity patterns obtained may not be relevant and need to be interpreted with care.

Lung lavage may provide a more accurate sample than nasal swabs but is more difficult to perform and generally impractical under feedlot conditions.

Laryngotracheal cultures using guarded equine swabs is reported to be a compromise in that relevant organisms are more likely to be obtained than from nasal cultures and although still somewhat difficult is easier than lung lavage.

For identification of viral agents involved in the BRD complex, fluorescent antibody (FA) examination of smears from nasal mucosa is preferable to virus isolation. Fluorescent antibody examination is faster, cheaper, and more reliable than virus isolation. Bovine herpes 1 (IBR virus) and respiratory coronavirus are fairly reliably identified with this technique. Bovine respiratory syncytial virus (BRSV) and parainfluenza-3 virus (PI<sub>3</sub>) can often be found but false negative results

are frequent while bovine virus diarrhea virus (BVD) does not lend itself to reliable identification in the nasal cavity.

If one chooses to attempt virus isolation (VI) on swabs of the upper or lower respiratory tract, the swabs need to be calcium alginate-free cotton or dacron. Regular cotton swabs used for bacterial cultures can be inhibitory to many viruses. Swabs should then be shipped chilled but not frozen, in a viral transport media. Some viruses such as BRSV cannot be recovered from mail-in samples. An ELISA test on swabs or lung tissue is effective for BRSV diagnosis but is somewhat expensive.

It is always a good idea to contact the laboratory prior to obtaining samples to be sure just what specimens that laboratory prefers and how they would like them sent.

### Necropsy Specimens

Necropsy evaluation is a valuable tool for disease diagnosis and monitoring.

For collection of specimens a supply of plastic bags, Whorl-Pac or Zip-Lock bags, wide-mouthed plastic or glass containers with 10% neutral buffered formalin, sterile cotton swabs and containers for bacterial culture and calcium alginate-free cotton or dacron swabs for virus isolation are all that should be necessary. Specimens of lung or trachea for culture should be "fist sized" and from representative lesions. This specimen needs to be large enough that the laboratory can sear the surface and obtain a non-contaminated culture from the interior. Preferably specimens should be submitted chilled and not frozen.

Specimens for histologic examination should be thin slices not more than 1 cm thick so that formalin can penetrate, and large enough, at least several cm square, for the pathologist to observe the overall architecture. It is important that tissues be fixed in an adequate amount of formalin. A formalin:tissue

ratio of roughly 10:1 should be used for at least overnight, after which a smaller volume of formalin, or gauze sponges heavily soaked in formalin, can be used to keep the specimens moist while in transit. Inadequate fixation can seriously compromise the diagnostic value of a specimen and negate a lot of work and expense on the part of the veterinarian and feedlot owner or manager. Several specimens from representative lesions in various stages of development are helpful to the pathologist to get the overall picture.

Specimens for virus isolation or fluorescent antibody examination should be unfixed and selected from representative areas. Most laboratories prefer FA and VI specimens to be submitted chilled and not frozen. If the specimens for virus isolation will be more than 24 hours in transit, freezing on dry ice will result in greater isolation success. If specimens are submitted on dry ice, the ice should be tightly sealed since the fumes can lower pH in the container and thereby lower viability of enveloped viral agents such as IBR.

A written record of the clinical and necropsy observations is important to identify trends and compare cases over time and as an aid to the laboratory pathologist. A good written record is also an aid to one's memory if legal questions later arise.

A good way of shipping specimens is in a large insulated, Styrofoam box with adequate padding and refrigerant to hold specimens in place and keep unfixed tissues chilled until arrival at the laboratory.

In general, histologic examination is of limited value in differentiating the common "shipping fever-type" feedlot pneumonias but it can be helpful in giving a rough estimate of the age of lesions and in differentiating these bronchopneumonias from interstitial pneumonia and other respiratory conditions such as lung worms. VQ

## TRICHOMONOSIS

BY PETER J CHENOWETH  
Department of Clinical Sciences

Trichomonosis (previously known as trichomoniasis) is a contagious venereal disease in cattle caused by a protozoon, *Trichomonas foetus*. This disease appears to be widespread in beef herds in different parts of the U.S., even though it has yet to be shown to be a significant problem in Kansas. Despite this, surveys have indicated that 8% of bulls in both Oklahoma and Florida were infected and 15% of

herds in California. The disease can cause significant losses through both delayed breeding and pregnancy loss, as well as incurring costs of testing, treatment and replacements. Trichomonosis may be confused with vibriosis (campylobacteriosis) which may cause similar losses and even be present in the same herd.

