President’s Message

By now, you have carefully read your summer newsletter from cover to cover and you are aware of the fact that the volume of ET work done by our membership continues to grow as the industry expands and becomes an ever more integral part of almost every area of animal agriculture. It is good to see the strong numbers, and it bodes well for the AETA and its membership as we look ahead. Many thanks to Brad Stroud and his committee for the incredible work they have done.

There is a small list of things that are happening, or have happened, as we approach the year end. Dr. Randall Hinshaw and the GMO committee are wrapping up their work to reach agreements with the banks involved in that settlement. The Cooperator Committee, USLGE, and the Government Liaison Committee have all been involved in the development of an export protocol for Russia. The USDA is now handling the negotiation after input from all parties. The board is preparing for its winter meeting to be held in Kansas City. If you have issues or concerns that you feel need to be addressed there, please contact a board member or the AETA office and let us know.

The IETS meeting was in Denver in January. These are great events and they tend to connect you with new groups of people and many new ideas. The 2009 meeting will again be in the United States, in San Diego, California.

Do not forget to plan for our annual convention, to be held jointly with CETA in Kansas City in October 2008. The year 2009 will find AETA north of the border with CETA again, in an as yet undetermined city. As I have said before, you need to plan now to bring a new member to the 2008 meeting. If everyone brings a new member, the math becomes easy.

I hope everyone had a safe and very fun holiday season, and has a prosperous New Year. Be well and be happy.

David Duxbury, DVM

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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.

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 of a page). The advertising of 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 of a 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 website advertising.

A Closer Look Advertising Rates for 2008

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Ads are due the 15th prior to each issue month. Online ads are full color and print ads are black and white.
Contract terms: All amounts due on invoicing.

AETA accepts electronic or camera-ready ads for publication. Accepted ad formats include: PDF (preferred) or high quality .jpg, .tif, or .eps. Call regarding all other formats.
If you would like to advertise in the next issue, please contact AETA at aeta@assochq.org or 217-398-2217.

Save These Dates!

AETA & ACTE

2008 AETA & CETA/ACTE Joint Annual Meeting
Westin Crown Center Hotel
Kansas City, Missouri
October 16–18, 2008

Future Meetings of Interest!

USLGE

2008 USLGE Annual Meeting and Marketing Conference
San Antonio, Texas
February 5–7, 2008

ICAR

16th International Congress on Animal Reproduction
Budapest, Hungary
July 13–17, 2008

AABP

41st Annual Convention
Charlotte, NC
September 25–27, 2008

WEVA

10th Congress
Moscow, Russia
January 28–February 1, 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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Foreword by Darrel DeGrofft, DVM

Given the increasing average age of many of our members in AETA, and some of my personal interactions with several of my colleagues over the course of time, I wanted to share an article written by my daughter, an RN currently working in clinical pharmaceutical research, about my experience with prostate cancer last year. I believe that being proactive about our health is important, and in sharing this information, I hope it will make us all think a bit more about the need to take a bit of time to go see a physician—for any reason. Please share this article with anyone you wish.

Best wishes to you all in your future health,
Darrel

Have you ever wondered, “what if?” or “had I only . . .?” Americans today have so much control over our health, yet we are not as proactive as we have the opportunity to be. We often ignore our chances for early detection of many diseases because we “feel fine.” Being proactive is imperative because reacting often isn’t enough.

On July 10, 2006, a biopsy of the prostate showed that my Dad, Darrel DeGrofft, had a Gleason score of 7—prostate cancer (commonly misspelled as “prost-rate”). According to prostate.com, “Statistics show that during his lifetime, a man has about a 1 in 10 chance of being diagnosed with prostate cancer. Over 200,000 new cases of prostate cancer are diagnosed annually in the US.” The proactive health care options available today, and the fact that my Dad had been diligent about his health care and testing over the course of time, led to his ability to make educated and calm choices about his health. He wasn’t reacting to a disease that was now out of control and life-threatening.

Prostate cancer is a very slow-growing cancer in most men, and many die with it, not of it. Dad was in to the doc at least yearly for a little blood draw (prostate-specific antigen, or PSA) and a digital rectal exam (DRE). For those of you who know him, his travel schedule is hectic, and finding time for dinner at home is often a challenge, but he made sure that his doctor appointments were included in his schedule and not overlooked, because without his health, he really has nothing.

