RESEARCH UPDATE IN ARTIFICIAL INSEMINATION
AND SEMEN PROCESSING
Michael Kaproth, Ph. D.
Genex Cooperative/ Cooperative Resources International
Email: mkaproth@crinet.com

INTRODUCTION

Giving updates on research efforts in our industry is an activity which provides a view of the future. While valuable, this is best when put in perspective to where we are now, and where we have been. In 1976, fresh from my graduate program in Minnesota with Professor E.F. Graham, I entered the artificial insemination (AI) industry at Minnesota Valley Breeders Association (MVBA) in New Prague, MN. The Cryobiology Lab at Minnesota, representing the quality and depth seen at many US universities at that time, well prepared groups of new graduate students, many of whom found their way into Andrology and AI.

PERSPECTIVE

What did we new graduates find (1976)?

Then: Post-thaw semen quality. Mean values for post-thaw semen quality were relatively modest, in the range of 40 to 50 % post-thaw motility (warm water thaw) and 35 to 45 % post-thaw motility (cold water thaw).

Then: Straw package. The customer didn’t get a uniform product. In the US, versions of 0.5 mL straws and the ampule divided the market. The 0.25 mL straw package was only available for rare imported supplies of continental beef breeds. Eastern AI sent 0.5 mL straws into the field in bottom-only cane storage. Midwest Breeders processed in both 0.5 mL straws and the unique 0.75 mL Magic Wand, which combined the AI gun and cryopreserved semen in one package. Pelleted frozen sperm was used internationally. Straw freezing was done in large open tanks with static or moving liquid nitrogen (LN) vapor, and in laminar flow freezers at ABS and Atlantic Breeders.

Then: Semen processing. Semen extenders varied, included many homemade formulas for egg yolk citrate, and the newly popular egg yolk tris and TEST formulas. Whole milk extender was used at several AI centers in the US, with skim milk-egg yolk used abroad. Commercial extenders, including the popular Plus-X extender, featured both one-step and two-step versions. Quality control (QC) assays included stop-motion photography, morphology, metabolism, and acrosomes. But often pre- and post-thaw motility alone determined the acceptability of a straw batch.

Then: Antibiotics and organisms. Certified Semen Services® (CSS, Columbia, MO) had not yet taken on antibiotic cocktail effectiveness. Penicillin and streptomycin controlled the easy-to-control microorganisms; but mycoplasmas, ureaplasmas, (and in one-step extenders, campylobacter fetus) were not being effectively controlled. To address this, minocin, polymixin, and linconspectin were being added at individual AI centers, according to their preferences and in-house research outcomes. Some AI centers
vaccinated bulls for IBR virus; other centers tested and removed positive bulls.

**Then: AI service.** Professional inseminators generally provided twice daily AI service, according to the AM/PM rule, responding to breeding request calls placed at their home or local dispatch office. Herdsmen inseminators seldom had extensive training or experience. Using a thermos, ampules were thawed in ice water, and straws in body temperature water. Fertility was considered excellent, likely due to careful observation of signs of estrus and modest milk production. Only first services were calculated for research, the service associated with optimal fertility, generally following Dr. Trimberger’s rule for a 60-d voluntary waiting period (VWP). Eastern AI introduced straws for AI whose semen processing allowed pocket thawing to be an alternative to warm water thawing of straws.

**Then: Research topics in this era.** New procedures were developed for handling semen in these new straw packages. The low-density lipoproteins (LDL) fraction of egg yolk and protein fraction of milk were identified as effective components of sperm protection in cryoprotection. Uterine factors responsible for sperm capacitation were utilized to achieve successful in vitro fertilization. Daily milk progesterone testing was found to be able to determine the estrus and pregnancy status.

Cooperating AI Centers and academics introduced the CSS antibiotic cocktail, enabling today’s effective control of microorganisms. Extensive isolation and testing procedures enabled the permanent status of AI centers as negative for viruses of interest. Careful records and trials revealed that once-daily AI service was equivalent to twice-daily AI service. Sorting sperm for gender used technologies based on differences in specific gravity and swimming rates.