In bulls, *T.foetus* occurs on the penis and prepuce, localizing within epithelial crypts and smegma such that older bulls tend to become chronic carriers. No clinical or pathological

signs are reported in bulls. *T.foetus* can survive freezing and thus be transmitted with AI. It can also exist for varying periods on bedding. It is very contagious; 80-90% of females bred to a carrier bull will become infected.

In females, the organism colonizes the vagina, uterus and oviduct. It produces vaginitis and endometritis with a mild discharge. It does not interfere with fertilization but either directly or indirectly starts to adversely affect the fetus from about day 50 day of gestation, with

death occurring between then and approximately 100 days. Pyometra occurs infrequently in affected females. Infertility for 2-5 months is a common sequel, after which females regain fertility along with some degree of convalescent immunity. Females may occasionally retain infection through pregnancy although most of these will eliminate the organism at the first or second estrous cycle post partum. The most obvious sign in the herd is often females returning to estrus 3-4 months after they were bred. In other herds, the first indication of a problem may be a lowered calf crop. On occasion, the initial sign may be an increased number of pyometras at pregnancy test. A tentative herd diagnosis for trichomonosis may be obtained from herd records which show strung-out calvings and/or open females. However, definitive diagnosis depends upon detection of the causal organism. This is best done from the prepuce/penis of mature bulls, using a technique such as the In-pouch7, or Diamond=s media as the preferred culture media.

Risk factors for trichomonosis in herds include commingling of cattle from different herds or backgrounds, use of older bulls, poor fencing and the continued presence in the herd of older or cull females. In bulls, carrier status appears to be related to the development of crypts within the penile and preputial epithelium. Such development is largely age related, although breed differences occur in the age at which some bulls may become carriers. For example, a small number of young (1-2 years) bulls have been identified as infected in Colorado and Florida.

Losses due to *T.foetus* in cattle breeding herds may be substantial. In Oklahoma, annual calf loss in 1979 was estimated at \$2.5 million. A simulation model estimated a reduction of 14 to 50% in annual calf crop when bull prevalence was between 20 and 40%, with net return per exposed cow being reduced by 5 to 35 percent. In large herds where bull prevalence was from 0-35.9%, cow performance measures were adversely affected by large numbers of infected bulls.

When trichomonosis is diagnosed in a herd, a number of options exist. These range from doing nothing and living with the economic consequences, to one or more of the following:

1. AI with semen from an accredited AI center.
2. Vaccination (females only).
3. Cull open cows and older bulls.
4. Institute "clean" vs "dirty" herds.
5. Test and remove positive bulls.

In some situations, a combination of strategies may be best, such as vaccination of older

animals and establishment of a Aclean@ herd with young animals. A restricted breeding season is useful in controlling trichomonosis as it improves early detection as well as reducing the chance of exposure.

Test and removal of infected bulls, which depends upon a reliable testing method and good animal identification and control, was employed successfully in an extensive beef herd in Florida. Here, the mean prevalence of *T.foetus* infected bulls was 11.9%. Test sensitivity of the In-pouch7 diagnostic system was estimated at the following for consecutive tests; 73%, 90%, 96% and 99%. Although this was lower than other published estimates for this technique, it approximates average diagnostic sensitivity for trichomonosis using different techniques in the A.I. industry. Given these data, there is still a greater than 10% chance that an individual bull harboring the infection will test negative. Although a single test is probably adequate to detect the presence of *Trichomonas* in a herd if several bulls are infected, three negative tests at weekly intervals are necessary to ensure that an individual bull is clean.

Given these data, there is still a greater than 10% chance that an individual bull harboring the infection will test negative. Although a single test is probably adequate to detect the presence of *Trichomonosis* in a herd if several bulls are infected, three negative tests at weekly intervals are necessary to ensure that an individual bull is clean.

A number of reasons can be advanced for lowered test sensitivity. For *T.foetus*, false positives are considered to be rare in experienced laboratories, with the possible exception of confusion with an enteric *T.foetus*-like organism as discussed below. False negatives, however, can occur even when an otherwise effective culture media is employed. Problem areas include the following:

**Restraint and Sampling**—a preputial smegma sample should be obtained from the region of the glans penis and the adjacent preputial membrane. This should be as free from contamination as possible. These conditions are not always easy to fulfil, especially when fractious bulls are examined in poor facilities. Bulls which have recently completed natural or AV service may have a reduced population of trichomonads and a minimum 4 day interval without sexual activity prior to sampling has been advised. In bulls with large sheaths, standard insemination pipettes may not be sufficiently long to reach the preferred sampling site.