He had a baseline PSA drawn about 6 years ago, giving his doctors a realistic measurement specific to him, compared with only the laboratory normal values. At these yearly visits (or as often as every 6 months), the DRE was normal. The PSA, however, spiked every so often, and after 5 years of some spikes, the doctor opted to do a biopsy (this is an in-office procedure at the urologist’s office). Dad agreed. In spite of “feeling fine” and “no different from yesterday,” on July 10, 2006, Dad’s biopsy was performed and showed that he was positive for cancer of the prostate. The urologist estimates it had already been present for at least 4 to 5 years.

So after evaluating the treatment options—and there are a few to consider (please speak to your doctor about potential options)—Dad opted for a prostatectomy as soon as possible. On September 8, 2006, the surgery was performed in Fort Collins, Colorado, at the Poudre Valley Hospital with the only robotic surgery option in the state of Colorado at the time. I am pleased to report that not only was the surgery highly successful, but also that the pathology reported a fully encapsulated prostate and the Gleason score was downgraded!

Now? Well, he had PSAs drawn about every 3 months for a year, every 6 months thereafter (until year 5), and then back to once a year again. Because prostate cancer is a malignant tumor, it can spread rapidly throughout the body, so patients are watched to ensure there is no spread in spite of full encapsulation.

A Few Facts

From prostate.com: “Prostate cancer is the second leading cause of cancer death in men. But the good news is that survival rates have improved a great deal. In the past 20 years, 5-year survival rates for all stages of prostate cancer combined have increased from 67% to 99%.”

Risk factors include age, ethnicity, family history, diet, and obesity. So who should be tested? According to the American Cancer Society, men aged 50 and older with a life expectancy of at least 10 years, and those over the age of 45 who are in high-risk groups (such as African American men and men with a family history of prostate cancer) should have a PSA blood test and a DRE once every year. (If you have a family history or are unsure of your family history with regard to prostate cancer, you should consult your physician right away, regardless of your current age, so that you can discuss options that are right for you, because testing may be done earlier in some cases based on that history.)
Many of you reading this article are men, who, based on statistics, go to the doctor much less often than women. I wanted to write this article because we all know a person in our lives at risk for prostate cancer—and I happen to know more than one who has fought this disease. Dads, uncles, brothers, husbands, sons, nephews, cousins, and friends . . . and many men are not diligent about their health care and early detection of potential issues. Going to the doctor can literally save your life! I asked Dad if I could share his story because his cancer was caught early, and I hope that in some small way, this will encourage you or someone you know to be proactive about their health. Cancer is a big and scary word, and I hope that by sharing this story someone else just might have a health issue caught that much sooner too.

**A Few Reference Sites for Prostate Cancer**

General information:
www.prostate.com  
http://www.cancer.gov/cancertopics/factsheet/detection/PSA
http://www.prostatecancerfoundation.org/
http://www.mayoclinic.com/health/prostate-cancer/DS00043

Sites regarding robotic surgery:
http://www.henryfordhealth.org/19147.cfm
http://www.davinciprostatectomy.com/video.html
https://vic.pvhs.org/pls/portal/docs/PAGE/PVHS/PVHS_DOCUMENT_MGMT2/NEWS_REPOSITORY/NEWS%20500TH%20ROBOTIC%20SURGERY%20PERFORMED%20AT%20PVH.PDF

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**Ask John . . .**

**Question:** I was recently asked by a practitioner about misshapen zonas that he was encountering in some of his bovine embryo recoveries. He wanted to know if anything he was doing during the recovery process was responsible for distorting the zonas, or perhaps it had something to do with the embryo filter.