**Where are we today?**

**Now: Post-thaw semen quality.** Mean values for post-thaw semen quality have been elevated by about 10 percentage points compared to values seen earlier. This is a remarkable achievement. Correspondingly, minimum values for acceptable semen quality also have been raised.

**Now: Straw package.** Packaging settled on straws, with 0.25 and 0.50 mL featured. Straws are available as goblets of 0.25 mL straws intended for tank-bottom storage, 0.25 mL and 0.5 mL straws distributed in upper and lower goblets per cane, or straws frozen in modified aluminum canes which also serve as goblets.

**Now: Semen processing.** Some extenders feature only non-animal products, using soybean phospholipids. Extenders can also feature supplements such as antioxidants. Extenders featuring LDL components are available in limited amounts. Milk extenders continue to be utilized. The flow cytometric sorting of semen to provide offspring of desired gender (sorted semen) is now a large part of our industry. New assays for sperm function and quality are utilized: Computer assisted semen analysis (CASA), sperm counting instruments (e.g. Nucleocounter™), and flow cytometry to determine cell function and chromatin integrity.

**Now: AI service.** Inseminators generally provide once daily AI service, and in synchronized herds, teams respond to timed AI by breeding large numbers in a short time. Inseminators can be employees of AI companies or herdsmen with extensive experience. Synchronization enables
effectiveness of AI where signs of estrus are not strong. Genex’s semen processing, allowing flexible thawing (pocket thawing, warm water thawing), continues to obtain breeding outcomes indicating these methods are highly effective, and equivalent.

Fertility in heifers continues to be excellent; however inseminations in cows, likely due to high milk production, and lack of estrous expression achieve mediocre outcomes with indications that up to 30% of fertilized eggs fail to develop. Voluntary waiting periods have been shortened and frequently greater fertility is associated with inseminations after the first service. National systems for estimating Sire Conception Rate (SCR) and Daughter Pregnancy Rates (DPR) are utilized as tools in sire selection. Genomic evaluations are bringing bulls into active service much earlier in life, and allow quick removal of sires with undesirable genetic traits.

**Introduction to today’s research.** The foregoing introduces the topic of current research topics in the context that a great deal has been accomplished.

**RESEARCH IN PROCESSING AND PACKAGING**

**Semen freezers**

Of great interest are advances in the area of straw freezers. We know that sperm are not now being frozen at optimum rates, due to inexactness of controlling the freezing curves for individual straws and groups of straws, and the physical geometry of the straw.

**Turbo freezer.** A newly developed (MiniTube of America, Inc., Verona, WI) straw freezer cools racked straws from end-to-end (crimped tip to cotton plug) with a horizontal laminar flow of nitrogen vapor, allowing a precise freeze rate in individual straws. Older static freezers have variable rates and offer little control to correct for stresses such as the heat of fusion. Most conventional programmable freezers will freeze stacks of horizontally racked straws from top down, resulting in a range of slightly differing freeze rates for individual straws. With more precise controls, we can freeze at optimal rates and apply interventions to mitigate stresses and limit cryopreservation damage.

**Rotating drum freezer.** A newly developed (IMV Technologies U.S.A., Maple Grove, MN) straw freezer uses a rotating drum where a straw batch is precisely frozen while tumbling, ensuring sperm will stay suspended within the straw prior to freezing. In effect, this raises the bar one more notch in preciseness of controlling the freezing rate. Early comparisons of similarly prepared straws frozen conventionally or in the rotating drum showed post-thaw motility increased by 4.2%. (Camus personal communication, 2012).

**Directional freezer.** A type of directional freezer utilizing laminar flow of cooling vapor has been used since introduced in the 1960s by Jondet. Now directional freezing is used, producing a uniform wave of ice formation sweeping along the straw in a manner that protects the sperm contained within from a specific type of ice damage which occurs when ice crystals are randomly produced (Arav and Natan, 2012). At Cogent AI Center (UK) straws (0.25 mL) of sorted sperm frozen in this process (Harmony Freezing™, IMT International, Chester, UK) are described as being of higher quality on thawing. Hayakawa et al. (2007) evaluated the use of the directional freezer for sorted sperm in
0.5 mL straws. Directional freezing showed higher viability and acrosomal integrity than the control; however, there was no effect on motility and progressive motility.

