



RELATIONSHIP BETWEEN QUALITY PARAMETERS AND FERTILIZING ABILITY OF CRYOPRESERVED SEXED BULL SPERM*

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Abstract

The aim of the experiment was to assess the correlation between sperm quality parameters and field fertility after AI with sex-sorted (X-bearing) bull semen. A total of 32 ejaculates from 26 Holstein-Friesian bulls were analyzed to assess sperm motility parameters (CASA), viability (SYBR-14/PI), apoptotic-like changes (YO-PRO-1/PI), chromatin structure (SCSA), and ATP content. In order to determine sperm fertilizing ability, 816 heifers and 727 cows were inseminated. Ultrasound diagnosis of pregnancy was performed on day 35 after insemination. For each ejaculate, the percentage of pregnant females was calculated separately. The results revealed that the pregnancy rate ranged from 20.0 to 85.7% for heifers and from 7.7 to 66.7% for cows. On the basis of the pregnancy rate (PR) obtained, the ejaculates were divided into 3 groups: high PR (about 25% of ejaculates), medium PR (about 50% of ejaculates) and low PR (about 25% of ejaculates). Significant differences were detected for amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) between high- and low-fertility ejaculates in heifers. Pearson's correlation analysis showed a significant relationship between the BCF and the pregnancy rate for heifers ($r = 0.53$, $P < 0.01$) and there was a trend towards significance for ALH ($r = -0.37$, $P = 0.07$). There was no relationship between the sperm quality parameters and pregnancy rate of cows. In conclusion, the present study identified markers of sexed bull sperm function that were related to the fertility of inseminated heifers.

Key words: bull, sex-sorted semen, fertility prediction, flow cytometry, computer-assisted sperm analysis (CASA)

Currently, the sorting of fluorescently stained cells by flow cytometry is the only proven (fast and reliable) method for the sex sorting of spermatozoa for commercial application (Johnson, 2000; Garner, 2006). This technology is constantly being improved and efforts are being made to minimize the negative impact of sorting steps on semen quality (SexedULTRA technology, Sexcel), but pregnancy rates for sexed bull semen continue to be lower than those for unsexed semen (DeJarnette et al., 2011; Maicas et al., 2019; Oikawa et al., 2019; Maicas et al., 2020).

The reasons for reduced fertility are lower sperm count in the insemination and sperm damage during sorting (Frijters et al., 2009; Lenz et al., 2016; Vishwanath and Moreno, 2018). Both of these factors are crucial for most bulls, but it is still unclear which one plays a greater role (Seidel, 2014). It was shown, however, that conception rates differ considerably between bulls when using low doses of sexed semen (DeJarnette et al., 2010, 2011).

Research aimed at linking semen quality to its fertilizing ability is carried out using increasingly technolo-

gy-intensive methods. The studies to date have assessed parameters such as sperm viability (Gillan et al., 2008; Christensen et al., 2011), cell membrane integrity (Oliveira et al., 2013; Ahmed et al., 2016), capacitation (Alm et al., 2001; Gillan et al., 2008), acrosome status (Christensen et al., 2011), mitochondrial activity (Ahmed et al., 2016), IVF (Ward et al., 2001), mucus penetration ability (Al Naib et al., 2011), levels of reactive oxygen species (Sellem et al., 2015), and chromatin/DNA integrity (Gillan et al., 2008; Ahmed et al., 2016). Although many of these studies showed a correlation with fertility, no individual trait assessed *in vitro* is a sufficiently reliable predictor of fertility. Sellem et al. (2015) demonstrated that a combination of computer-assisted semen analysis with flow cytometric analysis (viability, chromatin and acrosome status, oxidative stress, mitochondrial activity) could better account for the differences in fertility (but only in 40%). Holden et al. (2017) suggested that other markers may have to be used to determine the fertility of sexed compared to unsexed semen.

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The nature of the damage to sexed sperm has not been sufficiently elucidated, but there are many possibilities, including stretching of the sperm tail as droplets are formed at the nozzle opening and continued binding of the Hoechst 33342 solution to sperm post-fertilization, slowing down progression of the first cell cycle between fertilization and first cleavage (Seidel, 2012). Previous research has shown reduced sperm motility after sexing, damage within the cell membrane and acrosome, and lack of chromatin damage (Boe-Hansen et al., 2005; Suh et al., 2005; Mocé et al., 2006; Carvalho et al., 2010). Unfortunately, the effect of damaged sexed sperm on fertility can only be partly compensated by increasing their numbers per insemination dose (DeJarnette et al., 2010, 2011). It is therefore evident that additional studies are needed, taking into account various quality parameters, allowing for more accurate determination of sexed semen quality *in vitro*. The aim of this study was to determine the relationship between sexed sperm quality parameters (determined by CASA, flow cytometry, and luminometry) and fertility of inseminated females.

