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Sperm quality variables as indicators of bull fertility may be breed dependent

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ABSTRACT

A means of discriminating among bulls of high fertility based on sperm quality is needed by breeding centers. The objective of the study was to examine parameters of sperm quality in bulls of known fertility to identify useful indicators of fertility. Frozen semen was available from bulls of known fertility (Viking Genetics, Skara, Sweden): Swedish Red (n = 31), Holstein (n = 25) and Others (one each of Charolais, Limousin, Blonde, SKB). After thawing, the sperm samples were analyzed for motility (computer assisted sperm analysis), plasma membrane integrity, chromatin integrity, acrosome status, mitochondrial activity and reactive oxygen species. A fertility index score based on the adjusted 56-day non-return rate for > 1000 inseminations was available for each bull. Multivariate data analysis (Partial Least Squares Regression and Orthogonal Partial Least Squares Regression) was performed to identify variables related to fertility; Pearson univariate correlations were made on the parameters of interest. Breed of bull affected the relationship of sperm quality variables and fertility index score, as follows: Swedish Red: %DNA Fragmentation Index, $r = -0.56$, $P < 0.01$; intact plasma membrane, $r = 0.40$, $P < 0.05$; membrane damaged, not acrosome reacted, $r = -0.6$, $P < 0.01$; Linearity, $r = 0.37$, $P < 0.05$; there was a trend towards significance for Wobble, $r = 0.34$, $P = 0.08$. Holstein: Linearity was significant $r = 0.46$, $P < 0.05$; there was a trend towards significance for Wobble, $r = 0.45$, $P = 0.08$. In conclusion, breed has a greater effect on sperm quality than previously realized; different parameters of sperm quality are needed to indicate potential fertility in different breeds.

1. Introduction

Pregnancy rates in cattle have been decreasing for several years (Puglisi et al., 2010), probably due to a number of factors, including sperm quality. Concomitantly there have been several changes in sperm cryopreservation protocols, for example, by altering the cryo-extenders (e.g., Leite et al., 2010; Röpke et al., 2011) and adjusting pre-freezing equilibration times (Leite et al., 2010; Shahverdi et al., 2014). Quality control protocols at commercial semen collection stations state thresholds for certain sperm quality

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Table 1
The Least Squares Mean ± S.E. proportions of motile, plasma membrane intact, morphologically normal bull sperm, and those with low and high mitochondrial membrane potential, grouped according to fertility index score (least 10%, $n = 8$; greatest 10%, $n = 13$).

Fertility index	TM (%)	PM (%)	Live (%)	%DFI	Normal morphology (%)	Low MMP (%)	High MMP (%)
bottom 10% (≤93)	51 ± 4*	47 ± 4*	40 ± 6	5.4 ± 0.8	89 ± 3	71 ± 10	23 ± 8
Top 10% (≥103)	64 ± 3*	59 ± 3*	47 ± 5	3.8 ± 0.7	86 ± 3	59 ± 8	28 ± 6

Note: *Different within a column, $P < 0.05$.
TM = Total Motility, PM = Progressive Motility, %DFI = DNA Fragmentation Index, MMP = Mitochondrial Membrane Potential.

Table 2
Least Squares Mean (± S.E.) proportions of reactive oxygen species in bull sperm, grouped according to fertility index score (least 10% $n = 8$; greatest 10%, $n = 13$).

Fertility index	Membrane intact, SO negative	Membrane intact, SO positive	Membrane damaged, SO positive	Membrane intact, H2O2 negative	Membrane intact, H2O2 positive	Membrane intact, H202 negative	Membrane intact, H202 positive
bottom 10% (≤93)	30 ± 4	19 ± 2	51 ± 4	48 ± 4	1.4 ± 2.1	51 ± 4	0.2 ± 0.7
Top 10% (≥103)	39 ± 3	20 ± 2	41 ± 3	55 ± 3	3.8 ± 1.7	39 ± 3	1.7 ± 0.5

Note: SO = superoxide, H2O2 = hydrogen peroxide.

Table 3
Least Squares Means (± S.E.) kinematics of bull sperm according to the fertility index score (least 10%, $n = 8$; greatest 10%, $n = 13$).

Fertility index	VAP (μm/s)	VCL (μm/s)	VSL (μm/s)	STR	LIN	WOB	ALH (μm)	BCF (Hz)
bottom 10% (≤93)	64 ± 4	125 ± 8	45 ± 3	0.70 ± 0.02*	0.35 ± 0.01*	0.51 ± 0.01*	5.1 ± 0.3	22 ± 0.8
Top 10% (≥103)	64 ± 3	116 ± 6	48 ± 2	0.74 ± 0.01*	0.41 ± 0.01*	0.55 ± 0.01*	5.1 ± 0.2	24 ± 0.6

Note: *statistically significant within a column, $P < 0.05$. VAP = average path velocity, VCL = curvilinear velocity, VSL = straight line velocity, STR = straightness, LIN = linearity, WOB = wobble, ALH = amplitude of lateral head deviation, BCF = Beat cross frequency.

Table 4
Interaction between fertility and breed of bulls (Least Squares Mean ± S.E.) in sperm quality.

Fertility index	Breed	%DFI	LIN
bottom 10% (≤93)	SRB	7.32 ± 0.96*	0.38 ± 0.02
Top 10% (≥103)	SRB	3.29 ± 0.72*	0.40 ± 0.01
bottom 10% (≤93)	HOL	3.75 ± 1.24	0.33 ± 0.02*
Top 10% (≥103)	HOL	4.28 ± 1.08	0.43 ± 0.02*

Note: *indicates statistically significant within a breed, p < 0.05.%DFI = DNA fragmentation index, LIN = Linearity. SRB = Swedish Red Breed; HOL = Holstein.

variables so that sub-standard ejaculates are discarded before freezing or at the post-thaw assessment. There is a lack of consensus, however, on which variables of sperm quality to use. Traditionally sperm motility is used as an indicator of fertility but it is now thought that this measure is not sensitive enough on its own to select ejaculates that would result in acceptable fertility for freezing if used (Foote, 2003). Similarly, other sperm quality variables are not useful in isolation to predict the fertility of a semen sample (Mocé and Graham, 2008), although combinations of assays have been suggested to provide a more precise indicator of the potential fertility of an ejaculate (e.g., Holt, 2009).

Although sperm concentration and viability in the raw ejaculate, together with post–thaw viability, were advocated as being the variables of choice to judge sperm quality (Christiansen et al., 2011), they apparently do not provide sufficient discrimination to be useful in practice. This raises the question of which variables to evaluate. Some authors have suggested using a range of sperm quality variables to establish fertility indices (e.g., Rodríguez-Martínez, 2003; Oliviera et al., 2014). Plasma membrane integrity, chromatin integrity and sperm motility were related to fertility in Swedish Red bulls (Januskauskas et al., 2003), and Puglisi et al. (2010) considered that plasma membrane integrity was related to zona-binding capacity. Karoui et al. (2012) observed that values of 7% to10% in the sperm chromatin dispersion test were related to lower fertility. Plasma membrane integrity, acrosomal integrity and mitochondrial function were correlated with pregnancy rate in Nellore cows (Oliveira et al., 2014). Other variables have been used to assess the quality of semen after cryopreservation, such as production of reactive oxygen species (ROS) and chromatin integrity (Anzar et al., 2011; Morrell et al., 2014; Goodla et al., 2014). In a preliminary study with a mixed group of 24 Holstein and Swedish Red bulls, Nongbua et al. (2014) reported relationships between chromatin integrity and bull fertility, as well as plasma membrane integrity and fertility, whereas total and progressive motility were not indicative of fertility.