**Answer:** I am rather sure that nothing practitioners normally do during the embryo recovery process has any influence on the shape of the zona pellucida (ZP) of either unfertilized ova or embryos. The ZP is a glycoprotein that is laid down around the oocyte during what is called the secondary follicle stage, over a period of weeks, prior to the follicle forming an antrum. When one works with embryos by using a micromanipulator, it becomes clear that the ZP is extremely elastic. Zona pellucida-clad embryos act just like little rubber balls, and the ZP snaps right back into its original shape when distorted by mechanical pressure. We have all seen unfertilized ova that are very flat. I believe these are degenerating and were probably round in shape at the time of ovulation. Sometimes we see morulae or early blastocysts with misshapen ZP, especially pear-shaped and sometimes with a nipple-like protuberance. I believe that these ZP were formed inside a secondary follicle that was not quite spherical and, in some cases, had a little cavity that allowed a protuberance to be deposited.

************************************************************************

Questions for “Ask John” may be addressed to:
askjohn@assochq.org
This year’s meeting will be held at the historic Menger Hotel in San Antonio, Texas, February 5-7, 2008 and will have a slightly different schedule.

On Tuesday, February 5, 2008, we will have a training program for new members and those wanting additional information on USLGE international market development program management. The training session will be from 1:00 p.m. – 4:30 p.m., with the evening free.

Attendees not participating in the training program will want to arrive Tuesday afternoon or evening as the USLGE general program will start at 8:30 a.m. on Wednesday, February 6, 2008 and will have various presentations during the day and an evening dinner program.

On Thursday, February 7, 2008, the Specie groups will meet in the morning and review specie funding requests for the FY09 UES submission. The Specie groups will report their discussions and the USLGE Annual Meeting will be held at 1:30 p.m., followed by a USLGE Board of Directors re-organization meeting. Members will be able to schedule departures after 4:00 p.m.

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ICAR 2008

16th International Congress on Animal Reproduction
13–17 July 2008
Budapest, Hungary
www.icar2008.org

The Hungarian Society for Animal Reproduction and the Local Organising Committee cordially invite you to participate in the 16th International Congress on Animal Reproduction to be held 13–17 July 2008 in Budapest, Hungary. An interesting and varied social program will enhance the opportunities for delegates to network with associates as well as to enjoy the Hungarian cultural heritage. Fascinating pre- and postconference tours will be arranged to highlight the best of what Hungary has to offer. Furthermore, registered accompanying persons are warmly welcomed to join the special programs and tours organized for accompanying persons and also to join the social events of the congress. The theme of the congress is animal reproduction, including animal physiology, animal pathology, and reproductive technology. The ICAR Congress—held in every four years—is a successful world-class event and exhibition that attracts more than 900 international scientists and practitioners who want to stay current on the latest issues of animal reproduction.

A detailed scientific program of the plenary sessions and symposia is available on the congress Web site. Please also have a look at the information on registration fees, hotel reservations, and pre- and postcongress tours and social activities. Online registration is available! You can register for the ICAR 2008 Congress on the Web at http://www.icar2008.org/online/.

If you are interested in future progress of the organisation, you only have to give your contact information via the Web site and we will keep you informed.

Looking forward to meeting you in Hungary in 2008,
Professor Dr. László Solti, president of the Local Organising Committee

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2008 AETA Annual Meeting
October 16 - 18, 2008

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To find out more about this incredible breakthrough in pregnancy rates or to find a participating practitioner please visit www.ultimategenetics.com.

![Advantage Technology Embryo Armor™](image)

These data are a compilation of 16 separate experiments utilizing Advantage Technology Embryo Armor™ in a double blind study to compare pregnancy rates of treated embryos to control, non-treated embryos. 301 embryos were flushed from cows treated with Advantage Technology Embryo Armor™ and frozen prior to transfer to recipient cows, and were compared to 253 control frozen embryos. The difference between treated embryos and non-treated embryos was 13% in favor of treatment. 768 embryos were flushed from cows treated with Advantage Technology Embryo Armor™ and transferred fresh to recipient cows, and were compared to 412 control embryos. The difference between treated embryos and non-treated embryos was 10% in favor of treatment.

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Product Information and Use

Instructions for use:

1. Embryo Armor™ is kept frozen until use at -20°C as a 1 ml concentrate in capped plastic storage tubes.
2. When ready to use, thaw by warming to room temperature or accelerate thawing in a warm water bath.
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4. The tube can be inverted or kept upright and the cap can be pierced with the needle or removed.
5. Transfer product into flush medium bag via LuerLok® connection or needle entry. To assure complete transfer of product some flush medium can be pulled back into the syringe, used to rinse out the tube, then put in the bag.
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8. Collect the protected embryos and perform follow-up standard procedures.