Material and methods

Animals

For this study, 32 commercially available ejaculates of 26 Holstein-Friesian bulls aged 1 to 6 years were used. The selection of bulls was made based on the assumed breeding goal, according to the conducted genetic balance. Sperm sorted by SexedULTRA™ and SexedULTRA™ 4M technology were used. Sex-sorted (X-bearing) bull semen was analyzed to assess sperm quality and used for artificial insemination of heifers and cows at experimental stations in Kołbacz and Chorzów.

Ethical approval

All of the procedures that involved animals did not require approval by the local ethics committee.

Computer-assisted sperm analysis

Cryopreserved semen was thawed in a water bath at 38°C for 40 s. Post-thaw sperm motility was measured by computer-assisted sperm analysis (Sperm Class Analyser, S.C.A. V5.1, Microptic, Barcelona, Spain). Thawed sample (1 µL) was placed in a Leja counting chamber (Leja Products B.V., GN Nieuw-Vennep, The Netherlands) on a heated stage (38°C). Each sample was measured twice, 3 fields were evaluated, and at least 1000 cells were counted in each analysis. Instrument settings were 25 frames per second and 25 images per sample. Regarding the analysis settings, sperm with straightness >70% were considered progressively motile, whereas sperm with VAP >15 µm/s were classified as motile. The measured motility parameters included progressive motile sperm (10⁶ sperm per 0.25 mL straw) and

motile sperm (10⁶ sperm per 0.25 mL straw), curvilinear velocity (VCL, µm/s), straightness (STR, %), linearity (LIN, %), wobble (WOB, %), beat cross frequency (BCF, Hz), and amplitude of lateral head displacement (ALH, µm).

Sperm viability

Spermatozoa were stained using a sperm viability kit (Live/Dead Sperm Viability Kit, Life Technologies Ltd., Carlsbad, CA, USA) (Szczęśniak-Fabiańczyk et al., 2021). Aliquots of 1000 µL of diluted samples (5 × 10⁶ sperm/mL in PBS) were pipetted into cytometric tubes, and 5 µL of SYBR-14 working solution was added. The working solution was obtained by diluting a commercial solution of SYBR-14 in distilled water at a ratio of 1:49. Samples were mixed and incubated at 37°C in the dark for 10 minutes. Then, the cells were counterstained with 5 µL of PI 3 minutes before analysis. Measurements were carried out on a CytoFlex flow cytometer (Beckman Coulter, Brea, CA, USA). A total of 10,000 events were analyzed for each sample. Three subpopulations of cells were observed on dot plots of PI/SYBR-14 fluorescence: dead spermatozoa (PI+/SYBR-14-), moribund spermatozoa (PI+/SYBR-14+), and live spermatozoa (membrane-intact spermatozoa) (PI-/SYBR-14+).

Membrane integrity

The Vybrant Apoptosis Assay Kit #4 (Molecular Probes Inc., Eugene, OR, USA) was used to detect changes in plasma membrane permeability to YO-PRO-1 (Trzcińska et al., 2015). A total of 2 × 10⁶ thawed sperm was diluted in 1 mL of PBS (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 1 µL of YO-PRO-1 (100 µMol/L) was added. The tubes were gently mixed and incubated for 20 min at room temperature, and 2 µMol/L propidium iodide (PI) was added to each tube. Detection of green fluorescence was set with a YO-PRO-1 band-pass filter (525/40 nm) and red fluorescence was measured using a PI band-pass filter (690/50 nm). Fluorescence measurements were carried out using a CytoFlex cytometer (Beckman Coulter, Brea, CA, USA). At least 10,000 spermatozoa per sample were evaluated. The results are presented as the percentage of viable spermatozoa with membrane permeability characteristic of early stages of apoptosis (YO-PRO-1+/PI-).

Sperm chromatin structure assay

The sperm chromatin structure assay (SCSA) was performed using flow cytometry (Evenson and Jost, 2000). Briefly, individual sperm samples (5 × 10⁶ cells/mL) were incubated with HCl solution (pH = 1.5) for 30 seconds on ice. Then, samples were stained with acridine orange for 3 minutes on ice. The green (normal DNA) and red (fragmented DNA) fluorescence signals were collected from 10,000 spermatozoa. Fluorescence measurements were carried out using a CytoFlex cytometer (Beckman Coulter, Brea, CA, USA). Based on a histogram of the fluorescence ratio red/(red + green),

spermatozoa with fragmented DNA (DFI) were calculated.

