A Closer Look

AMERICAN EMBRYO TRANSFER ASSOCIATION

"THE VANGUARD OF THE EMBRYO TRANSFER INDUSTRY"

American Embryo Transfer Association

VOLUME 23, NO.2 SPRING 2008

President's Message

AETA President David Duxbury

I have to say a few words about spring as I write. It is an obligatory part of this newsletter, and spring does conjure up many positive thoughts as nature brings us longer days, green grass replaces snow, and the ice disappears from our lakes. But, I live in Minnesota, so, by way of a reality check, let me tell you that we had a snowstorm this weekend (April 25) and the temps are below freezing this morning, there is mud everywhere, and most of the lakes are still covered with ice. But, I'm not a cynic, and it really is a happy time.

As we move through the seasons, I have a few news items from the AETA board. Our management organization, the Federation of Animal Science Societies (FASS), has announced some important changes. Probably the most significant of these is that Dr. Jerry Baker has decided to leave his CEO position. Dr. Baker has been a very positive force for the AETA board, and he will be missed. I have spoken with the president of the FASS board, Dr. Gary Hartnell, regarding the change, and he is well aware of our organization's needs. He will keep us informed as their board addresses the FASS management changes. You may also have noticed that we have a new FASS staff member handling AETA affairs. Kathy Ruff comes to us with a strong background in event planning, and she will transition into her role with the help and guidance of Keely and Vicki as they assume other roles at FASS. Keely will continue to serve our organization in her role as director of IT.

Everyone should have received their membership directory by now, many thanks to the membership committee, Kathy Ruff, and the staff at FASS for their continued efforts. Please notify Kathy or a membership committee person if you notice errors or omissions.

The board had a meeting by conference call early in April to discuss several topics. We had been reviewing eastern city and state options for our 2010 annual convention, and we have chosen the Embassy Suites Hotel and Golf Club in Charlotte, North Carolina, as the meeting site. The dates will be October 13-18, and our Canadian counterparts will be joining us there.

AETA VISION STATEMENT: The main purpose for our April meeting was to discuss and approve the AETA vision statement, which was created on the basis of work done during our winter meeting. Remember that at our winter meeting, the board spent 2 days with a facilitator, reviewing the organization, its 25 year history, as well as its current form and the myriad of forces that might shape the future. Our goal was to come together to create a vision statement for the AETA and

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What's Inside . . .

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AETA Headquarters Directory

Vicki Paden,
AETA Administrative Assistant

As the AETA Administrative Assistant, Vicki works with the AETA members on day-to-day issues. She updates the AETA membership database, processes memberships, renewals, meeting registrations, orders, claims, invoices and responds to e-mail. She is also the helpful, friendly voice on the other end of the phone when you call the AETA line.

Kathy Ruff
Executive Assistant/Event Coordinator
(aeta@assochq.org)

As the Executive Assistant/Event Coordinator, Kathy will be working with the AETA members on everything from day-to-day issues, membership questions and renewals, meeting registrations, Japan certificates, as well as planning the Board of Directors' meetings and annual conventions. Kathy will also serve as the coordinator for the AETA newsletter, *A Closer Look*. Kathy is looking forward to working with AETA and its membership.

Newsletter Advertising 2008

Publication Schedule and Deadlines

The AETA newsletter is published four times per year and is mailed to all AETA members. Distribution is between 350-400 professionals in the animal embryo transfer industry.

Members – Advertise *FREE* with us*!*

Members wishing to place an advertisement related to sale of practice, buying and selling of used equipment, or employment opportunities may do so free of charge (up to 1/8 page). The advertising information (i.e., short courses, seminars, books, etc.) that is clearly to the benefit of the greater good of the AETA membership, and not considered to be of a commercial nature, may also be advertised free of charge (up to 1/8 page). Standard rates on any advertisements over 1/8 page shall apply. Any advertising request, which does not fit within these guidelines, shall be brought to the Newsletter Committee for approval. The same rationale shall apply to any Web site advertising.

A Closer Look Advertising Rates

Business Card Size:	$50 per issue
1/4 Page Ad:	$75 per issue
1/2 Page Ad:	$150 per issue
Full Page Ad:	$200 per issue

Ads are due to the AETA office as set forth below. Online ads are full color and print ads are black and white.

Payment terms: Advertiser agrees to pay the contract amount in full prior to the start date. This fee is nonrefundable and will not be prorated should the Advertiser decide to discontinue the display of the advertisement at any time prior to the end of the contract period.

Issue	Due Date
Winter 2008	February 26, 2008
Spring 2008	April 14, 2008
Summer 2008	July 11, 2008
Fall 2008	October 13, 2008
Winter 2009	December 29, 2008

The advertiser is responsible for providing all information and digital artwork to meet specifications. AETA reserves the right to determine the suitability of all ads submitted for distribution and to reject advertising that does not meet its editorial or digital criteria. Ads must be in PDF or high-quality JPEG, TIF, or EPS graphic files. Changes to ads may be made after each issue unit only. If you would like to advertise in the next issue, please contact AETA at aeta@assochq.org or call 217-398-2217.

Save These Dates!

AETA & CETA/ACTE	❖	2008 AETA & CETA/ACTE Joint Annual Meeting Westin Crown Center Hotel Kansas City, Missouri October 16–18, 2008
AETA & CETA/ACTE	❖	2009 AETA & CETA/ACTE Joint Annual Meeting Hilton Bonaventure Hotel Montreal, Quebec, Canada September 16–19, 2009
AETA & CETA/ACTE	❖	2010 AETA & CETA/ACTE Joint Annual Meeting Embassy Suites Hotel Charlotte-Concord, NC October 13–16, 2010

Future Meetings of Interest!

5th Sino-US Dairy Center Seminar	❖	Joint Program of the Babcock Institute and China Agriculture University Beijing, Harbin, China June 6–7, 2008
ICAR	❖	16th International Congress on Animal Reproduction Budapest, Hungary July 13–17, 2008
AABP	❖	41st Annual Convention Charlotte, NC September 25–27, 2008

NOTICE TO READERS
Articles published in *A Closer Look* are not necessarily peer-reviewed or refereed. All statements, opinions, and conclusions contained in the articles in *A Closer Look* are those of the author(s) and are not necessarily those of the American Embryo Transfer Association unless specifically approved by the AETA Board of Directors.

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President's Message . . .continued from page 1

to define the work to be done in the near term as we continue to keep the organization current and vital.

The vision statement is intended to be a rich description of our organization when it is at its best, running on all cylinders, meeting its goals, and fulfilling the mission to serve a diverse membership. The results of work done in crafting the vision will be the foundation for work to be done in the next 3 to 5 years as the AETA continues to evolve and sharpen the focus on the needs of it's members.

The vision statement is provided here as well as on our Web site. I want to thank Dr. Richard Whitaker and Dr. Allen Rushmer for their commitment to writing the drafts. Please read the statement and think about how the AETA organization helps you in your professional endeavors, both on a personal level and in your business. Remember that if you ever have ideas that might help the AETA serve you better, or questions about a topic, you should feel free to call anyone on the board or a committee. That's how the organization works best. If you would like to serve the AETA in some capacity, please don't wait to be asked.

Thanks very much to all AETA members for your continued support of your organization. Don't forget to make plans to invite a new member to the meeting in Kansas City. We all work very hard to make these meetings good, but it is the people that attend them that make them great!