**Transport and Culturing**—the temperature of the transport/culture media should be as

close to ideal culture temperature (37°C) as possible. Where this cannot be assured, samples should be transported to a laboratory incubator as rapidly as possible. Temperature extremes, contact with atmospheric oxygen, and contamination should be minimized.

**Interpretation**—technicians need to be trained to identify the telltale movements of trichomonads, and to be diligent in searching for them. On occasion, confusion may be caused by the presence of an enteric trichomonad which can resemble *T.foetus*. Tentative observations on this *T.foetus*-like organism indicate that it is possibly a commensal organism which can be isolated from bulls which would not be expected to test positively for *T.foetus* (e.g. young, virgin bulls). Here, definitive diagnosis is dependent upon use of electron microscopy, or specific staining, to define fine structures, which include 4 anterior flagellae and numerous dark-staining bodies in the cytoplasm.

### New Diagnostic Tests.

A number of laboratories have worked on a test for *T.foetus* using DNA probes and PCR Amplification Systems. Although these have shown great promise in improving diagnostic sensitivity and specificity, this method is still to be used as a frontline test by most diagnostic laboratories. VQ

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## Evaluating Stress of Calves Weaned at Three Different Ages

ANDREA BUENO, TODD G. CAPPEL, CHUCK STORY, MARK DRAGASTIN, RICK RASBY, EDD CLEMENS

Trials were conducted to evaluate the effects of weaning calves at 150, 210 and 270 days of age (i.e. August, October and December, respectively). A total of 75 Angus x MARC II heifers calves were used in this study. Heifers were bled on the day of weaning and again at 2, 7, 14 and 28 days after weaning. Blood was analyzed for differential WBC, cortisol, T3 and glucose. Weight changes were recorded. The data suggests October weaned calves (210 days) had both greater blood cortisol and glucose at days 7, 14 and 28 post-weaning and greater weight gains when compared to calves weaned at 150 and 210 days of age.

Source of article can be located in the 1998 Nebraska Beef Report. VQ

## Arthritis and Respiratory Disease

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Lameness in stocker cattle is a common condition. Injury and conditions such as foot rot can cause lameness in stocker cattle. However, infectious arthritis is a severe condition in which, clinically the animals present with a single leg or multiple leg lameness. Swellings may be observed in the soft tissues of the limbs, along tendon sheaths or in joints. The response to antibiotic and ancillary therapy is very poor with a case fatality rate approaching 80%. We have incorporated I.V. Tylan 200 in our treatment protocols along with our regular respiratory treatment on any animal that is demonstrating some arthritic lameness. Results have been equivocal. Animals may make some improvement and a very few may actually recover, however, the end result is usually death or severe chronic disease. Animals typically are 2 to 3 weeks post arrival with the entire group having gone through an expected respiratory disease outbreak beginning at 2 days to 10 days after arrival. Etiologic agents such as *Haemophilus somnus* and *Mycoplasma bovis* have been implicated in outbreak situations. Other agents can be involved as well, *Actinomyces pyogenes*, *Streptococcus* sp., and *Staphylococcus* sp. for example. In one recent example an injection site blemish lay-

ing adjacent to the sciatic nerve in the lower part of the round caused the lameness. The important message is that not all animals in one location or even within a pen will necessarily have the same organism or condition causing the problem. The arthritis is more a symptom of cattle that have experienced a bacteremic episode and are now showing the result. It is also important to necropsy as many of the animals as possible to demonstrate to owners the fact that cattle will have different conditions. Necropsies are also important to demonstrate the extent of chronic respiratory disease, to make the point that early treatment is imperative, that some disease conditions occurred prior to arrival and that the crisis does not mean an autogenous bacterin is necessary to "solve" the problem.