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Easy as 1-2-3!

1. Thaw and add the Embryo Armor™ to the collection/flush medium.
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Visit us on the web at www.ultimategenetics.com
Pregnancy Rates with Embryos Vitrified in 0.25-mL Straws

George E. SEIDEL, Jr. and David J. WALKER

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Abstract. Vitrification is an effective approach to cryopreserving preimplantation bovine and equine embryos. Dozens of protocols have been published that differ in types and concentrations of cryoprotectants, timing of procedures, and the nature of the vitrification container. However, only a few studies have involved transferring the hundreds of embryos per treatment needed for thorough evaluation of pregnancy rates. Here we present a vitrification procedure designed to be practical under field conditions. Cryoprotectant is added to embryos in two steps, and embryos are vitrified in 0.25-mL straws. Diluent containing 1 M galactose is aspirated into 0.25-mL straws, then air, and then vitrification solution and embryos followed by air and more diluent. These columns are mixed post-warming so that embryos can be directly transferred nonsurgically into recipients. Both vitrification solutions and diluent contain no animal products. Promising pregnancy rates have been obtained with both Bos taurus and Bos indicus embryos, but larger scale testing under field conditions is needed.

Key words: Bovine, Cryoprotectant, Embryo, Vitrification


U

itrification is an approach to cryopreservation with a number of theoretical advantages and disadvantages compared to conventional freezing of embryos. Characteristics of vitrification have been reviewed numerous times, including a recent, thorough review by Vajta and Nagy [1]. The main comparative advantages of vitrification are its low cost, simplicity, rapidity, and lack of ice crystal formation. The main disadvantages are exposure of cells to very high concentrations of cryoprotectants and the non-equilibrium nature of the process, which requires precise control of timing and other aspects of some steps. There are tradeoffs; for example, the cryoprotectant concentration can be lowered by increasing cooling and warming rates and perhaps independently by using very small volumes [1]: To keep cryoprotectant concentrations as low as possible, a plethora of approaches and devices have been developed to speed cooling and warming rates, including open-pulled straws, cryoloops, cryotop, etc. [1]. Approaches using these methods have been quite successful, particularly in experimental situations. They also have been used with excellent success in some commercial contexts, (e.g. Xu et al. [2]). However, vitrification is not in widespread use commercially; for example, less than 0.2% of bovine embryos cryopreserved commercially in the United States in 2005 were vitrified [3]. Part of the reason for this low rate of use is that conventional freezing procedures for embryos have become a mature, easy to use, robust technology.

The conventional freezing process requires about 1 hour, mostly due to the requirement of cooling in the range of 0.5 C/min from about −6 C to about −30 C. This usually is done via microprocessor-controlled equipment, but can be done by simple approaches such as carefully supervised lowering of freezing containers into the gas phase of liquid

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nitrogen storage tanks. While cooling slowly is a nuisance, we have learned to live with it. The huge advantage of conventional cryopreservation of bovine embryos with ethylene glycol as the cryoprotectant comes with thawing in the field, which requires about 30 sec and a simple water bath, plus the embryo is ready to transfer without the need for a microscope to change containers. This works particularly well in cattle and horses because nonsurgical embryo transfer procedures on the farm fit together with simplicity and rapidity of preparing embryos for transfer. Using glycerol as the cryoprotectant and 0.5 to 1.1 M sucrose for an in-straw dilution is an alternative procedure for conventional cryopreservation that also allows direct transfer [4].

There is ample proof that very rapid cooling and warming rates are beneficial when vitrifying in vitro-produced embryos and especially oocytes [1], which is why devices such as the open-pulled straw have been developed. However, very few studies (e.g. Park et al. [5]) have compared these systems with 0.25-ml straws as the vitrifying container. Recently, a large study examined vitrification using a cold metal surface for embryos produced in vitro with sexed sperm [2]. Transfer of these embryos yielded pregnancy rates similar to fresh, unsexed, in vitro-produced embryos and not much lower than in vivo-produced embryos frozen conventionally, 40.9, 41.9, and 53.1%, respectively. Although systems for very rapid cooling rates have yielded high in vitro survival rates and excellent pregnancy rates, essentially all of these approaches require subsequent transfer into a different container, usually the 0.25-ml plastic straw, for nonsurgical transfer, which not only takes time, but also a skilled technician and a microscope. This is not much of an issue if a laboratory is nearby, or if huge numbers of embryos are being transferred. However, these requirements are a nuisance for the on-farm environment in which the vast majority of embryos are transferred directly using 0.25-ml straws. In this review, we will limit consideration to studies in which embryos were vitrified in 0.25-ml straws and for which pregnancy rates are available.

