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Application of Technology in Male Reproduction

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The reproductive performance of an individual beef herd is extremely important and plays a major role in its financial success. Currently, most commercial beef cows and a large proportion of purebred beef cows are bred by bulls. To improve genetics within a herd, the use of artificial insemination (AI) utilizing proven purebred bulls is of utmost importance (Barth, 1993). Success with AI has been highly dependent on accurate heat detection, timing of insemination, use of high quality semen, and proper insemination technique. This objective of this note is to address how sperm functional parameters, timing of insemination, extenders influences fertility, and in addition discuss what new technology in male reproduction means to the producers.

Sperm functional parameters and fertility

Breeding soundness evaluation in bulls is performed to assess and categorize their potential breeding ability. Based on physical examination, scrotal circumference, and semen parameters, bulls are classified into three categories: satisfactory, questionable and unsatisfactory for breeding potential (Kennedy et al., 2002; Farin et al., 1989; Chenoweth et al., 1996). Color and volume of an ejaculate, and motility (gross and progressive) and morphology of sperm are the usual parameters used for semen evaluation. Among numerous variables involved in selecting a bull for natural service or cryopreservation of semen, certain crucial parameters are more indicative of semen quality. In field conditions, evaluation of motility and morphology of a semen sample are common methods for estimating breeding potential (Correa et al., 1997; Januskauskas et al., 1996). However, the usefulness of these parameters to accurately measure fertility of a semen sample is limited. The ultimate goal of semen evaluation is to predict the fertilizing capacity of an ejaculate. It is generally accepted that conventional sperm characteristics are not well correlated with the fertilizing capacity of sperm and that both inter- and intra-assay variability of these characteristics are high (Christensen et al., 1999). Hence, it is challenging for veterinarians to predict fertilizing capacity, as there is no single sperm parameter that accurately predicts fertility in vivo. Therefore, advanced evaluation techniques of semen are needed to increase the odds of achieving an accurate diagnosis (Christensen et al., 1999; Amman et al., 1993). Researchers have used additional laboratory assays to accurately predict the fertilizing potential of a semen sample. Among these are assays that evaluate sperm DNA Fragmentation Index (DFI), sperm membrane integrity and other sperm organelles (Ballachey et al., 1988; Januskauskas et al., 2001 & 2003). It should be noted that individual laboratory assays, which evaluate a single parameter, are not effective predictors of the fertility; however, a combination of several assays may provide a better prediction of fertility.

The extent of sperm DFI measured by the sperm chromatin structure assay (SCSA) using flow cytometry is a useful index in evaluating semen (Evenson et al., 1999; Evenson et al., 2000 & 2002; Gillan et al., 2005; Boe-Hansen et al., 2005). This parameter is highly repeatable and provides important biological information about sperm DNA defects for diagnostic and prognostic purposes for both human and animal subjects (Evenson et al., 1999, 2000, & 2002). The Sperm DNA variables were significantly related to male fertility in numerous species, including human (Larson et al., 2000; Bungum et al., 2004) and bull (Ballachey et al., 1988; Januskauskas et al., 2001; Boe-Hansen et al., 2005). A wide variation in these parameters was also observed among ejaculates of bulls with lower fertility potential (Bochenek et al., 2001). High rates of DNA damage in sperm have been associated with impaired preimplantation development of the embryo,

implantation failure and increased rates of early embryonic death (Aitken and Baker, 2006; Benchaib et al., 2003). The pathophysiology of DNA damage is due to a number of factors active at the testicular and post-testicular levels (Fisher et al., 2003).

A spermatozoon consists of a plasma membrane, acrosome membrane, mitochondrial membrane and other organelles. Sperm competency requires that each of these membrane compartments should be intact (Grahan et al., 1990). Sperm membranes are extremely susceptible to various kinds of damage, including cryoinjury and therefore the status of these should be an indicator of the success of cryopreservation. Sperm plasma membrane integrity (PMI) and mitochondrial membrane potential (MMP) is a meaningful tool to determine fertilizing capacity of the sperm. Sperm plasma membranes are semi-permeable; this helps to maintain the chemical gradient of ions and other soluble components across the membranes. Specific plasma membrane proteins facilitate transport of extracellular glucose and fructose into the sperm and these substrates are indispensable energy source substrates (Marin et al., 2003; Schurmann et al., 2002; Mukai et al., 2004). In the fertilization process, sperm enter the ooplasm by sperm plasma membrane fusion with oolemma. Most of the sperm plasma membranes are lost during the incorporation process and sperm chromatin is exposed to ooplasm. If the plasma membrane is not functionally intact, then the sperm fail to fertilize an egg. Sperm mitochondria provide energy to the sperm. Any changes in mitochondrial membrane potential could be a good indicator of sperm motility. Sperm population with high mitochondrial membrane potential is a good indicator of the fertility.