VQ

## Effects of Dietary Copper on Cellular and Humoral Immunity and Performance of Growing Calves

M.S. DAVIS, G.E. CARSTENS, J.C. BRANUM, R.E. MOCK, A.B. JOHNSON

In order to examine the rate of copper (Cu) repletion and its effect on immunocompetence of calves during the postweaning period, newly weaned Simmental x Angus calves were blocked by liver Cu (average  $23 \pm 3.85$  ppm DM), and randomly assigned to treatment diets containing 0, 10, and 50 ppm supplemental Cu. The basal diet consisted of corn (50%), cottonseed hulls (25%), molasses (5%), and a protein/mineral supplement (20%) and contained 5.6 ppm Cu. Supplemented Cu was a 1:1 mix of inorganic (CuSO<sub>4</sub>) and organic (Availa CuR) Cu. Treatment diets were fed individually for 75 days postweaning. Liver biopsies were performed on days 14, 28, and 42 to determine liver Cu concentrations. Calves were vaccinated with a 4-way modified live vaccine on days 14 and 28 and weekly serum samples assayed for infectious bovine rhinotracheitis virus (IBRV), parainfluenza type 3 (PI3), bovine viral diarrhea (BVD), and bovine respiratory syncytial virus (BRSV) antibody titers. Cell-mediated immune (CMI) responses were determined on days 14, 28, and 42 by measuring skin swelling responses to intradermal injections of phytohemagglutinin (PHA) at 6, 12, 24, and 48 h post-PHA injection. Liver Cu concentrations of 50-Cu calves were already 4.2-fold higher ( $P < .01$ ) than 0-Cu calves on day 14 ( $29, 54, \text{ and } 507 \pm 6.2$  ppm DM) and were 6.9-fold higher than the

0-Cu calves on day 42 ( $64, 166, \text{ and } 507 \pm 18.7$  ppm DM). Dry matter intakes ( $6.23, 6.24, \text{ and } 6.03 \pm .21$  kg/d) and ADG ( $1.20, 1.23, \text{ and } 1.14 \pm .08$  kg/d for 0-, 10-, and 50-Cu calves, respectively) over 42 d were not affected ( $P > .1$ ) by treatment. Antibody titers were analyzed using a log<sub>2</sub> conversion of the calves that sero-converted. The 0-Cu calves had higher ( $P < .05$ ) average IBRV titers through d 42 compared to 50-Cu calves ( $2.13, 1.86, \text{ and } 1.77 \pm .10$ , respectively). Antibody titers for BRSV, BVD, and PI3 did not differ ( $P > .1$ ) through d 42. Average PHA-induced CMI response was most affected by treatment on d 14. However, average CMI response tended ( $P < .1$ ) to be greater in 50-Cu calves than 0-Cu calves on both day 28 ( $12.6, 12.8, \text{ and } 13.1 \pm .18$  mm) and day 42 ( $12.5, 13.0, 12.9 \pm .16$  mm, for 0-, 10-, and 50-Cu calves, respectively). Results indicate that repleting Cu-deficient calves at a faster rate by feeding 50 ppm Cu diets may enhance cellular, but not humoral immune responses.

Co. State Beef Program Report. 1999.VQ

## Climate Affects Calf Birth Weights and Calving Difficulty

GENE DEUTSCHER, DAVE COLBURN, REX DAVIS

A six-year study was conducted to investigate effects of winter temperatures on two-year-old cows ( $n = 285$ ) and their subsequent calf birth weights and calving difficulty in the spring. The winter of 1992-93 (coldest) was 11°F colder than the winter of 1994-95 (warmest). The coldest winter was followed by calf birth weights that were 11 pounds heavier with 29 percent greater calving difficulty compared to the warmest winter. Our results indicated that as average winter temperatures decreased 1°F, subsequent calf birth weights increased 1 pound and calving difficulty increased 2.6 percentage points. When blankets were placed on cows before calving, hide temperatures increased slightly, but calf birth weights were unchanged. VQ

# Various Protocols for Synchronization of Estrus or Ovulation Using GnRH and Prostaglandin<sup>1</sup>

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## Summary

The intent of this paper is to summarize data on 4,383 cows from several studies and to clarify synchronization of estrus or ovulation using GnRH and prostaglandin (PGF) with the Ovsynch, CO-Synch, and Select Synch protocols. The Ovsynch protocol resulted in higher ( $P < .05$ ) pregnancy rates than Syncro-Mate-B (54% and 42%, respectively), but requires handling cows 4 times for injections and timed insemination. The CO-Synch protocol requires handling cows only 3 times, and results in similar ( $P > .1$ ) pregnancy rates as the Ovsynch protocol to a timed insemination (54% and 58%, respectively). The addition of 48-hr calf removal between the PGF and 2nd GnRH injection facilitates animal handling and improves ( $P < .05$ ) pregnancy rates to the Ovsynch and CO-Synch protocols approximately 8%. The Select Synch protocol differs from the Ovsynch and CO-Synch protocols in that it is used to synchronize estrus rather than ovulation. The Ovsynch, CO-Synch, and Select Synch protocols all resulted in similar ( $P > .1$ ) pregnancy rates, and all were capable of inducing estrous cycles in anestrus cows. Combinations of the Select Synch with the CO-Synch protocol allow a producer to take advantage of high pregnancy rates to insemination after observation of estrus and utilize timed insemination among non-responding cows. However, delaying the 2nd injection of GnRH and timed insemination beyond 48 hr following the PGF injection appears to decrease pregnancy rates.