David Duxbury, DVM

THE AETA VISION STATEMENT

The American Embryo Transfer Association embraces its responsibility as the resource for embryo transfer in the United States. This authority is developed and supported through our commitment to excellence in several broad areas.

Education is our first priority. AETA improves the quality of all ET practice by providing a direct link between the science laboratory and field applications. We support diverse learning opportunities through our annual meeting and wet labs, extensive web based services, and our printed newsletters. By developing and supporting "best practices" we avail this broad experience to all practitioners.

Our second commitment is to develop and maintain high industry standards. It is documented with several standards of quality, culminating with the gold standard: Certified ET Practitioner.

As the AETA delivers on Education and Standards of Quality we become the "voice" for our diverse membership. We honor that responsibility with commitment to collaboration with other associations, scientific societies, and government regulatory agencies at all levels.

The AETA can deliver all of the above value propositions to our membership only if we embrace the fundamentals of a sound organization. Management is important. We are self governed through an empowered Board of Directors and many working committees. All of our administrative and financial practices are sound and transparent. Yet the core of our association is anchored with our members…their integrity, their sound judgment, their joy, camaraderie, and commitment to each other. These make the soul of the American Embryo Transfer Association

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Ask John . . .

Comment/Question:
This week, I received a shipment of 12 embryos from Germany. Recovery to onset of freezing was one hour except for one embryo that was cultured for 24 hours and then frozen. Stages were: six 4's and five 6's except for the embryo cultured for 24 hours, which was a 7. Does anyone else do this? What is the conception? Culture media? The only reason I can guess that this embryo was cultured for 24 hours was that it was of inferior quality immediately postcollection?

Response:

I am not aware that there has ever been a published study involving controls on the issue of culturing low-quality embryos to see if their "quality" improved, followed by freezing. I very firmly believe that embryos do not improve in quality due to further culture in any medium. The appearance may change and a perceived improvement may take place, but does quality really improve? I think not. Bovine late morulae freeze just fine. There is no need to culture them to blastocysts before freezing. I have cultured many poor-quality bovine morulae in simple holding media and also in bicarbonate-buffered culture medium in a CO_2 incubator. It is not uncommon for these embryos to progress to blastocysts. However, if one looks carefully at these embryos, all the junk, such as cellular debris and extruded cells, is hidden between the trophoblast and the zona. I don't believe that these embryos have improved in quality.

I think there is pretty good evidence that successful cryopreservation is more sensitive to embryos being held outside the cow for extended periods than is fresh transfer. When kept in a decent medium, very high pregnancy rates can be achieved with holding fresh embryos for at least 24 hours. In contrast, we don't have great data on holding embryos prior to cryopreservation. I published data indicating that 3 hours of holding did not affect subsequent viability, and I think there are indications that 5 or 6 hours is not a problem. Beyond that, I suspect that survival postthawing is a compromise. Personally, I would not pay for an embryo that was cultured overnight before cryopreservation!

John

****

Response from Dr. James Griffen:

Dr Stan Leibo brought a CO_2 incubator when he came to Rio Vista in 1980. We cultured many poor-quality embryos. An amazing number of these embryos became beautiful blastocysts, and we transferred them. They did not produce pregnancies of any consequence, and we discarded the program. I have no data or numbers. Something else we should have published.

****

Questions for "Ask John" may be addressed to:
Cell: 970-222-5302
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MARK YOUR CALENDAR!

2008 AETA & CETA/ACTE JOINT CONVENTION
THE WESTIN CROWN CENTER
KANSAS CITY, MISSOURI
OCTOBER 16-18, 2008

The planning for the 2008 AETA & CETA/ACTE Joint Conference in Kansas City, Missouri, is well underway. Topics ranging from increased pregnancies by using "anti-prostaglandin" product to nutrition to dominant follicle/ovulatory follicle regulation will be presented. Sessions for those with small ruminant and equine interests are being planned as well. Drs. Randall Hinshaw and William Beal will be providing a breakout session in ET 101 with a technical slant. This will be an excellent opportunity for practitioners, new and old alike, to get the latest information on the basics of ET. A practical wet lab is being planned for emerging technologies, and there will be an AETA certification forum. The tentative program outline is included on the next page.

The golf tournament has been scheduled for October 16 at Shoal Creek, and for those not interested in golf, there are many sites to take in that are within walking distance of the hotel. Whether you want to check out the 14-square block outdoor shopping and entertainment area at Country Club Plaza, or if you are looking for an eclectic gathering place, head to the Crossroads Art District. Regardless of your taste, there are a multitude of things to do on foot or within a 15-minute taxi cab ride.

The companion tours include learning to cook like the pros from a professional chef at the Culinary Center of Kansas City, learning a little history and marveling at the hidden treasures of the Arabia Steamboat Museum, and dining amongst gorgeous antiques at the beautifully restored Webster House.

The convention will be held at The Westin Crown Center, which is located in downtown Kansas City. The convention rates are as follows: $149.00 for a single/double; $159.00 for a triple; and $169.00 for a quad. There is a lot to see and do in Kansas City, so we invite you and your companions to visit and learn!

For information on the convention and AETA, please visit the AETA Web site at:

http://www.aeta.org/2008/

WE LOOK FORWARD TO SEEING YOU!

A. Ideta, K. Hayama, M. Urakawa and Y. Aoyagi (2008). Abstract 296. Frequent rectal palpations following superovulatory treatment affect sex ratio of embryos recovered by Holstein heifers. ***Reprod. Fertil. Dev.*** *20(1), pp 228. Copyright IETS 2008. Published by CSIRO PUBLISHING, Melbourne, Australia. http://www.publish.csiro.au/nid/45/issue/4030.htm*

FREQUENT RECTAL PALPATIONS FOLLOWING SUPEROVULATORY TREATMENT AFFECT SEX RATIO OF EMBRYOS RECOVERED FROM HOLSTEIN HEIFERS

A. Ideta, K. Hayama, M. Urakawa and Y. Aoyagi

Abstract

Skewing the sex ratio of offspring towards males or females is very important for the livestock industry. Many factors, such as maternal stress, have been suggested to affect the sex ratio (Pratt NC et al. 1989 J. Reprod. Fertil. 87, 763–769). In a recent study (Ideta A et al. 2007 J. Reprod. Dev. doi:10.1262/JRD.19035), the proportion of female embryos recovered from superovulated heifers in which ovulation patterns were observed by repeated transrectal ultrasonography tended to be higher than the expected ratio of 50:50 (66.7%, 26/39). To investigate this phenomenon, we repeated the experiment using a larger number of Holstein heifers. The superovulatory treatment began in the midluteal phase of the estrous cycle (days 8 to 10) and consisted of eight decreasing doses of FSH i.m. (total of 28 Armour units, Antrin R-10, Kawasaki-Mitaka, Kanagawa, Japan) for 4 days with treatment twice daily. Doses of 5 mL and 3 mL of a PGF2α analogue (Veterinary Pronalgon F Injection containing 5 mg mL–1 Dinoprost, Pfizer Animal Health, Tokyo, Japan) were administered i.m. to the animals along with the seventh and eighth FSH treatment, respectively. The heifers were divided into two groups. One group, the rectal palpation (RP) group (n = 9), received transrectal ultrasonography with rectal palpation at 4-h intervals from 36 to 76 h after the first PGF2α treatment. The other group, the Control group (n = 8) received no treatment. The heifers were artificially inseminated at 56 and 72 h after the first PGF2α treatment using frozen–thawed semen from one bull. Seven-day embryos were recovered nonsurgically. Grade 1 to 3 embryos (IETS classification) were selected for this study. Male and female embryos were separated using the loop-mediated isothermal amplification procedure (Hirayama H et al. 2004 Theriogenology 62, 887–896). Data were analyzed using ANOVA and chi-square test. The mean number of recovered ova and embryos was 15.7 ± 3.8 (RP) and 14.4 ± 2.2 (Control). There was no significant difference in the percentages of unfertilized ova (RP; 14.9 %, 21/141 and Control; 11.3% 13/115, $P > 0.05$), grade 1 embryos (RP; 51.1%, 72/141 and Control; 54.8%, 63/115, $P > 0.05$) and grade 1 to 3 embryos (RP; 65.2%, 92/141 and Control; 69.6%, 80/115, $P > 0.05$) between the two groups. The proportion of female grade 1 embryos in the RP group (66.7%, 48/72) was significantly higher than the expected ratio of 50:50 ($P < 0.01$). The female ratio of grade 1 embryos in the Control group was 50.8% (32/63). Furthermore, the proportion of female grade 1 to 3 embryos in the RP groups (66.3%, 61/92) was significantly higher than the expected ratio of 50:50 ($P < 0.005$). The female ratio of grade 1 to 3 embryos in the Control group was 51.3% (41/80). Results indicate that frequent ultrasound examinations and rectal palpations following superovulatory treatment may skew the sex ratio of embryos towards females in Holstein heifers.