**Encapsulation**

Recent news reports say that there now is time release semen for cattle AI. As background, Nebel and Saacke (2004) reported that bovine sperm (nonfrozen) quality was maintained over several days in capsules placed in the uterus. However, they reported these capsules were not retained in the cow. No mechanism initiated a coordinated release of sperm with respect to ovulation; rather, mechanical disturbances released sperm.

*Patented encapsulation.* A recent patent (US Patent 8,178,130) and research report (Otten, 2012) disclosed methods, in swine, of sperm encapsulation that have been developed which permits prolonged storage of highly concentrated cell numbers (SpermVital™, Geno Global Ltd., Hamar, Norway). This system allows, for nonfrozen sperm, reasonable numbers of cells to be placed in females up to a half day earlier than conventional AI. It was not clear whether the system was extensively tested with cryopreserved capsules of sperm, or mechanisms ensuring encapsulated sperm would be released at a time coordinated with ovulation, nor whether capsules were retained or lost over time.

*Swiss AI encapsulation.* Swiss Genetics reported an encapsulation effort which combines a cellulose capsule with an integrated cellulase enzyme (Webera et al., 2006). The activated enzyme will release sperm. They added a receptor for LH (ovulation-associated hormone) linked to a trigger which activates the cellulose enzyme. In this preliminary report, recovered sperm quality was unimpressive, but evident; survival of the capsule however appeared to be uneven.

### RESEARCH IN SEMEN ANALYSIS

There are several roles for advanced semen analysis.

- *First* is to confirm by means of objective testing that everyday semen processing and routine QC procedures are in control and functioning as planned.
- *Second* is to identify and remove semen batches which should not be used.
- *Third* is to identify bulls which should not be used (candidates for culling).
- *Fourth* is to identify those bulls who have both superior genetics and whose semen can safely be more greatly extended.

### Description of flow cytometric assays

As flow sorting continues to be developed, the AI industry will use these tools to efficiently evaluate varied physiological parameters of sperm. Large numbers of cells (2000 to 5000) are counted in less than 3 min.

- *Viability (Live/Dead) assays:* This assay tests plasma membrane integrity using 2 fluorescent stains: Sybr 14 and propidium iodide. The Sybr 14 penetrates all of the cells and binds with nucleic acids. The propidium iodide cannot pass through an intact plasma membrane but can pass through a damaged membrane.
- *Plasma integrity and acrosome membrane integrity:* This assesses
plasma membrane integrity plus acrosome membrane integrity. For this, PNA-FITC is used along with propidium iodide. The PNA-FITC binds with damaged acrosomal membranes, propidium iodide passes through cells with damaged plasma membranes.

- **Cell counting**: To ensure quality with respect to number of cells per straw.
- **Mitochondrial membrane potential**: This is a measure for normal polarization, indicating membranes are intact and functional. This is taken to be an observation thought to be closely associated to sperm motility.
- **Lipid peroxidation** (merocyanin): To measure the organization of the lipid membrane.
- **Sperm chromatin structure assay (SCSA)** and **terminal transferase dUTP nick end labeling (TUNEL)**: These are measurements of the integrity of the DNA.

**Known issues**: Egg yolk and milk extenders have particles, and molecules with potential for autofluorescence. In assay development, an extender may need to be validated by the addition of a DNA-specific stain to properly identify the cell population. Some lectins, as used in acrosome testing, bind to native molecules of milk. These assays cannot be used with milk processed straws without making stringent assay modifications. Centrifuging thawed samples to remove extender components can compromise sample integrity. In many complicated protocols, elapsed time between sample thawing and acquisition of assay outcomes and washing procedures may reduce the power of the assay to accurately describe sperm quality.