**Key Words:** Synchronization of Estrus, Synchronization of Ovulation, Artificial Insemination

## Introduction

In the 1997, 1998 and this year's CSU Beef Program Reports, we have written several articles involving the use of the Ovsynch, CO-Synch, and Select Synch protocols for synchronization of ovulation or estrus to facilitate the use of AI. The intent of this paper is to summarize some of that data and help beef producers identify the synchronization protocol that may best suit their needs. The primary differences between the three protocols are illustrated in figure 1. The CO-Synch and Select Synch protocols are variations of the Ovsynch protocol that was originally developed for synchronization of ovulation with-

out heat detection in dairy cows. All three programs and combinations of the programs are effective in beef cows. The Select Synch protocol differs from the Ovsynch and CO-Synch protocols in that it is used to -synchronize estrus rather than ovulation, and thus, still requires heat detection.

<sup>1</sup>The authors appreciate the donations of *Cystorelin (GnRH)* from Merial, LTD and *Lutalyse (PGF)* from Pharmacia & Upjohn as well as funding by the National Association of Animal Breeders.

There is unpublished data from Dr. Fricke's, lab at the University of Wisconsin, that half doses of GnRH were sufficient for estrous and ovulation synchronization in dairy cows. However, beef producers are advised against trying half doses in beef cows because, unlike dairy cows, more beef cows are anestrus (not cycling) at the time of synchronization. In addition, it is possible to decrease synchronization costs and obtain high pregnancy rates by using combinations of the GnRH/PGF protocols.

## Materials, Methods & Results

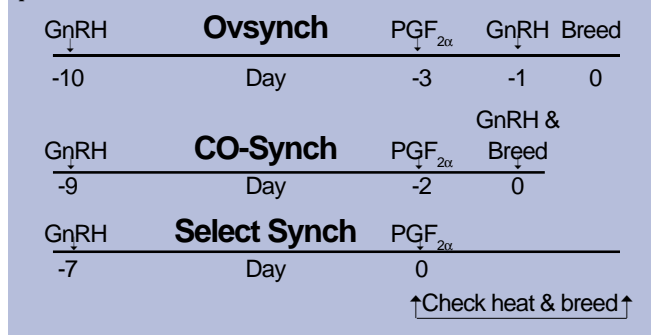
In order to evaluate the GnRH/PGF protocols in beef cows, we first evaluated pregnancy rates of cows that received the Ovsynch ( $n = 220$ ) or Syncro-Mate-B ( $n = 216$ ) protocol and were time inseminated. Timed insemination occurred at 24 hr after the 2nd GnRH injection or 48 hr after removal of the Syncro-mate-B implant. These cows were also exposed to 48-hr calf removal between the time

that the PGF injection and 2nd GnRH injection were given or from the time of Syncro-Mate-B implant removal until breeding. Pregnancy rates to the timed inseminations were higher for cows that received the Ovsynch protocol (54%) than for cows that received the Syncro-Mate-B protocol (42%).

With hopes of simplifying the Ovsynch protocol, we evaluated the CO-Synch Protocol to determine the effects of a timed insemination at the time of the 2nd GnRH injection rather than handling cows a 4th time for insemination. The pregnancy rates (54%) of cows receiving the CO-Synch protocol ( $n = 606$ ) were not different ( $P > .1$ ) than the pregnancy rates (58%) of cows receiving the Ovsynch protocol ( $n = 639$ ). A subset of the cows ( $n = 469$ ) that received the Ovsynch or CO-Synch protocol were divided to receive 48 hr calf removal or no calf removal to evaluate its importance in achieving high pregnancy rates to a timed AI. Pregnancy rates of cows that received temporary calf removal (62%), regardless of whether it was the Ovsynch or CO-Synch protocol, were higher ( $P < .05$ ) than pregnancy rates of cows that did not receive calf removal (54%). This temporary calf re-

*continued on page 6*

Figure 1. Illustration of the Ovsynch, CO-Synch and Select Synch protocols.



An advantage that exists for each of these protocols is the ability of these protocols to induce estrous cycles in cows that have not resumed cyclicity on their own since calving. This would be a tremendous advantage for producers who wish to shorten their calving season and achieve higher pregnancy rates by using one of these protocols to allow more cows extra chances to conceive earlier in the breeding season. Each of the hormone treatments (GnRH and PGF) are administered as intramuscular injections, so individual animal restraint is not necessary.