Reproduction, Fertility and Development 20(1) 228 - 228
Full text doi:10.1071/RDv20n1Ab296

Send Us Your Current Email Address!

The AETA receives several trade leads from various sources including USLGE. Because of the timely nature of these announcements, the AETA will immediately forward them to the membership via e-mail. Therefore, it is important that you provide the AETA with your most current e-mail address. To supply us with or update your current email address, send your name and email address to aeta@assochq.org

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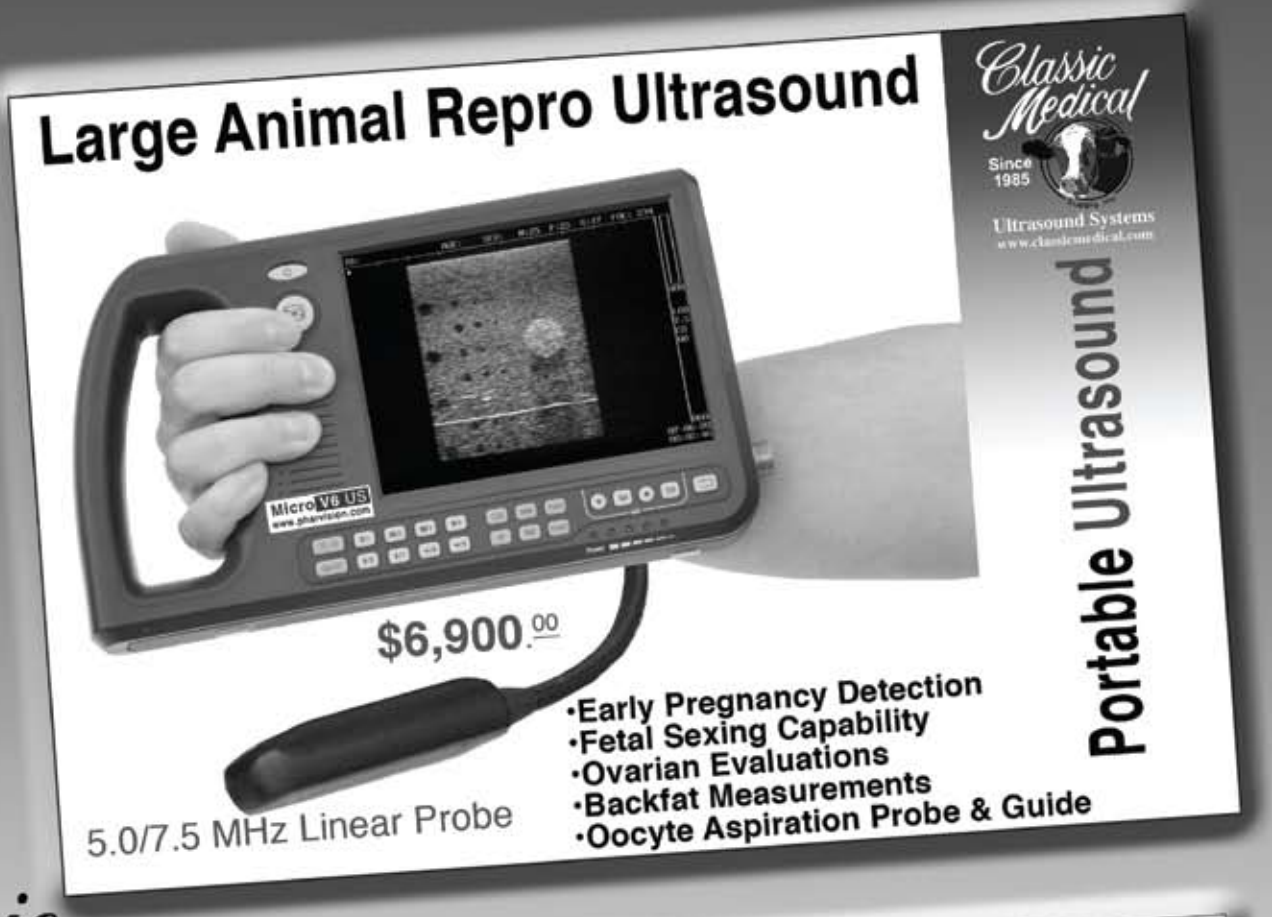

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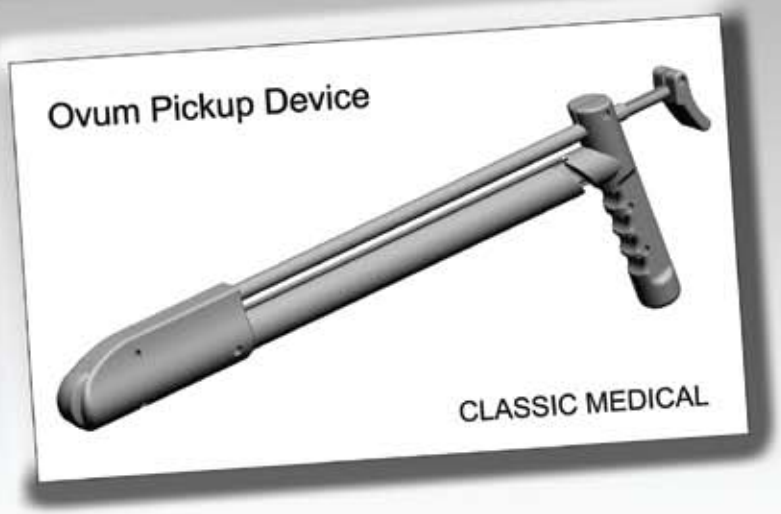