Review of Studies with Cattle

The published studies with in-straw vitrification of bovine embryos are summarized in Table 1. The majority of these studies involved sequential dilution of cryoprotectant after warming, and therefore do not meet the requirements of direct transfer. However, they do show that pregnancies can be obtained using conventional 0.25-ml plastic straws as the vitrification container. The first of these studies [6] resulted in similar pregnancy rates when in vitro-produced embryos were vitrified in ethylene glycol or transferred fresh. Most of the studies show promising results (Table 1) with small numbers of embryo transfers, although the experiments were done with vast differences in vitrification protocols.

In addition to studies in which embryos were thawed and exposed to sequential dilution, there are six published studies (Table 1) in which vitrified embryos were subjected to in-straw dilution and direct nonsurgical transfer. As with sequential dilution, these studies produced encouraging results. The most thorough study (van Wagendonk-de Leeuw et al. [7]) was done rigorously and is very convincing that pregnancy rates after vitrification in 0.25-ml straws were essentially identical to those with conventional methods of freezing embryos. They exposed embryos to glycerol in three steps before holding straws in liquid nitrogen vapor for vitrification and then immersion into liquid nitrogen. Straws were thawed in air for 10 sec and then 20°C water until ice disappeared. Sucrose diluent (1 M) was mixed with the vitrification solution by shaking straws to allow in-straw dilution. Embryos were then transferred nonsurgically into synchronized recipients. The pregnancy rate with vitrified embryos was 44.5% (N=393), while conventional freezing with glycerol and sequential dilution upon thawing resulted in a pregnancy rate of 45.1% (N=335).