**Sperm assays and induced acrosomal function: correlation to fertility**

Christensen et al. (2011) measured sperm motility, morphology, viability, DNA integrity, flow cytometric measures, and induced acrosome response in a fertility trial (195 bulls, 2 - 15 million sperm/straw, 75,000 AI outcomes. The semen was produced by very young bulls. The concentration of sperm in raw semen and its prefreeze viability, and post-thaw viability produced a perfect model for predicting fertility. Other measures of semen quality: acrosome response, sperm chromatin assay (DNA), and morphology were individually useful but did not provide additional predictive value past the above measures. Fertility outcomes using doses at 15 million sperm bore no relationship to outcomes at 2 million sperm.

**Flow sorting assays and their correlation to fertility**

In a recent study by Sellem et al. (2012), a similar project was undertaken. In this study, flow cytometric measures of acrosome integrity, oxidative damage, mitochondrial activity, chromatin integrity were combined with sperm motility values as measured by computer (CASA) and sperm morphology values. There were 50,000 AI outcomes. While measures of individual quality factors had low correlations with fertility, 2 models were found with high correlations with the fertility values. Correlation values reached as high as 0.69 for models which included all parameters.

**Semen analysis by CASA**

Historically, semen quality has largely been determined by the percentage of cells that are progressively motile. Methods to
determine this include subjective microscopic evaluation by a trained observer and more recently the use of CASA. Computer assisted semen analysis provides estimates of progressive and total motility; however these measures are still subjective due to their dependence on the choice of parameter values entered by the user. For that reason, lab-to-lab outcomes can be variable. Further, evaluation of sperm in extenders with particles (nearly all extenders) requires strobe uv light illumination of semen samples following sperm DNA staining with Hoechst. Most commonly in our industry, this is termed an IVOS evaluation.

CASA measurements. Multiple kinematic parameters are reported. These typically include 3 different velocity measures:

1. VCL is the point-to-point curvilinear velocity (actual path of the selected track).
2. VSL is the straight line velocity measured along the shortest distance between the beginning of the track and the end of the track.
3. VAP is the average cell path velocity.

Ratios include straightness (STR = VSL/VAP) and linearity (LIN = VSL/VCL). Additionally beat cross frequency (the number of times per second that the cell track crosses the cell path) and amplitude of the head movement across the path (ALH) are also measured.

There is substantial literature that ejaculates consist of a heterogeneous population of sperm cells that allows for the coexistence of different functional groups of spermatozoa in the ejaculate, and in the inseminate. CASA can track this heterogeneity as distinct sperm cell subpopulations, when user-determined cutoff points within the kinematic parameters define cell subpopulations of interest. There are as yet no reports combining subpopulation structure with measures of semen quality or fertility.

Sp100 Nucleocounter for verifying sperm numbers

The Nucleocounter is a widely used method for establishing sperm cells per mL or per straw in processed semen, and sperm cells per mL in neat semen (unextended), and to communicate these outcomes between labs. The instrument does not directly count sperm. The instrument works by staining the cell nucleus with a Hoechst stain in buffer; the mixed sperm/stain is pipetted into the instrument using a disposable cassette with a microfluidic channel and flows to the sampling chamber, where the stained sperm DNA will fluoresce under UV light. The instrument measures the total intensity of light and by an algorithm calculates a likely sperm per mL value consistent with prior experimental outcomes.

Known issues: The assay is subject to operator error during initial dilution of sperm with counting fluid, and judgment as to the dilution ratio. In an extensive comparison of counting methods, including the hemacytometer, Anzar et al. (2009) reported the Nucleocounter values agreed with the flow cytometer; but returned larger sperm concentration values than was measured by hemacytometer when the values came from concentrated semen. The reason for this is unknown. Kuster and Althouse (2010) provided an insight on instruments which use algorithms to correct for values resulting from cells travelling in microfluidic channels (CASA and
Nucleocounter cassettes and microfluidic slides as examples). An effect of cell distribution as a function of distance along the path has been determined. The Segre-Silberberg effect, when uncorrected for, introduces cell counting errors of up to 30%. Its values change according to sample viscosity, surface tension, pathway depth, cell size, and distance along the path. From these studies it seems reasonable to utilize more than one method for counting sperm.