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2008 AETA & CETA/ACTE JOINT CONVENTION TENTATIVE MEETING SCHEDULE

Wednesday, October 15	
8:00 AM – 11:00 AM	MEETING: CETA/ACTE Certification Committee
9:00 AM – 4:00 PM	MEETING: AETA Board of Directors
11:00 AM – 6:00 PM	MEETING: CETA/ACTE Board of Directors
1:00 PM – 5:00 PM	EXAM: CETA/ACTE Certification Exam
Thursday, October 16	
8:00 AM – 12:00 PM	SOCIAL: Golf Tournament at Shoal Creek SPONSORED BY: PARTNAR ANIMAL HEALTH, INC. & REPRODUCTION RESOURCES
9:00 AM – 12:00 PM	EXAM: AETA Certification Exam (for members seeking new certification)
12:00 PM – 5:00 PM	Registration
12:00 PM – 5:00 PM	Exhibit Set-Up
1:00 PM – 4:00 PM	WET LABS: ET 101 presented by Drs. Randall Hinshaw and William Beal Emerging Technologies presented by Drs. Charles Looney and Brad Lindsey
6:00 PM – 10:00 PM	SOCIAL: Preconference Social at Union Station SPONSORED BY: BIONICHE ANIMAL HEALTH USA, INC. & BIONICHE ANIMAL HEALTH CANADA, INC.
Friday, October 17	
7:00 AM – 7:00 PM	Exhibits
7:00 AM – 5:00 PM	Registration
7:00 AM – 8:00 AM	Continental Breakfast
7:45 AM – 8:00 AM	Introduction of Sponsors
8:00 AM – 8:45 AM	SESSION: Dominant follicle/ovulatory follicle regulation presented by Dr. Richard Pursley
8:45 AM – 9:45 AM	SESSION: Superovulation protocols presented by Drs. Reuben Mapletoft, Kirk Gray, and Richard Pursley
9:45 AM – 10:15 AM	Break SPONSORED BY: REPRODUCTION RESOURCES & MINITUBE OF AMERICA, INC.
10:00 AM – 2:30 PM	Companion Tour: The Culinary Center of Kansas City (includes lunch)
10:15 AM – 11:00 AM	SESSION: Increased pregnancies by using 'anti-prostaglandin' product presented by Dr. Mitch Hockett
11:00 AM – 12:30 PM	MEETING: AETA Annual Business Meeting
11:00 AM – 12:30 PM	MEETING: CETA/ACTE Annual General Meeting
12:30 PM – 1:30 PM	MEETING: CETA/ACTE New Board of Directors
12:30 PM – 1:30 PM	Lunch
1:30 PM – 2:15 PM	SESSION: Nutrition presented by Dr. Jose Eduardo Santos
2:15 PM – 3:00 PM	SESSION: Equine ET presented by Dr. Peter Sheerin
3:00 PM – 3:30 PM	Break SPONSORED BY: I.M.V. INTERNATIONAL CORPORATION
3:30 PM – 4:30 PM	SESSION: Financial Planning presented by Dr. Christopher Allen
4:30 PM – 5:15 PM	MEETING: AETA & CETA/ACTE Joint Convention Committee Meeting
5:15 PM – 5:45 PM	MEETING: AETA New Board of Director's Meeting
6:00 PM – 7:00 PM	Reception
7:00 PM – 9:00 PM	Awards Banquet & Entertainment AWARDS BANQUET SPEAKER SPONSORED BY: BIOGENICS, INC.

A Cyber Cafe will be open Thursday through Saturday until 5:00 PM in the Exhibit Hall sponsored by Pfizer Animal Health.

Saturday, October 18	
6:30 AM – 7:30 AM	AETA Past Presidents/Board of Directors' Breakfast
7:00 AM – 5:00 PM	Exhibits
7:00 AM – 5:00 PM	Registration
7:00 AM – 8:00 AM	SESSION: AETA Certification Open Forum
7:00 AM – 8:00 AM	Continental Breakfast SPONSORED BY: PETS, INC.
7:45 AM – 8:00 AM	Announcements
8:15 AM – 9:00 AM	SESSION: Sexed semen presented by Juan Moreno
9:00 AM – 9:45 AM	SESSION: IVF, sexed embryos and timed transfers presented by Dr. Jon Schmidt
9:30 AM – 2:30 PM	Companion Tour: The Best of Kansas City Tour *(*Arabia Steamboat Museum & Webster House with lunch*)*
9:45 AM – 10:15 AM	Break SPONSORED BY: PETS, INC.
10:15 AM – 11:00 AM	SESSION: Cryobiology and Cryopreservation presented by Dr. John Critser
11:00 AM – 11:15 AM	SESSION: USDA & APHIS Updates
11:00 AM – 12:30 PM	SESSION: CETA/ACTE Information Session
11:15 AM – 11:30 AM	SESSION: Cooperator Committee & USLGE Updates
11:30 AM – 12:30 PM	EXAM: AETA exam (for members renewing certification)
12:30 PM – 1:30 PM	Lunch
1:30 PM – 2:15 PM	SESSION: Genetics presented by Dr. Richard (Mark) Thallman
2:15 PM – 3:00 PM	SESSION: Small Ruminants presented by Dr. Gary Vannoy
3:00 PM – 3:30 PM	Break
3:30 PM – 4:15 PM	SESSION: Embryo Observations presented by Dr. Mike Kieler
4:15 PM – 5:00 PM	SESSION: Vet/PhD Open Forum

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Alternative approaches to setting up donor cows for superstimulation

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Abstract

Protocols that controlled follicular wave emergence and ovulation have had a great impact on the application of on-farm embryo transfer, as they permitted the initiation of superstimulatory treatments at a self-appointed time. However, the most commonly used approach for synchronization of follicular wave emergence involved estradiol, which cannot be used in many countries. Therefore, alternative treatments are required. Mechanical removal of the dominant follicle by ultrasound-guided follicle aspiration was effective, but required the use of specialized equipment and trained technical staff, which made it difficult to utilize in the field. Exogenous GnRH or pLH have also been used to induce ovulation of a dominant follicle, synchronizing follicular wave emergence, but their efficacy was dependent on the stage of the dominant follicle at treatment; thus, the emergence of the ensuing follicular wave may be too variable for superstimulation. An alternative approach could be initiating treatments at the time of emergence of the first follicular wave, but the need to synchronize ovulation may be a disadvantage in groups of donors at random stages of the estrous cycle. The final alternative may be to use FSH or eCG to initiate a new wave, without regard to the presence of a dominant follicle, followed by superstimulatory treatment at a predetermined time. All alternatives need to be thoroughly investigated in order to confirm their utility in the superstimulation of donor cows, regardless of the stage of the estrous cycle and without compromising ova/embryo production.