Recent Studies with Vitrified Bovine Embryos

We have done several studies [8, 9] built on the foundation of work cited in Table 1 and the work of others [10, 11] that have been designed to make vitrifying bovine embryos as simple and efficacious as possible. The procedure developed is similar to that of van Wagendonk-de Leeuw et al. [7], but with several important modifications. First, we used ethylene glycol instead of glycerol as the
### Table 1. Summary of published studies in which embryos were transferred following vitrification in 0.25-mL straws.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment</th>
<th>Cryoprotectant</th>
<th>Dilution</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tachikawa et al. 1993 [6]</td>
<td>Vitrified IVP</td>
<td>40% glycerol</td>
<td>Sequential Dilution</td>
<td>13% (1/8)₄</td>
</tr>
<tr>
<td></td>
<td>Vitrified IVP</td>
<td>50% ethylene glycol</td>
<td></td>
<td>25% (5/20)₄</td>
</tr>
<tr>
<td></td>
<td>Fresh IVP</td>
<td>-</td>
<td></td>
<td>23% (5/22)₄</td>
</tr>
<tr>
<td>van Wagendonk-De Leeuw et al. 1995 [18]</td>
<td>Vitrified In Vivo</td>
<td>46% glycerol</td>
<td>In Straw Dilution</td>
<td>43% (17/40)</td>
</tr>
<tr>
<td></td>
<td>Vitrified In Vivo</td>
<td>25% glycerol/25%propanediol</td>
<td>In Straw Dilution</td>
<td>24% (5/21)</td>
</tr>
<tr>
<td></td>
<td>Conventional In Vivo</td>
<td>9% glycerol</td>
<td>Sequential Dilution</td>
<td>59% (20/34)</td>
</tr>
<tr>
<td>Saha et al. 1996 [19]</td>
<td>Vitrified In Vivo</td>
<td>40% ethylene glycol</td>
<td>In Straw Dilution</td>
<td>60% (3/5)</td>
</tr>
<tr>
<td>van Wagendonk-De Leeuw et al. 1997 [7]</td>
<td>Vitrified In Vivo</td>
<td>48% glycerol</td>
<td>In Straw Dilution</td>
<td>44.5% (174/393)</td>
</tr>
<tr>
<td></td>
<td>Conventional In Vivo</td>
<td>10% glycerol</td>
<td>Sequential Dilution</td>
<td>45.1% (151/335)</td>
</tr>
<tr>
<td>Agca et al. 1998 [21]</td>
<td>Biopsied Vitrified IVP*</td>
<td>25% glycerol/25% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>44% (7/16)</td>
</tr>
<tr>
<td></td>
<td>Biopsied Conventional IVP</td>
<td>11% glycerol</td>
<td>Sequential Dilution</td>
<td>23% (3/13)</td>
</tr>
<tr>
<td></td>
<td>Biopsied Fresh IVP</td>
<td>-</td>
<td></td>
<td>50% (7/14)</td>
</tr>
<tr>
<td>Agca et al. 1998* [22]</td>
<td>Vitrified IVP</td>
<td>25% glycerol/25% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>38% (12/32)</td>
</tr>
<tr>
<td></td>
<td>Conventional IVP</td>
<td>10% glycerol</td>
<td>Sequential Dilution</td>
<td>26% (9/34)</td>
</tr>
<tr>
<td></td>
<td>Fresh Day 6 IVP</td>
<td>-</td>
<td></td>
<td>59% (47/80)</td>
</tr>
<tr>
<td></td>
<td>Fresh Day 7 IVP</td>
<td>-</td>
<td></td>
<td>54% (38/70)</td>
</tr>
<tr>
<td>Donnay et al. 1998 [23]</td>
<td>Vitrified IVP</td>
<td>25% glycerol/25% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>9% (1/11)</td>
</tr>
<tr>
<td></td>
<td>Vitrified IVP</td>
<td>10%glycerol/40% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>0% (0/11)</td>
</tr>
<tr>
<td></td>
<td>Vitrified IVP</td>
<td>25% glycerol/25%ethylene glycol</td>
<td>Sequential Dilution</td>
<td>44% (7/16)</td>
</tr>
<tr>
<td></td>
<td>Fresh IVP</td>
<td>-</td>
<td></td>
<td>36% (5/14)</td>
</tr>
<tr>
<td>Pugh et al. 2000 [25]</td>
<td>Vitrified IVP</td>
<td>20% ethylene glycol/20% DMSO/10% 1,3-butanediol</td>
<td>In Straw Dilution</td>
<td>22% (17/76)</td>
</tr>
<tr>
<td></td>
<td>Fresh IVP</td>
<td>-</td>
<td></td>
<td>22% (5/22)</td>
</tr>
<tr>
<td>Al-Katanani et al. 2002 [26]</td>
<td>Vitrified IVP / timed transfer</td>
<td>25% glycerol/25% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>6.5% (3/54)</td>
</tr>
<tr>
<td></td>
<td>Fresh IVP / timed transfer</td>
<td>-</td>
<td></td>
<td>19% (6/33)</td>
</tr>
<tr>
<td>Martinez et al. 2002 [27]</td>
<td>IVP Vitrified in 0.1 M sucrose</td>
<td>25%glycerol/25% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>50% (20/40)</td>
</tr>
<tr>
<td></td>
<td>IVP Vitrified in 0.3 M sucrose</td>
<td>25% glycerol/25% ethylene glycol</td>
<td>Sequential Dilution</td>
<td>40% (16/40)</td>
</tr>
<tr>
<td></td>
<td>Fresh In Vivo</td>
<td>-</td>
<td></td>
<td>65% (26/40)</td>
</tr>
<tr>
<td></td>
<td>Fresh IVP</td>
<td>-</td>
<td></td>
<td>51.4% (18/35)</td>
</tr>
<tr>
<td>Nedambale et al. 2004 [28]</td>
<td>Vitrified IVP</td>
<td>48% glycerol</td>
<td>Sequential Dilution</td>
<td>30% (3/10)</td>
</tr>
<tr>
<td>Wurth et al. 2004 [17]</td>
<td>Vitrified IVP</td>
<td>48% glycerol</td>
<td>In Straw Dilution</td>
<td>24% (20/85)</td>
</tr>
<tr>
<td></td>
<td>Conventional IVP</td>
<td>9% glycerol</td>
<td>Sequential Dilution</td>
<td>14% (5/35)</td>
</tr>
<tr>
<td></td>
<td>Fresh IVP</td>
<td>-</td>
<td></td>
<td>42% (51/121)</td>
</tr>
</tbody>
</table>

*Two embryos transferred/recipient; one embryo transferred ipsilateral and one contralateral to corpus luteum. Data are reported as pregnancies/embryo transferred.