ATP content

ATP was extracted from spermatozoa and assayed using a firefly luciferase bioluminescent assay kit (Cambrex Bio Science, Rockland, ME, USA). This ATP assay is based on the quantitative measurement of a stable level of light produced as a result of an enzyme reaction catalyzed by firefly luciferase. The amount of light generated by this reaction is measured in a luminometer, and it is directly related to the amount of ATP in the sample. Briefly, a 10 μ L aliquot of semen sample was mixed with 100 μ L cell lysis reagent and incubated at room temperature for 5 minutes to extract ATP from cells. Following the addition of 100 μ L ATP monitoring reagent, luminescence was measured using a Lumat LB9508 luminometer (Berthold, Bad Wildbad, Germany). The generated signal was compared to standard ATP dilutions. Sperm ATP content from each probe was assessed in duplicate. The results are presented as pMol ATP per 10^6 motile sperm.

Monitoring of sperm fertilizing ability

The fertilizing ability of X-bearing sperm was monitored based on the pregnancy rate of 816 heifers and 727 primiparous cows. Cows and heifers were kept in free-standing barns with free access to the feed table and

water. They were fed in the TMR system. The heifers were inseminated at a mean age of 13.6 months and the cows from the 50th day of lactation. Insemination was performed from September to June by experienced technicians, who remained the same throughout the study. Heifers and cows were inseminated during natural estrus. Only animals showing clear estrus symptoms were selected for insemination. No hormonal estrus synchronization was used. Pregnancy was tested by ultrasound 35 days after insemination. For each ejaculate, the percentage of pregnant females was calculated separately. Data were obtained from the experimental stations in Kołbacz and Chorzew.

Statistical analysis

Statistical analysis was performed using SAS version 9.1. on data, which were log transformed in the absence of a normal distribution. An analysis of variance was performed and the differences between the means were tested using Duncan's test. Pearson's correlations were computed to analyze the relationship between sperm quality parameters and pregnancy rate.

Results

The results of analysis of sexed semen are presented in Tables 1, 2, 3 and 4.

Table 1. Motility parameters of spermatozoa in sexed semen, grouped according to pregnancy rate of heifers (mean $\pm$ SEM)

Pregnancy rate	Sperm $\times 10^6$ per straw		VCL	STR	LIN	WOB	ALH	BCF
	progressive motile	motile						
37.9 $\pm$ 3.4 a (n = 7)	1.0 $\pm$ 0.2	1.2 $\pm$ 0.2	103.3 $\pm$ 3.1	90.4 $\pm$ 0.6	60.6 $\pm$ 1.5	66.5 $\pm$ 1.4	3.8 $\pm$ 0.2 a	10.9 $\pm$ 0.6 a
60.9 $\pm$ 1.9 b (n = 11)	1.3 $\pm$ 0.2	1.7 $\pm$ 0.2	98.2 $\pm$ 1.8	90.2 $\pm$ 0.4	61.4 $\pm$ 1.3	67.4 $\pm$ 1.2	3.6 $\pm$ 0.1 ab	11.6 $\pm$ 0.3 ab
77.8 $\pm$ 2.0 c (n = 7)	1.4 $\pm$ 0.1	1.6 $\pm$ 0.2	98.3 $\pm$ 2.5	90.8 $\pm$ 0.6	61.6 $\pm$ 1.7	67.1 $\pm$ 1.5	3.4 $\pm$ 0.1 b	12.8 $\pm$ 0.5 b

a, b, c – values in columns with different letters differ significantly ($P < 0.05$).

VCL, curvilinear velocity (μ m/s); STR, straightness (%); LIN, linearity (%); WOB, wobble (%); ALH, amplitude of lateral head displacement (μ m); BCF, beat cross frequency (Hz).

Table 2. Quality parameters of sexed semen (amount of ATP, viability, apoptotic-like changes, DNA fragmentation), grouped according to pregnancy rate of heifers (mean $\pm$ SEM)

Pregnancy rate	ATP pMol/ 10^6 motile sperm	Live (%)	Apoptotic sperm (%)	DFI (%)
37.9 $\pm$ 3.4 a (n = 7)	19.3 $\pm$ 4.9	33.1 $\pm$ 3.5	9.5 $\pm$ 3.7	1.3 $\pm$ 0.2
60.9 $\pm$ 1.9 b (n = 11)	14.4 $\pm$ 1.0	35.6 $\pm$ 3.7	10.0 $\pm$ 4.6	1.4 $\pm$ 0.3
77.8 $\pm$ 2.0 c (n = 7)	13.5 $\pm$ 0.7	37.7 $\pm$ 2.7	6.2 $\pm$ 3.5	1.4 $\pm$ 0.3

a, b, c – values in columns with different letters differ significantly ($P < 0.05$).