Keywords: Follicular development; Superovulation; FSH; GnRH; eCG

1. Introduction

Variability in superovulatory response and the time and effort required for treatment and estrus detection have been the primary limiting factors affecting the success of embryo transfer technology in genetic improvement programs. Although the recent development of protocols that controlled follicular wave emergence and ovulation have not eliminated the variability in superovulatory response, these treatments have had a positive impact on the application of commercial, on-farm embryo transfer, by permitting the initiation of treatments at a self-appointed time [1,2]. Furthermore, protocols that synchronized ovulation allow insemination of donor cows at a fixed-time, thereby avoiding the necessity of estrus detection during the superstimulatory protocol [2,3]. Thus, treatments were more ''user friendly'' and easier to implement by farm personnel, and were not critically dependent upon estrus detection efficiency. However, the most commonly used approach for synchronization of follicular wave emergence for superstimulation (i.e., estradiol treatment) cannot be used in many countries because of concerns regarding the effects of estrogenic substances in the food chain. The intention of this manuscript is to

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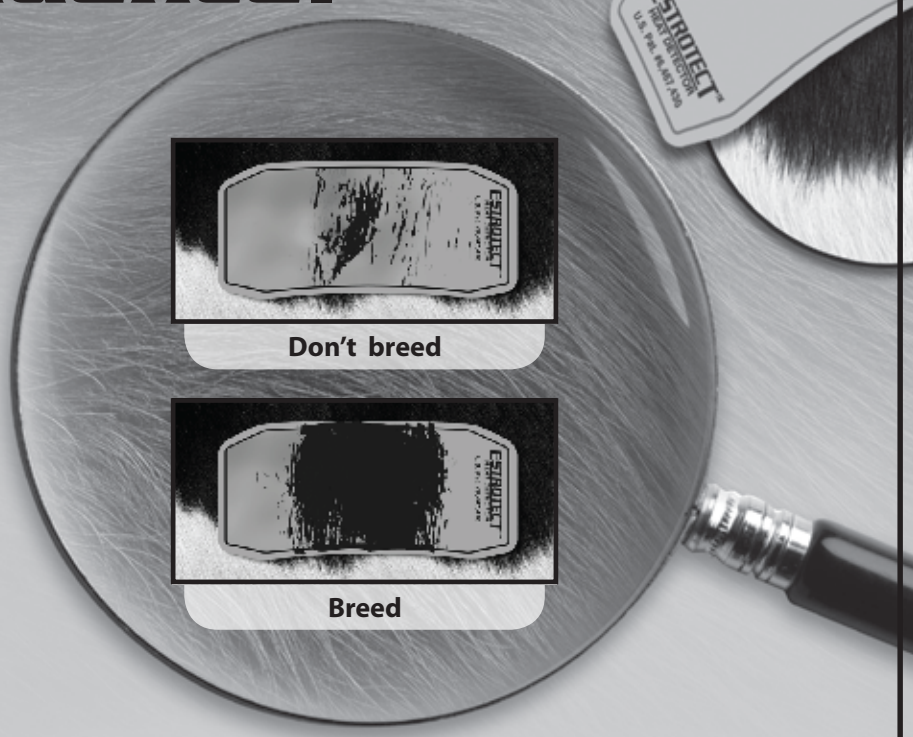

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briefly review the commonly used treatments for superstimulation of donor cows without estrus detection and to present preliminary information on potential alternative treatments that do not require the use of estradiol.

2. Manipulation of the follicular wave for superstimulation

It is now known that the traditional protocol for initiating ovarian superstimulation during mid-cycle (i.e., 8–12 days after estrus) [reviewed in 4] encompassed the time of emergence of the second follicular wave in cows exhibiting two- or three-wave cycles [5]. However, the superovulatory response was higher when gonadotropin treatments were initiated at the precise time of follicular wave emergence, rather than 1 or 2 days later [6,7]. Hence, the ability to electively induce follicular wave emergence would permit initiation of superstimulation at any time and eliminate the need for estrus detection, or for waiting 8–12 days to initiate gonadotropin treatments.

One approach to controlling the time of follicular wave emergence involved transvaginal ultrasound-guided ablation of all follicles $\geq$5 mm [8], or even just the largest two follicles [9,10]. Superstimulatory treatments may then be initiated 1–2 days later, at the time of emergence of a new follicular wave. Although this treatment was highly effective [reviewed in 2], it is difficult to implement ''on-farm''; therefore, a hormonal approach using estradiol and progesterone has been preferred. The most common hormonal treatment to synchronize the emergence of a follicular wave involved the administration of estradiol-17β (E-17β) or estradiol benzoate (EB) and progesterone, by intramuscular injection, at the time of insertion of an intravaginal progesterone-releasing device [reviewed in 2]. From a practical perspective, this approach was ideal for busy embryo transfer practitioners. However, the inaccessibility of these effective synchronization tools leaves many embryo transfer practitioners in a serious dilemma.

3. Alternative approaches for follicle wave synchronization and superstimulation

3.1. GnRH or pLH

It has been shown that GnRH will induce ovulation or luteinization of the largest follicle at the time of treatment [11], with emergence of a new follicular wave approximately 2 days later, but only when treatment resulted in ovulation [12]. Different ovulation rates after GnRH treatment at random stages of the estrous cycle have been reported in lactating dairy cows, dairy or beef heifers and beef cows. Pursley et al. [13] reported ovulation rates of 85% in lactating dairy cows and 54% in dairy heifers. In a more recent study, treatment of lactating dairy cows at random stages of the estrous cycle resulted in an ovulation rate of 62.4% in those given 25 mg pLH (Lutropin-V, Bioniche Animal Health, Belleville, ON, Canada) and 44.3% in those given GnRH (Fertiline; Vetoquinol N-A Inc., Lavaltrie, QC, Canada; $P < 0.01$) [14]. Other studies reported ovulation rates of 78 and 56% in beef heifers treated with pLH or GnRH, respectively ($P < 0.09$) [12]. Lactating beef cows appeared to be more similar to heifers than lactating dairy cows, with seldom more than 60% ovulating following administration of GnRH at random stages of the estrous cycle [15]. It has also been shown recently that circulating concentrations of progesterone affected LH release following the administration of GnRH in beef cattle [16]. Therefore, the interval from GnRH treatment to wave emergence may not be as consistent as required for superstimulation.

Attempts to synchronize follicular wave emergence for superstimulation with either GnRH or pLH have had limited success. In three successive experiments [17], GnRH or pLH treatments for the synchronization of follicular wave emergence for superstimulation resulted in fewer embryos than in control animals, or those in which follicular wave emergence had been synchronized with estradiol or follicle ablation. However, recent results from a commercial embryo transfer practitioner (Steel R., personal communication) have shown that giving GnRH 1.5 days after insertion of a controlled internal drug releasing (CIDR) device (1.38 g of progesterone, Eazy-Breed CIDR, Pfizer Animal Health, USA) and 60 h prior to initiation of FSH treatment resulted in a similar number of transferable embryos (5.5, $n = 95$) as in cows superstimulated 4 days after insertion of a CIDR and an injection of 5 mg E-17β and 100 mg progesterone (5.4, $n = 56$). Controlled and appropriately designed experiments must be done to confirm these encouraging observations.

An alternative to overcome the variable response in terms of follicular wave emergence following the administration of GnRH or pLH is to ensure that a viable growing dominant follicle is present at the time of treatment. Stage of development of the dominant follicle [12], or stage of the estrous cycle [13,18] at the time that GnRH was administered affected results. If GnRH was administered when the dominant follicle was immature or post-mature, ovulation may not occur and a new