One of the greatest obstacles to producer acceptance of the GnRH/PGF synchronization protocols has been the cost of GnRH. Between the 1996 breeding season and 1998 breeding season, the cost of a single dose of GnRH has dropped from approximately \$4.50 per head to approximately \$3.00 per head. Increased use by producers and competition between the companies that market a GnRH product may decrease the price of it a little more in the future. We have evaluated methods to decrease the costs of these protocols by using a less expensive hormone called human chorionic gonadotropin (hCG; Salverson et al., 1999), and will evaluate the effectiveness of half doses of GnRH during the 1999 breeding season.

**GnRH continued from page 5**  
 removal appears to result in a little earlier ovulation in most cows and thus may result in a tighter synchrony of ovulation. The number of animals that received the CO-Synch or Ovsynch protocol with or without calf removal and their timed AI pregnancy rates are listed in Table 1. The cows used in this study were also bled twice prior to synchronization to determine which cows were exhibiting estrous cycles at the time of synchronization and which cows were not cycling (anestrus). Comparing the prior estrual status of cows that either received calf removal or not with their pregnancy rates revealed that pregnancy rates of cyclic and anestrus cows were 70% and 58% if they received 48-hr calf removal and 62% and 48% if they did not receive calf removal. Thus, 48-hr calf removal is more important if producers believe a high percentage of their cows are not exhibiting normal estrous cycles at the time of synchronization.

Table 1. Timed AI pregnancy rates of cows by synchronization and calf removal treatments.

Synchronization Treatment	No. of Cows	Pregnancy Rate
CO-Synch + 48-hr Calf Removal	n= 119	63%
Ovsynch + 48-hr Calf Removal	n= 112	62%
CO-Synch	n= 115	55%
Ovsynch	n= 123	52%

Combined data that compared pregnancy rates of cows inseminated at a fixed time with the CO-Synch protocol (either 48 or 54 hr following the PGF injection) or following an observed estrus with the Select Synch protocol revealed similar pregnancy rates to both methods (43% and 42%, respectively). These studies were conducted at Kansas State University (54 hr timed AI; n = 823) and Colorado State University (48 hr timed AI; n = 169) during the 1996 and 1997 breeding seasons (Grieger et al., 1998; Thompson et al., 1998; Unpublished data). The conception rate of cows that received the Select Synch protocol was high at each location (70%), but the percentage of cows detected in estrus was low (59%). The synchronization response among cyclic (80%) and anestrus (47%) cows suggests that a higher pregnancy rate might be obtained in herds with a low percentage of anestrus cows (Thompson et al., 1998). The effects of 48-hr calf removal on synchronization and pregnancy rates to the Select Synch protocol have not been evaluated.

The results of combining the Select Synch and CO-Synch protocols to take advantage of high conception rates among cows bred following an estrus and acceptable pregnancy rates

following a timed insemination have yielded interesting results. During the 1997 breeding season, 696 cows within 3 locations received the Select Synch protocol and were artificially inseminated approximately 12 hr following observation of estrus for 72 hr following the PGF injection. At 72 hr post PGF injection, all cows that had not yet been bred were divided into two groups to be time inseminated at either 72 hr with a 2nd injection of GnRH or at 84 hr with a 2nd injection of GnRH. None of the cows at these locations were observed for estrus prior to the PGF injection. The percentage of cows that exhibited estrus within 72 hr following the PGF injection averaged 48%. The conception rate of cows that were bred following an observed estrus was 56%. The conception rate of cows that received a 2nd GnRH injection and were time inseminated at 72 or 84 hr following the PGF injection was 21% and 24%, respectively. Thus, the overall conception rates to AI were

44% or 45% for breeding by an observed heat with timed AI at 72 or 84 hr, respectively. During the 1998 breeding season, 682 cows at one location received the Select Synch protocol and were artificially inseminated approximately 12 hr following observation of estrus for 48 hr following the PGF injection. At 48 hr post PGF, the cows that had not yet been bred were divided into two groups to receive a 2nd injection of GnRH and be time inseminated at 48 or, 64 hr. None of the cows at this location were observed for estrus prior to the PGF injection. Only 18% of cows were observed in estrus by 48 hr post PGF injection, but their conception rate was high (68%). One thing that may have affected the low estrus response by 48 hr is the fact that heat detection on 682 cows at the same time makes it difficult to identify all cows that are in heat. The pregnancy rate of cows that were time inseminated 48 or 64 hr was 43% and 39%, respectively. Taken together, this data suggests that the optimal time for high pregnancy rates to a timed AI is 48 hr following the PGF injection when a 2nd GnRH injection is given at the time of AI. However, based on pregnancy rates of cows that are time inseminated com-

pared to those bred following an observed estrus, it would be desirable for most producers to breed a majority of their cows following an observed estrus.