**Detecting a sperm biomarker for good fertility and semen quality**

Kennedy (2011), in Sutovsky’s lab, has identified a potential sperm protein associated with good fertility and semen quality. This protein is a component of the sperm’s developing acrosome (post-acrosomal WW-domain binding protein, PAWP, located in the post-acrosomal sheath, PAS). The PAWP protein is likely released by the sperm into the oocyte during fertilization, and perhaps plays a role in oocyte activation. The hypothesis is that integration of this protein in sperm PAS is reflective of bulls’ sperm quality and fertility. Flow cytometric measurements found correlations between sperm PAWP levels and conventional semen and fertility parameters, indicating it is possible to use a PAWP-assay to determine acceptable PAWP content in individual sires.

**Relationship of sperm head shape to fertility**

Parrish et al. (2006) reported a series of innovative efforts to use an application of image analysis software to images of thawed bovine sperm head outlines. From these observations, important parameters are derived which then are included in modeling to predict sire fertility. Parrish’s group utilized image analysis of the perimeter of fluorescently stained sperm head. They obtained Fourier harmonic amplitudes which were then modeled against fertility (210 sires). The Low Fertility group was set at -1.77 (32 sires, as ERCR). Testing included total motility, progressive motility, sperm head abnormalities, SCSA, staining intensity, and Fourier harmonic analysis. One model containing all factors correctly placed all 32 sires belonging to the Low Fertility group. When head shape analysis is combined with additional semen quality tests, as Annexin V expression, TUNEL, and mitochondrial function, we are now able to detect and remove those ejaculates with heat stress damage, undetectable otherwise, and expected to have reduced fertility (Parrish, 2012).

**CRYOBIOLGY**

In both of the studies following, with low density lipoprotein (LDL)/cholesterol, and cyclodextrin/cholesterol, cryopreserved semen was produced with altered cholesterol to phospholipid ratios. I expect that changes in the cholesterol to phospholipid ratios will also alter the in vivo performance of the sperm with respect to timing of AI and its relationship to ovulation.

**LDL production**

These studies (Bailey, 2010; Parks, 2010) are representative of the current field: utilizing combinations of LDL egg yolk fractions, cholesterol as a means to enhance delivery of phospholipids to the sperm cell and modify phospholipid to cholesterol ratios in the sperm membrane. These studies evaluated the effectiveness of egg yolk and its LDL component as bull semen extender materials as it interacts with additional cholesterol. Post-thaw sperm survival was estimated with CASA and flow cytometric estimations of mitochondrial function. Use of these materials together in
cryopreservation improved freezability as measured by CASA beyond either material by itself, or for samples frozen with control extenders. The combinations however did not improve mitochondrial function. The significance of these efforts are the opportunities given in being able to choose the phospholipid species and cholesterol ratio of the membranes with a view towards improving sperm survival during cooling and subsequent freezing.

**The effect of membrane cholesterol**

In studies featuring the ram, but previously based on bulls, Moce et al. (2010) demonstrated that cholesterol can be added to sperm with cyclodextrin molecules. Cyclodextrins take up cholesterol and transport it according to concentration gradient to the sperm membrane and load it into the membrane. Cryopreservation with cholesterol loaded cyclodextrin (CLC) cells yielded thawed sperm samples exhibiting greater percentages of motile sperm compared to the control samples.

**PHYSIOLOGY OF BREEDING ANIMALS**

**Feeding bull calves for sperm production**

In a series of studies (Brito et al., 2007) the effects of bull nutrition during calfhood were studied. Early calfhood nutrition has permanent effects on GnRH pulse generator in the hypothalamus: low nutrition decreases LH secretion, whereas high nutrition increases secretion. Therefore, restricted feed during calfhood may affect the early gonadotropin rise and age at puberty in bulls, regardless of the nutrition provided after this critical period of development through the end of the study (70 wk). Can we give neonatal GnRH to get enhanced *adult testis growth*? Not likely. The authors cited earlier work by Chandolia where suppressing LH secretion with prolonged GnRH treatment during calffood was found to reduce paired testes weight at 50 wk of age.

