President’s Message
Spring 2003
Randall H. Hinshaw, D.V.M.

By now I am sure everyone is aware of the plight of the AETA. On April 11th Don Ellerbee informed the Executive Board that all of our funds had been misappropriated by GMO, the company hired to manage the affairs of the AETA since its inception. Your Board had an emergency meeting on April 12th and assumed control of the day-to-day operations. Legal counsel and an accounting firm have been hired to gather evidence for the Nebraska Attorney General’s Office. Currently, an audit is being done and the Attorney General has an ongoing investigation. The AETA was left without any funds and no professional management company. Your Board of Directors has been hard at work to keep the AETA functioning and to re-establish its financial well-being. The AETA administrative office has been temporarily relocated to Harrisonburg, VA and duties have been assigned to various board members and committee chairmen. The Board has turned its attention to returning the AETA to sound financial footing. Options considered included re-billing of membership dues and certification fees, a general assessment of the membership, pre-payment of future dues and/or convention registration, and donations to a sustaining membership fund. For a number of reasons, the Board has chosen donations from the membership as the most appropriate action. Our goal is to raise $100,000, which we need for fiscal 2003 operating costs and estimated legal and accounting fees. In the first two weeks we have received about $21,000, which is well short of what is needed. With 282 regular members and 80 associate members, we need each member to contribute a minimum of $300. I would like each of you who have not contributed to reconsider your commitment to your association. For those of you who have contributed, I would like to say thank you for helping the AETA. The survival of the AETA depends on member participation and support. If our goal is not met, other action may need to be taken.

A search committee has been formed and is chaired by Steve Malin. Steve and his committee are actively pursuing new management companies, and a number of them have expressed interest in the AETA. We hope to have a new management company hired by mid-summer.

As you know, an isolated case of BSE was diagnosed on May 20th in northern Alberta. Although not affecting the Canadian export of embryos or semen, it is having a devastating economic impact on their cattle industry. Let’s hope it is a short-lived problem.

The program for the joint meeting with CETA in Calgary is complete. You will find a copy of the program in this newsletter. There are many opportunities to sightsee while in Calgary, and you can visit the tourism website at www.tourismcalgary.com for information. It is very important that we have a well attended meeting this year. Invite someone you know who is doing embryo transfer to our meeting.

I hope to see everyone in Calgary this September. If you have ideas or suggestions for the AETA, call or e-mail any member of the Board of Directors. You can find their phone number or e-mail address at www.aeta.org.

AETA Committee Chairmen for 2002-2003
Listed below are the AETA Committee Chairmen for 2002-2003.

If you would like to serve on one of these committees, contact the chairman or call the AETA office.

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Exhibit Committee.................................Dr. Pat Richards
Convention Program Planning Committee.....Dr. Larry Kennel
Cooperator Committee.............................Dr. Scott Armbrust
Government Liaison Committee..................Dr. Richard Whitaker
Manuals & Promotion Committee..............Dr. Thomas Borum
Newsletter Committee..............................Dr. John Hasler
Nominating Committee.............................Dr. Darrel DeGroff
Professional Review Committee..............Dr. Randall Hinshaw
Statistical Information Committee.............Dr. Steve Hughes

2003 AETA/CETA Convention
Westin Hotel
Calgary, Alberta, Canada
September 3-6, 2003
Officers & Board of Directors
2002-2003

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Email: cginc@hotmail.com

TRYPsin ACTIVITY AFTER PROLONGED REFRIGERATED STORAGE

N.M. Loskutoff, K.A. Morfelcl, and E.G. Crichton
Center for Conservation and Research, Henry Doorly Zoo,
Omaha, NE 68107-2200, USA