₄Two embryos transferred/recipient; data are number of live calves born/embryo transferred.

* IVP, in vitro-produced embryos.
cryoprotectant because it is the more permeable cryoprotectant for bovine embryos. Second, cryoprotectant is added in only two steps, 3 min in 5 M ethylene glycol and 45 sec in 7 M ethylene glycol. Third, we have used galactose instead of sucrose for in-straw dilution of cryoprotectant after warming because of the lower viscosity of that monosaccharide. Fourth, we have developed media that contain no animal-derived products, primarily by replacing serum or albumin with polyvinyl alcohol, which has the advantage of chemically defined consistency and freedom from possible viral contamination.

To develop a practical vitrification procedure, we dealt with issues of labeling, preventing straws from cracking, and preventing embryos from sticking to the straws when using chemically defined components. We also have used a commercially available base medium to simplify procedures. Most of our work on developing these procedures used in vitro-produced blastocysts as the model, but we also have tested procedures with in vivo-produced embryos, which incidentally appear to be more robust than in vitro-produced embryos, and survive vitrification procedures very well [8].

Initial attempts to apply our laboratory-tested procedure into the field revealed weaknesses in the procedure. For instance, practitioners had trouble with damaging straws during direct plunge of straws into liquid nitrogen, and we originally replaced BSA with hyaluronan for synthetic media, resulting in viscous media and sticking of embryos in straws. We have continually worked to refine the procedure to make it more user-friendly. Initial pregnancy rates following transfer of in vivo-produced Bos taurus embryos were lower than expected with average pregnancy rates at two sites of 22% (21/95). These poor results were in part due to the procedural problems discussed above. Recent, unpublished pregnancy rates using the modified procedure for in vivo-produced Bos indicus embryos at two sites have been encouraging, resulting in an average pregnancy rate of 43% (21/49); only five Bos taurus embryos were transferred resulting in three pregnancies.

Our currently recommended procedure is as follows: Syngro® (AB Technology, Pullman, WA) + 0.1% additional PVA is used as the base medium. First, expose embryos to 5 M ethylene glycol in base medium for 3 min. Then transfer embryos in 1 μL or less into a 15-μL drop of vitrification solution (base medium with 7 M EG + 18% w/v Ficoll 70 + 0.5 M galactose) for 45 sec. During exposure to vitrification solution, a column of diluent (1 M galactose in base medium) is aspirated followed by air, more diluent, air, vitrification solution plus the embryo, and diluent, leaving an air space at the end of the straw (Fig. 1). Straws are then plugged. Labeled 1/2 mL straws are flattened and placed over the cotton plug, and after 45 sec the straw is placed embryo down into a plastic goblet previously placed into liquid nitrogen (goblet is not completely submerged into liquid nitrogen) (Fig. 2). Straws are held in vapor for 1 to 3 min and then plunged into liquid nitrogen. Straws are thawed in air for 8 sec, and then in 37 C water for 15 sec, held at the cotton-plugged end and shaken 4 times like a clinical thermometer to mix diluent and vitrification solution. Embryos can be held at ambient temperature or in 37 C water after mixing until transfer, which should be done between 5 and 10 min after mixing.

Studies with Equine Embryos

Analogous procedures have been developed for equine embryos that have many of the same features as the bovine system described, but also some important differences [12–15]. We will not review these procedures in detail, but point out that...
a mixture of glycerol and ethylene glycol is used as the cryoprotectant, and that as for conventional freezing of equine embryos, results are poor for blastocysts ≥300 μM in diameter. However, for the smaller blastocysts, published pregnancy rates have been around 60% (15/26 (58%)) [12]; 5/7 (77%) [13]; 28/40 (70%) [14]; 6/11 (55%) [15]). When implementing a vitrification procedure, it is important to follow procedures in detail. In part due to the non-equilibrium nature of vitrification procedures, deviations in timing and other aspects are not well tolerated.