**AMH testing to determine females with enhanced ovarian function and fertility**

A new service offered through Minitube of America, Inc. is the measurement of AMH anti-Müllerian hormone. Research suggests that circulating levels of this hormone are in direct proportion to the ovarian reserve in that female. The size of the ovarian reserve should reflect her inherent fertility. A single blood test for serum AMH is possibly a means to determine if fertility is likely to be suboptimal in young adult cattle. The female has to have had one cycle prior to measurement.

**Heat Shock Protein 70 as a component of the ejaculate changes with season and is associated with elevated semen quality**

Patterson (2011) studied samples of neat semen to determine the association of Heat Shock Protein 70 (*HSP70*, a beneficial chaperone molecule which mitigates cell stress) and records for semen quality and production across seasons. HSP70 is expressed during spermatogenesis. Bulls were grouped by their haplotypes for HSP70. These groups significantly differed with respect to maintaining sperm production and semen quality during hot months. Using HSP70 haplotypes with marker-assisted management may be beneficial in selecting sires with desirable hot climate reproductive traits.
AI PRACTICES

Best practices for using multiple straws

The current concept for using groups of straws, as reviewed by the National Association of Animal Breeders (NAAB), is that it is a safe practice to thaw and use straw groups when straws are protected from environmental factors (solar/heat/cooling) between thawing and AI, and straws are used within 10-15 min of thawing. In a new report (Oliveira et al., 2012), this topic was examined to determine reproductive outcomes for using groups of straws under tropical field conditions in Brazil. Investigators followed the usage of 3 bulls (3 batches / bull and 1000 total inseminations), thawing one cane, 10 straws, at one time. All straws were deposited in cows over a span of 1-7 min, and fertility outcomes were recorded according to the sequence.

With respect to fertility, one bull did not maintain his level of fertility for his final (9th, 10th) straws relative to his fertility for earlier straws. The other 2 bulls maintained unchanged fertility over these 7 min. Lab observations (comparing 1 min and 7 min incubation in thaw bath before measurements) were contradictory: the bull with the seeming decline in fertility maintained all semen quality parameters during incubation, while another bull maintaining high fertility throughout the AI sequence, exhibited slight declines in semen quality. The third bull did not decline in quality or fertility. The essence of the take home message from the investigators: we should be concerned about protecting thawed straws from overheating conditions during AI. Perhaps this is what happened in this case. In practice, it is not possible to predict how individual bulls’ cryopreserved semen will react to stressful environmental conditions. It will always be important to protect straws from environmental factors between thawing and AI.

Determine effectiveness of herd estrous detection by milk progesterone

A portable instrument (eProCheck™) by MiniTube of America Inc. offers an opportunity to accurately gauge a herd’s level of effectiveness in the timing of AI with respect to ovulation. For each cow inseminated, a milk sample is measured and her progesterone level is determined. Follow-up samples from the same cow taken later, reveals her status at AI, and current status:

- Her AI was too early
- Her AI was right on
- Her AI was late
- Already pregnant at first AI
- She is now open
- She is now pregnant

FATE OF SPERM IN THE COW

More is now known about the bovine sperm reservoir created in the cow following AI, populated by sperm, and becomes, at ovulation, the functional sperm population available for fertilization.

Seminal plasma proteins needed for sperm reservoir release

Inseminated sperm attached to the oviductal epithelium are the bovine sperm reservoir. In vivo, it takes more than 6 hr for bull sperm inseminated by natural service to become capacitated. Sperm undergo capacitation in order to be able to fertilize eggs. When capacitated, bull sperm begin to loosen their binding to oviductal epithelium. This loss of binding releases sperm from the storage reservoir and they can resume
The Dairy Cattle Reproduction Council does not support one product over another
and any mention herein is meant as an example, not an endorsement.

moving to the site of fertilization. Hung and Suarez (2010) described the release of sperm might be accounted for by a loss of 1 of 3 seminal plasma proteins (BSP PDC-109) which happens during capacitation. Binding is restored in capacitated sperm by adding back PDC-109 to sperm. They found that for fresh bull sperm, each BSP protein responds differently to capacitation. Why? The function of differential responses of the 3 BSP proteins may be to regulate the gradual release of sperm from the oviductal storage reservoir and direct their subsequent movement toward the egg. This is an elegant picture of how sperm behave following natural service: with sperm release triggers at differing times allowing a steady population of sperm to meet the oocyte. This group determined (Ardón and Suarez, 2012) that processing and cryopreservation changes the abundance of binding proteins associated with sperm.