According to the Manual of the International Embryo Transfer Society (Stringfellow and Seidel, eds., 3rd Edition. 1998), it is important to store trypsin as a frozen stock and to prepare working solutions immediately before their use for disinfecting embryos of specific pathogens. However, it is evident that many practitioners do not follow such guidelines and some are known to re-use trypsin solutions for prolonged periods. Since it is not possible to enforce or ensure that trypsin solutions are prepared properly, there is a potential risk that disease transmission will occur via embryo transfer if the embryos were processed using trypsin solutions that were unknowingly inactive. The objective of this practical study, therefore, was to test the activity of trypsin after prolonged refrigerated storage. Aliquots of 0.25% trypsin (T-4549, Sigma Chemical Co., St. Louis, MO, USA) were immediately frozen (to serve as a positive control) and the remaining volume was placed in a conventional refrigerator. Aliquots of the refrigerated and frozen (freshly thawed) working solutions were removed weekly, warmed at 38°C for 30 min., and then used to dissociate confluent Buffalo rat liver (BRL) cell monolayers cultured in Falcon 35 mm 6-well plates. The time taken from the point BRL cells were initially affected (cells rounding and lifting) to the time that >90% were dissociated and detached from the surface of the culture dishes were recorded and compared between the refrigerated and frozen-thawed trypsin aliquots. After 5 months of the weekly exercise, the refrigerated trypsin solution would dissociate and detach the confluent BRL cell monolayers within the same timeframe from initial exposure as the freshly prepared (frozen-thawed) trypsin aliquot (P > 0.05, t-test). Table I.

In conclusion, it is evident by the results of this study that trypsin activity, as measured by the time taken to dissociate and detach >90% of confluent BRL cell monolayers, is not depleted after prolonged refrigerated storage of up to 5 months. In practice, however, it is important to stress that proper aseptic handling be maintained to avoid the risk of contaminating refrigerated trypsin solutions with psychrophilic bacteria and fungi.

Continued on next page
Table 1
Mean times for confluent BRL cell monolayers to detach after exposure 2.5% trypsin stored at -20°C vs. 4°C for 5 months.

<table>
<thead>
<tr>
<th>Trypsin storage (°C)</th>
<th>Initial time(s)</th>
<th>Final time(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20</td>
<td>41.6 ± 4.6</td>
<td>238.4 ± 23.2</td>
</tr>
<tr>
<td>4</td>
<td>36 ± 3.3</td>
<td>236.2 ± 22.2</td>
</tr>
</tbody>
</table>

(Initial = first sign of cells rounding and lifting; final ≥ 90% cells detached).

EFFECT OF THE INTERVAL BETWEEN CIDR AND ESTRADIOL BENOATE ADMINISTRATION AND INITIATION OF FSH INJECTION ON THE SUPEROVULATORY RESPONSE IN JAPANESE BLACK CATTLE

T. Nishisouzu¹, M. Sugawara¹, S. Aoki¹, O. Dochi¹, M. Kishi², and H. Koyama¹

¹Department of Dairy Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan,
²Embryo Transplantation Laboratory, Snow Brand Milk Products Co. Ltd., Tomnkomai, Hokkaido, Japan