Lipid peroxidation is an important pathophysiological process occurring in numerous diseases and stress conditions and results in a series of degradative processes affecting the organization and function of cellular components. Many studies have investigated the possible effect of this process on the loss of sperm functional parameters. Results from series of studies (Kasimanickam et al., 2006 & 2007)we conducted showed sperm lipid peroxidation and bull fertility were negatively correlated ($r = -0.78$; $P < 0.05$). The DFI and lipid peroxidation were positively correlated ($r = 0.86$; $P < 0.001$), whereas PMI and lipid peroxidation ($r = -0.78$; $P < 0.05$) and total progressive motility and lipid peroxidation ($r = -0.83$; $P < 0.01$) were negatively correlated.

This relates to -

(i) the chance of siring calves was low for a bull with higher sperm lipid peroxidation
(ii) the chance of siring calves was low for a bull with higher DFI
(iii) the chance of siring calves was high for a bull with a higher PMI and
(iv) the bulls with higher sperm lipid peroxidation were more likely to have a high DFI and low PMI.

It is evident that the reduced fertility was due to the lipid peroxidation, and impairment in the sperm DNA and sperm plasma membrane. The relationship of lipid peroxidation with sperm DNA fragmentation and plasma membrane integrity was a cause and effect relationship. It infer that the deleterious effect of sperm lipid peroxidation imposed on the PMI and sperm DNA resulted in loss of bull sperm fertilization potential, whereas sperm motility was an independent factor (essential to load the sperm reservoir).

Extenders and antioxidants

Damaging effects of lipid peroxidation on sperm include morphological defects, reduced motility and poor fertilizing ability (Kasimanickam et al., 2006 & 2007). The seminal plasma has been endowed with an array of antioxidants that can protect the sperm integrity against the negative impact of oxidants. However, cryopreservation causes extensive chemical and physical damages to sperm membranes, which are attributed to alterations in the transition from the lipid phase, increases in lipid peroxidation of the membrane induced by reactive oxygen species (ROS), and mechanical stress on cell membranes due to osmotic stress and temperature changes. In recent years, the addition of antioxidants to bovine sperm or to the extenders has been shown to protect

sperm against the harmful effects of ROS and to improve post-thaw sperm motility, viability and fertility. The important antioxidant substances include superoxide dismutase (SOD), catalase, glutathione peroxidase, while non-enzymatic agents are alphatocopherol, ascorbic acid, pyruvate, taurine, hypertaurine and urate.

Sire effect on AI pregnancy

Numerous studies with embryo quality, pregnancy and non-return rate have investigated the optimal time of AI relative to the stage of estrus and concluded that 12–16 h after the onset of estrus is the optimal time for breeding (Schiewe et al., 1987; Pursley et al., 1998; Dalton et al., 2000 & 2001,Satori et al., 2004). Macmillan and Watson (1975) studied the effects of the time interval from estrus to AI on non return rates of sires (Macmllan and Watson, 2004). They have selected groups of sires with different fertility to AI cows at different stages of estrus and concluded that the fertility varied between sires within each estrus to AI interval. The lack of a decline in non-return rate at early insemination among above average fertility sires compared to average and below average fertility sires indicates sire fertility is closely associated with sperm longevity in female reproductive tract. This indicates that fixed time AI (AI) may magnify the differences in sire fertility due to variation in time from AI to ovulation (Darjarnette et al., 2004). This fertility difference can potentially be utilized to select a specific sire for a particular synchronization program which may result in a higher pregnancy rate. A study was conducted to determine the sire effect on the pregnancy outcome in beef cows synchronized with progesterone based Ovsynch, CO-Synch or Select-Synch AI protocols (Kasimanickam et al., 2008). Three Angus sires with more than 300 breedings were evaluated for differences in pregnancy outcome from 1868 inseminations. The sire 2 had poorer pregnancy rate following progesterone based synchronization Ovsynch and CO-Synch programs compared to Sire 3 (Table 1). No sire differences were observed in AI pregnancy for cows in Selectsynch-CIDR group (Table 1). So there was evidence that there are differences in sire fertility when they were used in synchronized AI protocols. However the readers are cautioned that only 3 bulls were used in this study.

Table 1. Effect of sire (N=3) on the pregnancy outcome in progesterone based Ovsynch, CO-Synch and Select-Synch protocols in beef cows (N=1868).

	Sire 1		Sire 2		Sire 3		Total	
	N	PR[1]	N	PR	N	PR	N	PR
Ovsynch-CIDR	452	53.9[ab]	322	47.0[a]	495	54.9[b]	1269	50.1
CO-Synch-CIDR	200	50.5[ab]	124	43.2[a]	275	56.6[b]	599	52.6
Select-Synch CIDR[1]	114	58.8[a]	63	50.1[a]	139	55.4[a]	316	55.7

[ab] different superscripts within the row are different ($P < 0.05$).
[1]PR – Pregnancy rate;
[2]Cows showed estrus observed by activated Kamar on day 9 before 1500 h