Repeat breeder cows have abnormal cycles of epidermal growth factor

Katagiri et al. (2012) reported that uterine endometrial epidermal growth factor (EGF) concentrations may be a useful indicator of fertility in problems cows: exhibiting elevated EGF in fertile cows, and depressed levels in poor, or repeat breeder cows. Infusing the vagina with seminal plasma on the day of estrus restored the pattern of elevated EGF cycles seen in fertile cows. In limited fertility trials remarkable fertility improvements were seen for repeat breeder cows receiving the seminal plasma treatment. It was conjectured that elevated EGF modulates uterine cytokine levels.

EMBRYO QUALITY

An estimated 30% of fertilized oocytes do not go on to produce a successful pregnancy. Are there male-specific factors involved in establishing the quality of the resulting embryo?

Sperm factors introduced at fertilization

Within the past decade it has come to be realized that sperm contain and deliver large numbers of factors into oocytes. A sperm-borne protein, PLC ζ (zeta), initiates a cascade of calcium oscillations facilitating oocyte activation (Ito et al., 2011). Studies by Gur and Breitbart (2008), suggest that sperm cells may use stable, long-term mRNA transcripts to synthesize novel proteins. These mRNA transcripts might be used to synthesize proteins in the female reproductive tract during storage in the sperm reservoir and during capacitation. In addition, the mRNA might be transported to the egg and participate in the zygote development. Card et al. (2012) reported the first complete transcriptome of sperm mRNA. Genes involved in all phases of physiology and maturation are represented. Until the developing embryo itself begins to transcribe mRNA, it is dependent on mRNA provided by the oocyte and by the sperm. It has been conjectured that an impaired or incomplete male contribution would result in an embryo which may not be fully competent to survive through the transition to embryonic transcription.

Fetal loss due to homozygosity of genetic defects

We are seeing rapid discoveries of new recessive defects through genomic technologies. For suspect alleles, a lack of adult homozygous individuals indicates fetal loss. Five haplotypes were discovered
The Dairy Cattle Reproduction Council does not support one product over another and any mention herein is meant as an example, not an endorsement.

(VanRaden et al., 2012). Two of the founder bulls are popular sires Chief and Soldier. Mating of heterozygotes should be avoided.

Y are you not pregnant

McDaneld et al. (2012) reported detection of misplaced Y-associated DNA in beef females. Females were grouped by reproductive success, and the poor fertility group was found to have a high number of cows with Y-chromosome DNA as detected by a 700,000 SNP marker assay. The findings were confirmed by PCR, which revealed that 21 - 29 % of females were positive for Y chromosome amplicons.

CONCLUSION

We have challenges of low pregnancy rates and high rates of embryonic mortality. Herd reproductive management teams understand and use recent advances to control ovarian and uterine function. As a consequence, fertility has been partially restored. The other partners in reproductive performance, technologies such as artificial insemination and semen processing, offer improved products for AI and wider and more intelligent use of superior genetics for reproduction as well as strategies to enhance herd reproductive performance even further.

Research is improving post-thaw sperm quality, allowing us to establish functional uniformity between bulls and batches, and allowing better defined sperm function. However, at this time, additional research is needed to fully utilize genomic opportunities to select sires and dams with superior genetic makeup for reproduction and to eliminate sires that should not be used. The problem of high rates of embryonic mortality has to be further addressed through tools of molecular biology. Advances derived from these technologies will undoubtedly provide producers with additional tools to further improve the reproductive efficiency.

REFERENCES