Conventional superovulation programs begin with the injection of FSH at 8-13 days after estrus. Recently, intentional superovulation became possible by using CIDR and estradiol benzoate (EB), without the necessity of estrus detection. The objective of this study was to investigate the effect of the interval between CIDR and EB administration and the initiation of FSH injection on the superovulatory response in Japanese Black cattle. Japanese Black cows (n = 27) were randomly allocated to one of three treatment groups. Cows received an intravaginal progesterone device (CIDR-B, InterAg, New Zealand) combined with 2 mg EB at a random stage of the estrous cycle (Day 0). Superstimulatory treatments were initiated on Days 5 (Group A), 6 (Group B), or 7 (Group C) with a total dose of 18 or 24 mg FSH via twice daily i.m. injections for 3 days in decreasing doses. PGF₂α (PG) was administered in the morning (25 mg) and afternoon (15 mg) of the last day of FSH injection. The CIDR-B was removed at the time of the second PG injection. Two days after the PG injection, cows were injected i.m. with 100 μg GnRH, and were artificially inseminated (AI) the next morning. Ova/embryos were collected non-surgically 7 days after AI. The follicular dynamics of the ovaries were observed during FSH injection and at the time of AI by means of ultrasonography. Follicles were classified according to diameter into small (≥ 3 mm), medium (4–7 mm), and large (≥ 8 mm) categories. Data were analyzed using ANOVA. The results are presented in Table 1. There were no differences in the mean number of follicles. However, the number of small and middle follicles at the time of FSH injection in Groups B and C was fewer than those of Group A (P < 0.05), and the number of large follicles at the time or AI in Groups B and C showed a tendency to be more numerous than those of Group A. There were no differences among groups regarding the mean number of CL. Total ova/embryos and the number of viable embryos the Group B showed a tendency of being more numerous than those of Groups A and C. These results suggest that superovulatory response in cows may be superior when the interval between FSH and CIDR-B/EB is 6 days.

Table 1
Effects of interval between CIDR-B and EB administration and initiation of FSH injection on superovulatory response in Japanese Black cattle.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of donors</th>
<th>No. of corpora lutea</th>
<th>Total ova/embryos</th>
<th>No. of viable embryos</th>
<th>No. of unfertilized ova</th>
<th>No. of degenerated embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
<td>7.9 ± 2.1</td>
<td>5.9 ± 2.6</td>
<td>3.2 ± 1.2</td>
<td>0.9 ± 0.5</td>
<td>2.0 ± 1.5</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>11.9 ± 1.5</td>
<td>10.8 ± 1.6</td>
<td>5.6 ± 0.9</td>
<td>2.8 ± 1.8</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>11.1 ± 1.9</td>
<td>6.6 ± 1.4</td>
<td>2.6 ± 1.2</td>
<td>0.9 ± 0.5</td>
<td>3.1 ± 1.1</td>
</tr>
</tbody>
</table>

Values were means ± S.D. Values with different superscripts (a, b and c) tended to differ (P < 0.1).
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<td>Solutions for Embryo Holding, Freezing, Thawing, and IVF</td>
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<tr>
<td>BoviPro™ TL Hepes (500 ml)</td>
<td></td>
</tr>
<tr>
<td>EquiPro™ Recovery (2.0 liter)</td>
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## 2003 CETA/ACTE & AETA
### JOINT CONVENTION PROGRAM