Conclusions

An increased interest in transferring in vitro-produced bovine embryos was observed in 2005 [16]. With increased production of embryos in vitro, an efficient cryopreservation method will be demanded. Vitrification of in vitro- or in vivo-produced bovine embryos results in acceptable pregnancy rates, and vitrification may be more appropriate for in vitro-produced embryos [17]. While it is easy to implement complex vitrification procedures in a laboratory setting, practical, user-friendly procedures, particularly after warming, would be attractive if they are reasonably robust, and pregnancy rates are not compromised. Vitrification in 0.25-mL straws coupled with in-straw dilution techniques would facilitate broader application of vitrification as an alternative to conventional cryopreservation. The procedure that we have developed appears promising, but needs additional testing under field conditions. To date, only van Wagendonk de Leeuw et al. [7] have convincingly shown that vitrification plus in-straw dilution in 0.25-mL straws can be used commercially with similar pregnancy rates to conventional cryopreservation. The recent study by Xu et al. [2] convincingly showed the potential for vitrification of in vitro-produced bovine embryos with rapid cooling devices before transfer, and placing the embryos into straws post-warming. Vitrification is not likely to replace conventional methods for freezing in vivo-produce bovine embryos in the near future, primarily because processing and loading straws or other devices with single embryos becomes time consuming when large numbers are done at one time. However, vitrification is much simpler and faster than conventional procedures if only a few embryos need to be cryopreserved at a time, which happens frequently with bovine embryos and almost always with equine embryos. In addition, the cost of equipment is very low, which is attractive for persons cryopreserving limited numbers of embryos.

Acknowledgements

Numerous students and colleagues have contributed to these studies. We especially acknowledge the technical assistance of Zell Brink. We appreciate sharing of unpublished results from Alta Genetics (Brian Shea), Ovagenix (Charles Looney), Wesley Larkin, and Webb ET Services (Jimmy Webb). This research was funded in part by Bioniche Life Sciences and in part by USDA Regional Project W-1171.

References

7. van Wagendonk de Leeuw AM, den Daas JHG,


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Successful superovulation of cattle by a single administration of FSH in aluminum hydroxide gel

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Abstract
We investigated whether Al-gel could adsorb and release FSH effectively in vitro and in vivo, and whether a single administration of FSH in Al-gel could successfully induce superovulation (SOV) in cattle. Porcine FSH (pFSH; 30 mg) was mixed with 5 mL of Al-gel; 99.98 ± 0.01% of pFSH was adsorbed by the gel and 71.6 ± 1.1% of the adsorbed pFSH was subsequently released in the presence of BSA. In cattle given a single i.m. treatment of 30 mg of pFSH in 5 mL of Al-gel, the numbers of CL, total ova recovered, and transferable embryos per cow were not significantly different from conventional (twice daily for 4 d) pFSH treatment (12.3 ± 1.7 versus 11.7 ± 1.8, 10.0 ± 2.5 versus 9.3 ± 1.7, and 8.6 ± 2.3 versus 8.0 ± 1.8, respectively, mean ± S.E.M.); plasma pFSH concentrations were increased for 4 d, indicating sustained release from the Al-gel. Five cows were given 30 mg pFSH in 5 mL of Al-gel i.m. on five occasions (once every 2–3 months); there was no significant difference among treatments for the number of CL (12.4 ± 3.8, 13.8 ± 4.8, 9.0 ± 1.9, 9.8 ± 3.0, 12.0 ± 2.1), total ova recovered (12.0 ± 3.8, 12.6 ± 5.1, 6.8 ± 1.9, 7.6 ± 1.8, 11.4 ± 2.5), and transferable embryos (11.4 ± 3.9, 10.4 ± 5.8, 6.6 ± 2.1, 4.8 ± 1.4, 10.4 ± 2.6). In conclusion, a single i.m. treatment of 30 mg pFSH in 5 mL Al-gel effectively induced SOV in cattle.

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