DFI, spermatozoa with fragmented DNA.

Table 3. Motility parameters of spermatozoa in sexed semen, grouped according to pregnancy rate of cows (mean $\pm$ SEM)

Pregnancy rate	Sperm $\times 10^6$ per straw		VCL	STR	LIN	WOB	ALH	BCF
	progressive motile	motile						
12.7 $\pm$ 2.0 a (n = 6)	1.1 $\pm$ 0.2	1.5 $\pm$ 0.2	101.8 $\pm$ 3.7	90. $\pm$ 0.9	59.4 $\pm$ 2.6	65.2 $\pm$ 2.3	3.8 $\pm$ 0.3	11.4 $\pm$ 0.6
33.5 $\pm$ 1.7 b (n = 11)	1.5 $\pm$ 0.2	1.9 $\pm$ 0.3	99.2 $\pm$ 2.5	89.7 $\pm$ 0.5	60.2 $\pm$ 1.1	66.5 $\pm$ 1.0	3.6 $\pm$ 0.1	11.5 $\pm$ 0.5
50.6 $\pm$ 3.8 c (n = 6)	1.5 $\pm$ 0.3	1.9 $\pm$ 0.4	99.1 $\pm$ 4.0	90.2 $\pm$ 0.6	62.0 $\pm$ 1.4	68.0 $\pm$ 1.1	3.5 $\pm$ 0.2	12.3 $\pm$ 0.4

a, b, c – values in columns with different letters differ significantly ($P < 0.05$).

VCL, curvilinear velocity ($\mu\text{m/s}$); STR, straightness (%); LIN, linearity (%); WOB, wobble (%); ALH, amplitude of lateral head displacement (μm); BCF, beat cross frequency (Hz).

Table 4. Quality parameters of sexed semen (amount of ATP, viability, apoptotic-like changes, DNA fragmentation), grouped according to pregnancy rate of cows (mean $\pm$ SEM)

Pregnancy rate	ATP pMol/ 10^6 motile sperm	Live (%)	Apoptotic sperm (%)	DFI (%)
12.7 $\pm$ 2.0 a (n = 6)	18.5 $\pm$ 5.1	29.5 $\pm$ 3.7	7.3 $\pm$ 3.3	1.2 $\pm$ 0.2
33.5 $\pm$ 1.7 b (n = 11)	14.3 $\pm$ 1.9	42.3 $\pm$ 3.1	9.7 $\pm$ 3.6	2.2 $\pm$ 0.8
50.6 $\pm$ 3.8 c (n = 6)	12.9 $\pm$ 2.0	35.1 $\pm$ 3.8	9.9 $\pm$ 6.3	1.3 $\pm$ 0.3

a, b, c – values in columns with different letters differ significantly ($P < 0.05$).

DFI, spermatozoa with fragmented DNA.

On the basis of the pregnancy rate (PR) obtained for heifers, the 25 ejaculates were divided into 3 groups: low PR (about 25% ejaculates, $n = 7$, range from 20.0 to 47.3), medium PR (about 50% ejaculates, $n = 11$, range from 50.0 to 69.2) and high PR (about 25% ejaculates, $n = 7$, range from 72.2 to 85.7). Significant differences were detected for amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) between high- and low-fertility ejaculates in heifers ($P < 0.05$). No significant differences were detected for the other variables. In the case of cows, 23 ejaculates were also divided into 3 groups: low PR (about 25% ejaculates, $n = 6$, range from 7.7 to 20.0), medium PR (about 50% ejaculates, $n = 11$, range from 23.1 to 41.3) and high PR (about 25% ejaculates, $n = 6$, range from 44.4 to 66.7). No statistical differences were detected for sperm quality parameters between groups.

Pearson's correlation analysis showed a significant relationship between the BCF and the pregnancy rate for heifers ($r = 0.53$, $P < 0.01$) and there was a trend towards significance for ALH ($r = -0.37$, $P = 0.07$). There was no relationship between the sperm quality parameters and pregnancy rate of cows.

Discussion

The field fertility of sexed spermatozoa has been extensively discussed in many review articles (Seidel, 2012, 2014; Vishwanath and Moreno, 2018; Reese et al.,

2021), in which the focus was mainly on the relative fertility of sex-sorted compared to unsorted spermatozoa. The fertility of sex-sorted bull semen has always been lower than that of conventional semen, while compensatory measures that would normally increase the fertility of bulls with reduced fertility, such as increasing the number of sperm or the percentage of spermatozoa with better morphology, did not produce the expected improvement for sexed semen (Frijters et al., 2009; De-Jarnette et al., 2011).