### WEDNESDAY, SEPTEMBER 3
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>8:00 a.m.</td>
<td>CETA/ACTE Certification Committee Meeting</td>
</tr>
<tr>
<td>11:00 a.m.</td>
<td>CETA/ACTE Board of Directors Meeting</td>
</tr>
<tr>
<td>1:30 p.m.</td>
<td>CETA/ACTE &amp; AETA Certification Examination</td>
</tr>
<tr>
<td>8:00 a.m.</td>
<td>AETA Board of Directors Meeting</td>
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### THURSDAY, SEPTEMBER 4
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>7:00 a.m.</td>
<td>Golf Tournament at 'Springbanks Link Golf Course'</td>
</tr>
<tr>
<td>1:00 p.m.</td>
<td>Registration</td>
</tr>
<tr>
<td>2:30 p.m.</td>
<td>Session: ET 101&lt;br&gt;Presented by: Dr. Roger Sauvé</td>
</tr>
<tr>
<td>2:30 p.m.</td>
<td>Wet Lab: Embryo Splitting &amp; Embryo Sexing&lt;br&gt;Presented by: Dr. Martin Darrow</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>Wet Lab: Ultrasound&lt;br&gt;Presented by: Dr. Bill Beal</td>
</tr>
<tr>
<td>7:00 p.m.</td>
<td>AB Technology &amp; Friends Cocktail Party</td>
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### FRIDAY, SEPTEMBER 5
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00 a.m.</td>
<td>Registration</td>
</tr>
<tr>
<td>7:00 a.m.</td>
<td>Breakfast (in Exhibit Area)</td>
</tr>
<tr>
<td>8:00 a.m.</td>
<td>Welcome and Announcements, Introduction of Exhibitors</td>
</tr>
<tr>
<td>8:15 a.m.</td>
<td>Session: USDA/APHIS Update&lt;br&gt;Presented by: Dr. George Seidel</td>
</tr>
<tr>
<td>8:45 a.m.</td>
<td>Session: Embryo Metabolism &amp; Bovine Embryo Quality&lt;br&gt;Presented by: Dr. George Seidel</td>
</tr>
<tr>
<td>10:00 a.m.</td>
<td>Coffee Break in Exhibit Area</td>
</tr>
<tr>
<td>10:45 a.m.</td>
<td>AETA Annual Business Meeting</td>
</tr>
<tr>
<td>10:45 a.m.</td>
<td>CETA/ACTE Annual General Meeting</td>
</tr>
<tr>
<td>12:30 p.m.</td>
<td>CETA/ACTE New Board of Directors Meeting</td>
</tr>
<tr>
<td>12:30 p.m.</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:30 p.m.</td>
<td>Session: Leptin DNA Markers&lt;br&gt;Presented by: Leigh Marquess</td>
</tr>
<tr>
<td>2:00 p.m.</td>
<td>Session: Donor Nutrition, Freezing Times and CIDR use &amp; reuse&lt;br&gt;Presented by: Dr. Bill Beal</td>
</tr>
<tr>
<td>3:30 p.m.</td>
<td>Coffee Break in Exhibit Area</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>Session: Practice Tips</td>
</tr>
<tr>
<td>6:00 p.m.</td>
<td>Social Hour in Exhibit Area</td>
</tr>
<tr>
<td>7:00 p.m.</td>
<td>Banquet</td>
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### SATURDAY, SEPTEMBER 6
<table>
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<th>Time</th>
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<tbody>
<tr>
<td>7:00 a.m.</td>
<td>Registration</td>
</tr>
<tr>
<td>7:00 a.m.</td>
<td>Breakfast in Exhibit Area</td>
</tr>
<tr>
<td>8:00 a.m.</td>
<td>Session: Update on Chronic Wasting Disease in Elk&lt;br&gt;Presented by: Dr. Glen Zobarth</td>
</tr>
<tr>
<td>8:45 a.m.</td>
<td>Session: Leadership: From Self Mastery to Business Results&lt;br&gt;Presented by: Collin Gollard</td>
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<tr>
<td>9:30 a.m.</td>
<td>Session: Male Fertility Parameters affecting Embryo Development &amp; More Evidence for a Direct Interaction between Prostaglandin F2alpha and Embryo Development&lt;br&gt;Presented by: Dr. Neal Schrick</td>
</tr>
<tr>
<td>10:30 a.m.</td>
<td>Coffee Break in Exhibit Area</td>
</tr>
<tr>
<td>11:00 a.m.</td>
<td>Session: Advances in Equine Superovulation&lt;br&gt;Presented by: Dr. Ed Squires</td>
</tr>
<tr>
<td>12:00 p.m.</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:30 p.m.</td>
<td>Session: Certification&lt;br&gt;All CETA/ACTE &amp; AETA Certified Practitioners must attend</td>
</tr>
<tr>
<td>2:30 p.m.</td>
<td>Coffee Break in Exhibit area</td>
</tr>
<tr>
<td>3:00 p.m.</td>
<td>Session: Practitioners Forum&lt;br&gt;Moderator: Dr. Reuben Mapletoft</td>
</tr>
</tbody>
</table>
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Exhibit Chairman: Dr. Martin Darrow, British Columbia

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Convention Chairman: Dr. Larry Kennel
Exhibit Chairman: Dr. Pat Richards

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