Sperm mRNA and Fertility

As mentioned, sperm quality evaluation in the laboratory typically includes assessment of the integrity of genomic DNA (gDNA), acrosome, plasma membrane, and mitochondria, as well as sperm-oocyte interactions (Davis et al., 1992; Madrid-Bury et al., 2005).The effects of paternal genes, sperm RNA, sperm and seminal plasma-specific biomarkers, and sperm DNA are associated with fertilization and early embryo development. Ejaculated sperm retain a complex, yet specific, population of RNAs (Miller et al., 2005; Boerke et al., 2007; Roncoletta et al., 2006; Wu et al., 2008). The development of a reliable method for routine isolation of high-quality RNA from bull sperm will be an important step to develop novel non-invasive approaches to evaluate bull fertility. It was recently proposed that these RNA transcripts may have important roles in sperm development, chromatin repackaging, genomic imprinting, and even zygote development (Miller et al., 2005). Hence, the heterogeneous RNA content of a spermatozoon could be used

for genomic analysis to assess semen quality, in terms of both spermatogenesis and fertility potential. Agreement between testicular and sperm profiles supported the idea that sperm RNA can be used to monitor past events during spermatogenesis; this could provide a non-biased, systematic approach to determine sperm dysfunction. Of the 5,000 distinct mRNA transcripts in sperm, 25% encoded for proteins involved in transcription and regulation of transcription (Miller et al., 2005 & 2006). Proteins present in sperm have distinctive functions and are essential in preparing sperm for fertilization in a timely manner. Understanding the function of individual sperm protein may explain male infertility. Selection of bulls with these biomarkers may lead to improved fertility (Arangasamy et al., 2011).

Table 3. mRNA abundances of sperm biomarkers in sperm and their association to fertility

Protein	Function	Association to fertility
CRISP2	Sperm capacitation and sperm-egg fusion	Positive
PEBP1	Sperm capacitation and sperm-egg fusion	Positive
CCT8	Indicator for the presence of immature cells	Negative
AK1	Motility	Positive
IB5	Fertilization and early embryo development	Positive
Doppel	Acrosome function and fertilization	Positive
TIMP2	Acrosome function and fertilization	Positive

CRISP2, Cysteine-Rich Secretory Protein 2; CCT8, Chaperonin Containing T-complex protein 1, sub unit 8; PEBP1, Phosphatidylethanolamine binding protein 1; AK1 - Adenylate kinase 1; IB5 - Integrin beta 5; TIMP2 - Tissue inhibitors of metalloproteinases 2;

Use of sexed semen in beef industry

The terms gender-biased, gender-selected and sexed semen are used interchangeably. These terms refer to sexed-sorted semen (using the Beltsville method) with the purpose of selecting X-chromosome-bearing sperm and consequently to produce a higher proportion of females calves (Seidel and Garner, 2002).

Sexed semen is available commercially in the dairy industry for almost a decade. There are apparent benefits of using sexed semen in dairy. It increases the chances of birth of heifer calves from about $5/10^{th}$ to $9/10^{th}$. This is especially significant in dairy, where the bull calves have virtually no value. However, the availability of sexed semen from beef bulls along with concerns about success of the technology has limited the use of sexed semen in purebred and commercial beef operation.

High sexed semen's cost compared to conventional semen is one of the main bottle-neck for use of sexed semen technology in beef cattle operations. In addition to lower prices, reasonable for high-value markets and competitive to low volume markets, educational effort required to promote proper use of sexed semen in synchronized breeding systems will change the perception that the technology is too expensive to be viable for beef cattle producers.

Points to consider for sexed semen use

1) Consider only if AI pregnancy rates in the herd with conventional semen are consistently $\geq 60\%$.

2) Select healthy cycling females with good body condition.
3) Inseminate only animals observed in heat. If using fixed-time AI, make sure a high percentage of the animal were in heat before fixed-time AI. We believe this is the advantage to the CO-Synch + 5 day CIDR protocol.
4) Be extremely careful with semen thawing and handling.
5) AI technician should be an experienced and proven one.

The reasons for sexed semen use
It will vary widely depending upon the operation. Each of the reasons listed below has advantage for using sexed semen.

To produce higher number of males for bulls for a seed stock producer or steers for a commercial unit. In a commercial situation where the sex ratio is skewed toward males, the value is in the difference between the weaning weight of the steers vs. heifers plus the higher selling price for steers.

Producing replacement females from a select population of the herd with high genetic merit
When a higher ratio of females is desired for replacements, the increased value of the maternal characteristics should be considered.

Production of females from virgin heifers to reduce calving difficulties
When the females are desired to reduce calving difficulties the value lies in the reduced losses in calves and virgin heifers during calving.

Aware of the issues with this technology

1. the cost of a unit of sexed semen would probably range from 1.5 to 2.25 times the present value of conventional semen - A baseline cost of unsexed conventional and sexed semen dose (*AIC*) can be set at $15 and $45, respectively (Olynk and Wolf, 2007), which indicates a premium of about $30 when using sexed semen compared with conventional unsexed semen.
2. low genetic diversity due to relatively small percentage of AI bulls are available as sexed semen.
   Estimates of the effect of use of sexed semen on the rate of genetic change vary widely (Weigel, 2004). Through increased selection of dams, the rate of genetic change has been expected to increase not more than 15% for sexed semen (Weigel, 2004).
3. low pregnancy rates - 10% to 20% lower with sexed semen compared to conventional semen (DeJarnette et al., 2009).
4. special recommendations and high variability in success.

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