We have conducted one other synchronization experiment that involves the Select Synch protocol with timed, insemination. This study was conducted during the 1997 breeding season and we have been reluctant to present the findings because we were concerned that they may not be repeatable and too risky for other producers to try, and because we haven't figured out how to interpret the results of the study. At this time, we still haven't repeated the study and still can't explain the findings so we are cautioning producers about adopting this protocol. Three hundred and thirty two beef cows at one location received the Select Synch protocol to synchronize estrus. Cows that expressed estrus during the 72 hr followed the PGF injection (n 169) were artificially inseminated approximately 12 hr after their first observed signs of estrus. At 72 hr post PGF, the remaining 163 cows were randomly sorted to be inseminated at 72 hr or 80 hr and either receive or not receive the 2nd GnRH injection at the time of AI (Table 2). A high percentage of cows exhibited estrus (51%) within the 72 hr following the PGF injection. The pregnancy rate of cows bred following an observed estrus was 61%. Unlike previous experiments, the timed AI (TAI) pregnancy rates were good for cows inseminated at 72 hr or 80 hr following the PGF injection (Table 2). Interestingly, the TAI pregnancy rate for cows that did not receive a 2nd injection of GnRH at the time of synchronization (51%) tended ( $P > . 1$ ) to be higher than the rates of cows that did receive a 2nd GnRH injection (47%). It is possible that the inherent fertility of the cows in this herd was greater than among cows from other herds. Also, fewer cows may have been anestrus in this herd than in other herds. In addition, different semen and inseminators were used for this herd, and it is possible that the semen was able to survive longer in the female reproductive tract. However, the data still suggests that the second injection of GnRH may be unnecessary. If this proves to be true, then the I cost of this protocol (approximately \$5.00 for synchronization drugs) makes it an economical

Table 2. Synchronization and pregnancy rates of cows by treatment.

Synchronization Treatment	No. of Cows	Pregnancy Rate
Select Synch + Estrus-AI	n= 169	61%
Select Synch + 72 hr TAI + GnRH	n= 46	54%
Select Synch + 72 hr TAI	n = 42	55%
Select Synch + 80 hr + GnPH	n = 41	39%
Select Synch + 80 TAI	n = 34	47%

method of synchronization for most commercial and purebred operations.

## Applications and Discussion

The synchronization protocol chosen by a producer should be based on his or her goals for the program as well as the amount of time and labor that can be committed to the program. However, these results show that the GnRH/PGF protocols are somewhat flexible and can be tailored to fit an individual operation. One important aspect to keep in mind when comparing synchronization protocols that include a timed AI with a protocol that doesn't is to make sure you are comparing pregnancy rates of both protocols. Pregnancy rates are defined as the number of cows pregnant divided by the number of cows exposed to synchronization. Conception rates are defined as the number of cows pregnant divided by the number of cows that were inseminated. With a timed AI program, the pregnancy equals the conception rate because all cows are inseminated. However, with a synchronization program that breeds cows only following an observed estrus, conception rates could be high, but result in a low pregnancy rate if only a few cows were observed in estrus. Conception rates of cows that are bred because they were observed in estrus are usually higher than conception rates of cows that are time-inseminated, but pregnancy rates are generally similar. Timed AI programs may be more expensive than programs that breed only following an observed estrus if the extra drugs and semen are more expensive than the savings in labor from accurate heat detection.

Cows in the above studies that received the Select Synch protocol were observed for signs of estrus for 5 days following the PGF injection. Since these studies were performed, we have observed that some Cows ((10%) may display estrus up to 30 hr prior to the PGF injection (Downing et al., 1998). The cows that exhibit estrus prior to the PGF injection have good fertility and should be inseminated following this estrus. These cows are usually females that were between day 14 - 17 of their estrous cycle at the time of administering the 1st GnRH injection and did not completely respond to the GnRH injection. Very few cows have been observed in estrus more than 30 hr prior to the PGF injection, so producers would not need to observe cows until the day prior to the PGF injection. It should be noted, that these cows and cows that display estrus within 24 hr following the PGF injection have little chance of conceiving to a timed AI at 48 hr following the PGF injection. Thus, it would be prudent for a producer to observe for estrus and AI any cows that display estrus within 24 hr prior to or after the PGF injection, even

when using the CO-Synch protocol.