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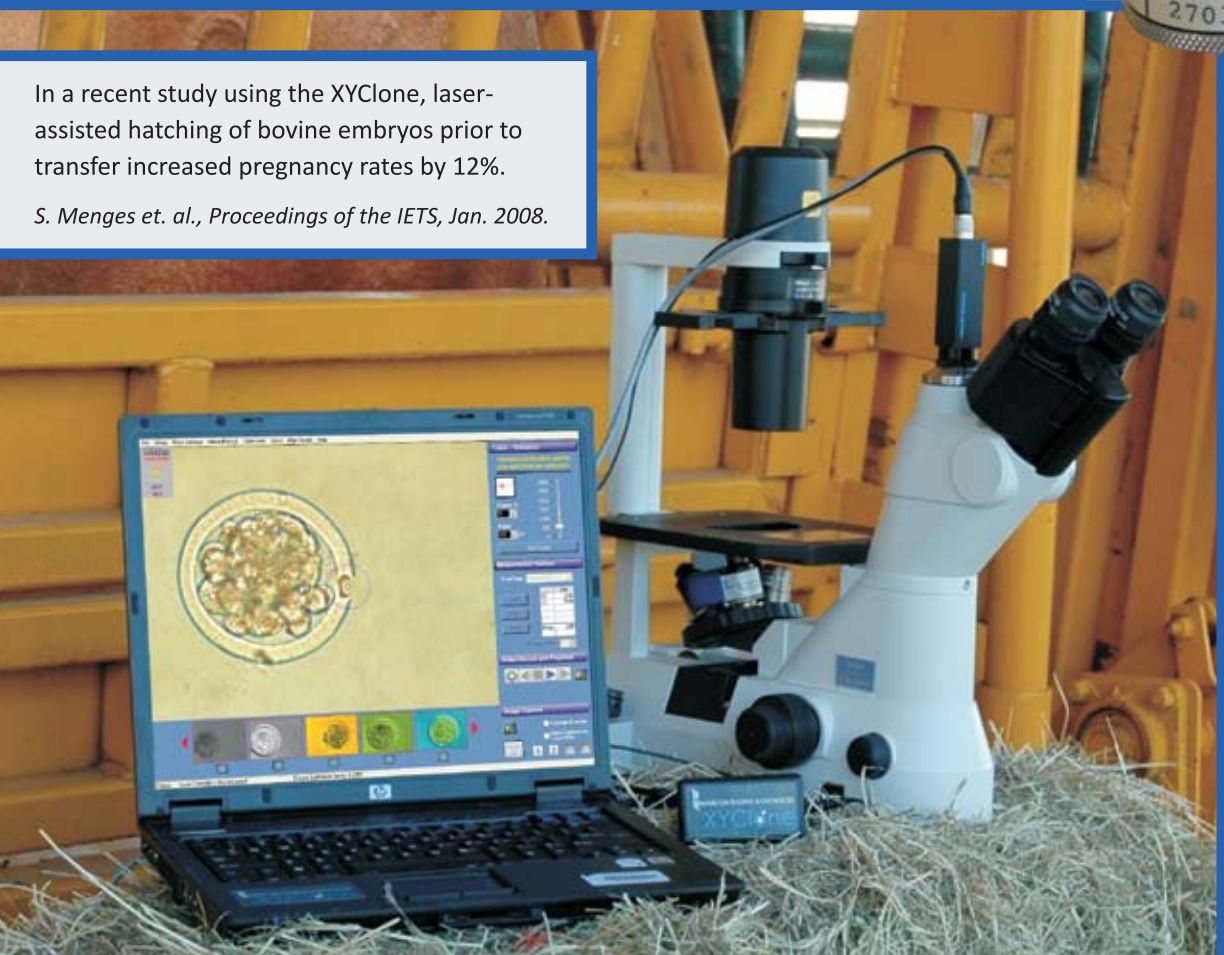

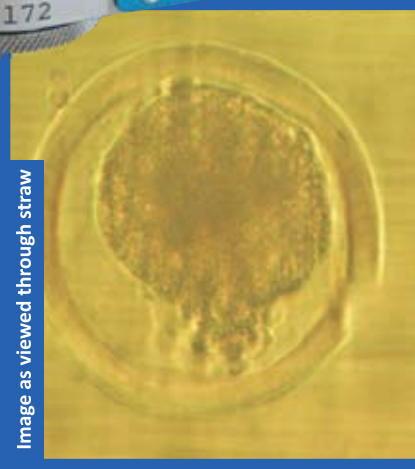

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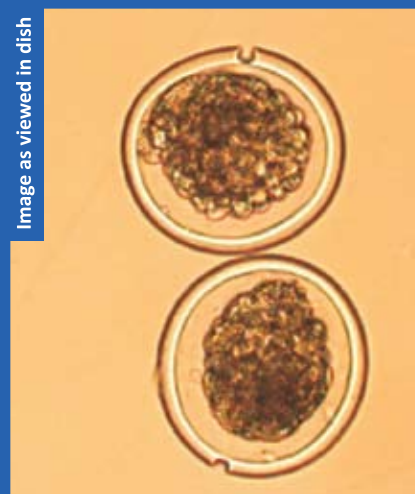

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follicular wave will not emerge [12]. It has been suggested that cattle will respond most consistently to GnRH-based protocols initiated between days 5 and 12 of the estrous cycle; this can be accomplished by using a PGF2α pre-synchronization treatment, with the last PGF2α given 12 days before the first injection of GnRH [18]. However, to our knowledge, the initiation of superstimulatory treatments subsequent to pre-synchronization and GnRH or pLH treatment has not been studied.

3.2. Superstimulation at emergence of the first wave

Another alternative is to initiate gonadotropin treatments at the time of emergence of the first follicular wave. The first follicular wave consistently emerges on the day of ovulation (or the day after the onset of estrus) in cattle [20]. Nasser et al. [7] reported that superstimulation can be initiated successfully at the time of emergence of the first follicular wave, and Adams et al. [6] showed that the superovulatory response did not differ whether gonadotropin treatments were initiated at the time of the emergence of the first or second follicular wave.

To avoid the need to detect estrus or ovulation, Nasser et al. [21] induced a synchronous ovulation in Nelore (*Bos indicus*) donor cows treated with an EB-CIDR protocol for 8 days and the administration of pLH 24 h after CIDR removal. Superstimulatory treatments were initiated 24 h later (at the expected time of emergence of the first follicular wave, i.e., ovulation) and donors did or did not receive a new CIDR during superstimulation. There was no difference in the number of transferable embryos in CIDR-treated cows, whether FSH treatments were initiated at the time of emergence of the first follicular wave (8.0 ± 1.8) or 4 days after the injection of 2.5 mg EB and 50 mg progesterone (Control group; 6.6 ± 2.0), but both were greater than when treatments were initiated at the time of emergence of the first follicular wave without the use of a new CIDR during superstimulation (0.2 ± 0.2; $P < 0.05$).

It may also be possible to synchronize ovulation prior to superstimulation by inducing ovulation of a persistent follicle with GnRH or pLH. It was possible to induce a persistent follicle with a previously used-CIDR for 7–10 days, with PGF2α at the time of insertion to regress the CL [19]. Administration of GnRH at CIDR removal resulted in ovulation and follicular wave emergence 1–2 days later.

We have recently evaluated a similar approach in a superstimulation treatment protocol [22]. Seventy Bonsmara donors (29 cows and 41 heifers) were randomly allocated into one of two treatment groups. Donors in the First Wave Group received a progesterone releasing device (1.56 g of progesterone, Cue-Mate, Bioniche Animal Health) along with a dose of PGF2α (0.150 mg D(+)-cloprostenol, Bioprost-D, Biotay SA, Argentina) at random stages of the estrous cycle. Cue-Mates were removed 10 days later, and a second PGF2α was administered at that time, followed by GnRH (0.050 mg Lecirelina, Biosin-OV, Biotay SA) 36 h later. Ovulation was expected to occur within 30 h after GnRH. On day 0 (36 h after GnRH), donors received a new Cue-Mate, and superstimulation treatment was initiated with a total dose of 200–260 mg (heifers) or 320 mg (cows) NIH-FSH-P1 of Folltropin-V (Bioniche Animal Health) in twice-daily decreasing doses over 5 days. PGF2α was administered with the last two Folltropin-V treatments and Cue-Mate devices were removed with the last. All donors received 12.5 mg pLH

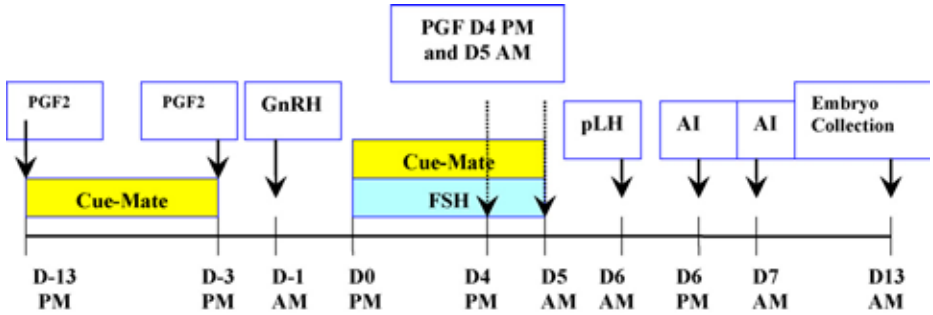

Fig. 1. Treatment schedule for synchronizing ovulation and superstimulating donor cows during the first follicular wave. Donor animals received a progesterone-releasing device (Cue-Mate), along with a dose of PGF2α. Cue-Mate was removed 10 days later and a second PGF2α was administered at the same time, followed by GnRH 36 h later. On day 0 (36 h after GnRH) donors received a new Cue-Mate and superstimulation treatment was initiated in twice-daily decreasing doses over 5 days. PGF2α was administered with the last two FSH injections and Cue-Mate was removed with the last FSH injection. Ovulation was induced with pLH 24 h after Cue-Mate removal, donors were AI 12 and 24 h later, and embryos were collected 7 days after pLH treatment.

Table 1
Superovulatory response (means ± S.E.M.) in Bonsmara cows and heifers treated with Folltropin-V during the first follicular wave, or 4 days after administration of estradiol benzoate

Main effects	No.	Total ova/embryos	Fertilized ova	Transferable embryos
Control	34	11.0 ± 1.4	6.3 ± 1.1	5.1 ± 0.9
Wave 1	34	8.4 ± 1.4	5.2 ± 1.1	3.7 ± 0.8
Cows	29	10.7 ± 1.6	6.7 ± 1.4	5.1 ± 1.0
Heifers	39	9.1 ± 1.2	5.0 ± 0.9	3.8 ± 0.7

Adapted from Carballo Guerrero et al. [22]. Means did not differ ($P > 0.20$).

24 h after Cue-Mate removal, with AI 12 and 24 h later. Embryos were collected 7 days after pLH treatment. The treatment schedule is shown (Fig. 1).

Donors in the Control Group received a Cue-Mate and 2 mg EB (Bioestradiol, Biotay SA) and 50 mg of progesterone (Lab Rio de Janeiro, Argentina) and superstimulation treatments were initiated 4 days later, with the same dosages of Folltropin-V as used in the First Wave Group. PGF2α administration, Cue-Mate removal, pLH treatment, AI and embryo collections were done as in the First Wave Group. Results from this experiment are shown (Table 1). It was not possible to pass the cervix with the collection catheter in two heifers in the Control Group and they were excluded from the analysis. There were no significant effects of treatment or donor category (cows versus heifers) on superovulatory response and embryo quality.

Although this study has shown that superstimulation on the first follicular wave was as efficacious as the "standard" estradiol superstimulation treatment protocol in beef cattle, the duration of the protocol (i.e., 26 days versus 15 days from Cue-Mate insertion until embryo collection) made it more time-consuming and difficult to implement. Therefore, a subsequent study was designed to shorten the Cue-Mate pretreatment to 5 days (Carballo Guerrero, personal communication). For this study, cows and heifers were randomly allocated into four treatment groups. Donors in Group 1 were treated similarly to the First Wave Group in the previous experiment. Donors in Groups 2 and 3 received PGF2α on day-8 and either a new Cue-Mate or a Cue-Mate in which one of the two progesterone impregnated pods (0.78 g of progesterone each) was replaced by a blank (no progesterone) pod (Cue-Mate 1 pod) for 5 days. PGF2α was administered at the time of Cue-Mate removal, followed by GnRH 36 h later. On day 0 (36 h after GnRH) donors in all three groups received a new Cue-Mate and superstimulation treatment was initiated with a total dose of 260 mg (cows) or 200 mg (heifers) of Folltropin-V, in twice-daily decreasing doses over 5 days. PGF2α administration, Cue-Mate removal, pLH treatment, AI and embryo collections were done as in the previous experiment. Donors in Group 4 (controls) were set up with EB as in the previous experiment. As this is an ongoing experiment, only preliminary results are presented. Superovulatory response did not differ among treatment groups (Table 2) and only one cow (in the group treated with Cue-Mates with one pod for 5 days) failed to ovulate within 36 h after GnRH treatment. Data suggest that any of these protocols involving the first follicular wave could be used to superstimulate groups of donors at a self-appointed time.

3.3. Down-regulation of the pituitary gland

It has been shown that following the administration of an experimental GnRH agonist in cattle, follicles grew to ~8 mm in diameter, when pulsatile LH release

Table 2
Superovulatory response (means ± S.E.M.) in Brangus cows and heifers treated with Folltropin-V during the first follicular wave synchronized by a 10- or 5-days pretreatment with a progesterone-releasing device, or 4 days after administration of 2.5 mg EB, 50 mg progesterone and insertion of a progesterone-releasing device

Group	No.	CL	Total ova/embryos	Fertilized ova	Transferable embryos
10-Days two pods[a]	9	13.9 ± 2.0	9.9 ± 2.9	4.7 ± 2.1	3.4 ± 1.6
5-Days two pods[a]	8	15.6 ± 2.0	12.3 ± 3.8	9.0 ± 3.5	6.1 ± 3.5
5-Days one pod[b]	7	15.7 ± 2.5	15.1 ± 3.3	10.4 ± 2.7	4.6 ± 1.1
Control	9	11.0 ± 2.3	12.9 ± 4.7	9.4 ± 3.6	8.2 ± 3.7

Means did not differ ($P > 0.4$).
[a] Cue-Mate devices with two silicon pods impregnated with 0.78 g progesterone each.
[b] Cue-Mate devices with one silicon pod impregnated with 0.78 g progesterone.

was inhibited, and to ~4 mm in diameter, when both FSH release and LH pulses were inhibited [23]. Similarly, when an anti-GnRH vaccine was administered [24,25], follicles grew to 3 mm, but not larger. The growth of follicles to a larger size resumed upon treatment with exogenous FSH and their growth rate in response to exogenous FSH was similar to controls [24]. Both approaches provided for the possibility of preparing donor cows that are in a constant state of readiness with follicles that never achieve dominance unless exogenous gonadotropins are administered.

D'Occhio et al. [26] developed a model in which two implants impregnated with the GnRH agonist, deslorelin, was inserted to desensitize the pituitary gland to GnRH and block the endogenous LH surge. Each implant released 20 μg of deslorelin per 24 h. Seven days after treatment at random stages of the estrous cycle, superstimulatory FSH treatments were initiated and 2 days later PGF2α was administered; 60 h after the PGF2α treatment, ovulation was induced with an injection of pLH [26]. This treatment protocol was compared to the EB-CIDR superstimulation protocol in Nelore cows and the number of transferable embryos did not differ [27]. Unfortunately, deslorelin implants are not commercially available for use in cattle.