This report does not include data on the GnRH/PGF systems in heifers. The Ovsynch protocol has not been evaluated in beef heifers, and the CO-Synch and Select Synch protocols have received little use in heifers. There are rumors that these protocols do not work in heifers based on some data out of the University of Florida that evaluated the Select Synch and Ovsynch protocols in dairy heifers. Last year Kansas State University (Unpublished data) and Colorado State University (Doherty et al., 1999) compared the Select Synch protocol to tie the MGA/PGF protocol with timed insemination at 72 hr following the PGF injection in any heifers not observed in estrus. The overall pregnancy rate appeared to be 5 ñ 10 percent lower for the heifers that received the Select Synch protocol. Thus, the Select Synch protocol may not be as effective as the MGA/PGF protocol, but for producers who are unable to use the MGA/PGF protocol, the Select Synch protocol may be an effective alternative.

One area that scientists and AI representatives could be criticized for in the past is "Over-Selling" a synchronization protocol. Right now, beef producers are hungry for something that will help make them money and may be quick to jump at every possible option. As mentioned in the introduction, the Select Synch, CO Synch and Ovsynch protocols all can induce cyclicity in anestrous cows. This is not to say that they will induce cyclicity in all anestrous cows, as GnRH is not a miracle drug. Good candidates for a GnRH/PGF synchronized AI program are cows that are at least 30 days since calving (the more days, the better), in moderate body condition and receiving adequate nutrition at the time of breeding. Producers using these guidelines who choose to adopt the Select Synch protocol and are willing to observe cows for 24 hr prior to the PGF injection should expect synchronization rates above 65% and conception rates between 55 to 70%. These synchronization and conception rates (65% and 70%, respectively) will yield a pregnancy rate of 46%. Producers who adopt the CO-Synch protocol as illustrated in Figure I should expect pregnancy rates of 45 to 60% depending on whether or not they employ 48 hr calf removal and breeding cows that show estrus early. While higher pregnancy rates are possible with these protocols, producers should approach estrous/ovulation synchronization protocols with the idea of being comfortable with achieving approximately 50% pregnancy rates.

Probably the best combination of the Select Synch and CO-Synch protocols for most situation with AI approximately 12 hr after an observed estrus to the CO-Synch protocol.

This way, the cows that show estrus early have a better opportunity to conceive to the insemination. In addition, 48-hr calf removal should simplify animal handling for the timed insemination, facilitate heat detection, and increase pregnancy rates by approximately 8%.

If a producer is interested in using 48-hour calf removal, there are a couple of things to keep in mind. There is a lot of data which shows that calf removal for this length of time has no effect on weaning weight. At the Rigden location, it snowed about 6 inches the first night that calves were removed from the cows. The following day, it rained. The calves that were removed from their dams actually appeared to be less stressed than the calves that remained with their dams. There were also no additional cases of scours or other health problems for the removed calves. So, while this period appears stressful to the calves, the noise is probably more stressful on the producer. It is possible, however, for calves nursing high milk producing cows to experience milk scours for a day or two after they are placed back with their mothers. When utilizing 48 hr calf removal it is important to provide calves with a clean area where they have access to clean grass hay and water. It is also important to make sure that pairs mother up well after returning calves to the cows, before turning them out to pasture.

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### Coming Events

January 29, 2000  
9th Annual Small Animal Medicine  
Conference on Cardiology  
*Guest speaker: Dr. Matthew Miller*  
Texas A&M University

February 19, 2000  
8th Annual Emergency Medicine  
Conference on Neurology  
*Guest speaker: Dr. Curtis Dewey*  
Texas A&M University

February 20, 2000  
Small Animal Internal Medicine  
Continuing Education Series, Spring 2000  
*Endocrinology*

March 5, 2000  
17th Annual Frank W. Jordan Seminar  
on Feline Infectious Diseases  
*Guest speaker: Dr. Michael Lappin*  
Colorado State University

March 11, 2000  
Veterinary Technicians Conference  
*Behavioral Problems*  
*Heartworm Giardia Lab*  
*Equine Wound Treatment*

March 12, 2000  
Small Animal Internal Medicine  
Continuing Education Series,  
Spring 2000  
*Parasites*

April 9, 2000  
Small Animal Internal Medicine  
Continuing Education Series,  
Spring 2000  
*Geriatric and Neonatal Diseases*

June 4-7, 2000  
62nd Annual Conference  
for Veterinarians

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