3.4. Superstimulation without induction of dominant follicle atresia

With current knowledge about the endocrine control of follicular wave dynamics in the cow, it should be possible to develop schemes that will synchronize follicular wave emergence in groups of cows at random stages of the estrous cycle. We know that we can standardize the luteal phase of the cycle with a progesterone-releasing device. To synchronize follicular wave emergence, it is a matter of causing regression of existing follicles and a surge in FSH to induce emergence of a new follicular wave, at which time gonadotropin treatments can be initiated. However, the synchronization of follicular wave emergence may not be the only requirement for successful superovulation. In a recent study, superovulatory response was most dependent on the numbers of follicles entering the wave; a simple ultrasound examination at wave emergence was highly predictive of the subsequent superovulatory response [28]. Therefore, cows with a low number of follicles entering the wave responded poorly, even when treatments were initiated at the time of follicular wave emergence.

An interesting concept that has emerged is that of "subordinate follicle breakthrough." During a normal follicular wave, subordinate follicles regress because of decreasing concentrations of FSH, caused by the secretions of the cohort and especially the dominant follicle (estradiol and inhibin). Small follicles require FSH to continue their growth; it was recently documented that follicles as small as 1 mm in diameter will commence growth under the influence of FSH [29]. Perhaps all that is required for superstimulation is to cause small follicles to grow to a diameter of 3 or 4 mm at which time the regular 4- or 5-days superstimulatory treatment protocol can be initiated. With a growth rate of 1 mm/day [29], adding 2–3 days to the superstimulation treatment protocol may provide sufficient time to recruit new follicles into the superstimulated cohort. The presence or absence of a dominant follicle may be of little consequence to the superovulatory response under these circumstances; the exogenous FSH replaces that being depressed by the secretory products of a dominant follicle.

Caccia et al. [30] reported that the administration of 500 IU of equine chorionic gonadotropin (eCG) 2 days before initiating FSH treatments tended to increase the superovulatory response, presumably by recruiting more follicles into the wave. More recently [31], the administration of 500 IU eCG 2 days before initiating FSH treatments (i.e., on day 2 of a progesterone/estradiol protocol with FSH treatments beginning on day 4) in cows with a history of poor embryo production (i.e., $\leq$3 transferable embryos per superovulatory treatment) resulted in a higher ($P < 0.01$) number of transferable embryos (3.6 ± 0.6), than when eCG was not given on day 2 of the superstimulatory treatment protocol (1.0 ± 0.2). Further studies are required to evaluate the possibility of using eCG-pretreatment in a superstimulatory scheme in which the follicular wave had not been synchronized.

An alternative treatment protocol using FSH only could involve the insertion of a progesterone-releasing device at random stages of the cycle and initiation of FSH treatments 2 or 3 days later (once progesterone concentrations had stabilized). The FSH could be given over 6 or 7 days, with the latter 4 days of the protocol not differing from that in current use. The total dose of FSH may not have to be increased; rather, the total dose of FSH could be divided over more treatments, with early treatments being low so as to recruit very small follicles into the wave.

In a preliminary study (Bó, unpublished) performed with cross-bred beef donor cows that were used in an embryo collection training program, this treatment regimen resulted in a superovulatory response similar to when donors were superstimulated 4 days after the

Table 3
Superovulatory response (means ± S.E.M.) in cross-bred beef cows treated with Folltropin-V for 4 days, initiated 4 days after EB plus progesterone administration and insertion of a progesterone-releasing device (Control Group), or treated with Folltropin-V for 6 days, initiated 2 days after the insertion of a progesterone-releasing device, without estradiol administration (6-days FSH Group)

Group	No. CL	Total ova/embryos	Fertilized ova	Transferable embryos
6-days FSH	12.3 ± 1.1 (n = 29)	6.9 ± 1.3 (n = 16)	4.7 ± 1.1 (n = 16)	3.6 ± 1.0 (n = 16)
Control	14.1 ± 1.2 (n = 26)	10.5 ± 2.3 (n = 15)	6.1 ± 2.0 (n = 15)	4.9 ± 1.7 (n = 18)

Means did not differ ($P > 0.2$).

treatment with EB. Briefly, cows in the treatment group (6-days FSH Group) received an intravaginal progesterone-releasing device (0.75 g of progesterone, Pro-Ciclar, Zoovet, Argentina) at random stages of the estrous cycle (day 0). On day 2, superstimulatory treatment was initiated with a total dose of 320 mg NIH-FSH-P1 Folltropin-V for 6 days (i.e., 10, 20, 60, 40, 20 and 10 mg bid). PGF2α was given twice on day 6, devices were removed on day 7, GnRH was given on day 8, and all cows were AI 12 and 24 h later. Embryos were collected 7 days after GnRH. Cows in the Control Group received 2.5 mg EB and 50 mg progesterone with the insertion of a Pro-Ciclar on day 0. On day 4, superstimulatory treatments were initiated with a total dose of 320 mg of Folltropin-V, but in this case it was administered in twice-daily decreasing-dose injections over 4 days (i.e., 70, 50, 30 and 10 mg bid). PGF2α administration, device removal, GnRH treatment, AI and embryo collections were done as in the 6-days FSH Group. Results are shown (Table 3). Due to the nature of these data (i.e., collected by trainees during embryo collection short courses), there were more cows in which CL were counted by palpation than those in which ova/embryo data were collected and considered for analysis. Therefore, the results presented must be considered as preliminary information and evaluated with caution. Regardless, the 6-days FSH initiated at random stages of the estrous cycle seemed feasible and efficacious.

It was thought that there was a risk, by chance, that 20% of animals treated with FSH at random stages of the cycle may be treated at the time of follicular wave emergence and the extra days of FSH treatment may affect oocyte quality. However, there was no indication that oocyte/embryo quality was compromised in the 6-days Group. It is unlikely that the oocytes in these follicles will deteriorate, providing progesterone concentrations remained high. It has been shown that oocytes will remain in the germinal vesicle stage for at least 120 h after the cessation of FSH treatments, when progesterone concentrations were maintained at >5 ng/mL [32]. Obviously, further studies are needed to confirm these preliminary findings.

4. Summary and conclusions

Incorporation of protocols designed to control follicular wave dynamics offered the convenience of being able to initiate superstimulatory treatments quickly and at a self-appointed time, without the necessity of estrus detection and without sacrificing results. However, estradiol, which has proven to be most useful for these purposes in the field, is being withdrawn from many veterinary markets around the world, leaving only follicle ablation as a reliable method to synchronize follicular wave emergence for superstimulation. Unfortunately, follicle ablation is difficult to utilize in the field. Although administration of GnRH or pLH to synchronize follicular wave emergence would appear to be too variable for superstimulation, pre-synchronization may improve response. An alternative currently available may be initiating FSH treatments at the time of emergence of the first follicular wave with the inclusion of a progesterone-releasing device (on the day of ovulation), but the duration of the treatment to synchronize ovulation in groups of donors at random stages of the estrous cycle may preclude the use of this approach. An exciting alternative may be to use FSH or eCG to recruit follicles into the wave, regardless of the stage of development of the dominant follicle, and to initiate the regular superstimulatory treatment protocol at a predetermined time (e.g., 2 or 3 days later).

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