Our study focused on the relationship between the quality parameters and field fertility of sexed semen. The main finding of our study is that the sperm motility parameters (ALH and BCF) were related to the pregnancy rate of heifers. This is consistent with earlier research showing motility parameters as markers of sexed semen fertility (Holden et al., 2017) and agrees with previous studies on the use of unsexed spermatozoa (Phillips et al., 2004; Gillan et al., 2008; Rabidas et al., 2012).

Farrell et al. (1998) indicated that total motility alone had modest correlation with field fertility, whereas if combined with other kinetic parameters (e.g. BCF and ALH as in our study) it yielded higher levels of correlation. Different results were obtained by Morrell et al. (2017) when analyzing the semen quality for the top and bottom 10% bulls based on the bull fertility index. Statistical differences were found for TM, PM, STR, LIN and WOB parameters but not for ALH and BCF.

Research shows that sperm motility parameters including ALH and BCF are related to the process of

hyperactivation and capacitation (Chamberland et al., 2001) and that motile sperm remaining after cryopreservation undergo premature capacitation (cryo-capacitation) (Bailey et al., 2000). The flagellum of an activated sperm, as is seen in freshly ejaculated sperm, generates a symmetrical, lower amplitude waveform that drives the sperm in a relatively straight line. In contrast, once sperm from most species become hyperactivated, the flagellar beat becomes asymmetrical and higher amplitude, which results in circular or figure-eight trajectories (Ishijima et al., 2002). Current evidence suggests that the role of activated motility is to aid in propelling the sperm through the female reproductive tract to the oviduct, whereas the role of hyperactivated motility is to help sperm detach from the oviductal epithelium, reach the site of fertilization, and penetrate the cumulus and zona pellucida of the oocyte (Stauss et al., 1995; Ho and Suarez, 2001). There is good evidence that both activated and hyperactivated motility are important for normal fertility (Turner, 2003). A variety of protocols, cryoprotectants and additives have been tried to protect sperm during cryopreservation with varied degree of success, but unable to prevent premature capacitation (Singh et al., 2014; Kumar et al., 2018). The mechanism of cryo-capacitation is not fully understood and is complicated as the onset of normal capacitation has yet to be elucidated. Significant differences demonstrated in our study for parameters related to the process of hyperactivation and capacitation (ALH and BCF), between high- and low-fertility ejaculates indicate that capacitation-like changes in spermatozoa may be a factor responsible for the decreased fertility of sexed semen.

The lack of correlations between sperm quality parameters and fertility for cows in our study suggests that such correlations may exist, but perhaps they are masked by low sample size or other factors, such as postpartum disturbances, typical of dairy breeds. It should be noted, however, that correlations in such a small sample size ($n = 23$ ejaculates) should always be interpreted with caution. However, we found that the other semen parameters analyzed were unrelated to fertility. It should be noted that all ejaculates used in this study had been assessed for typical/standard quality control parameters (sperm concentration, motility, and viability) at AI centers and again at sperm sex-sorting centers for the population of sexed spermatozoa. However, similar to other studies (Holden et al., 2017), we observed considerable variation in pregnancy rates (20.0 to 85.7% for heifers and 7.7 to 66.7% for cows), which indicates that these quality control parameters are limited in their ability to predict fertility. The very high results obtained with some sexed semen ejaculates in this study may be due to the fact that only animals showing clear estrus symptoms were selected for insemination. In addition, the results may be due to good herd management (including semen handling, semen placement during artificial insemination, feeding, and stable conditions) and also partly to the small number of inseminations. In typical field practice, conception rates of sexed semen are often biased by

preferential use in females exhibiting definitive signs of estrus, whereas females exhibiting questionable signs of estrus are inseminated with less expensive conventional semen (DeJarnette et al., 2011).

The lower fertilizing ability of sexed semen is attributed to various biochemical changes that take place in the semen during sperm sorting. The sexing procedure distinguishes more than 20 different sub-processes, including longer incubation time before staining, Hoechst 33342 fluorochrome staining, exposure to a laser beam to induce fluorescence, exposure to an electric field to separate into fractions, and centrifugation, all of which may lead to deterioration of the biological quality of sperm (Hollinshead et al., 2004; Suh et al., 2005). The multi-stage process of sexing involves rather drastic changes in the environment of the sex cells, and altogether these stages contribute to even lower sperm quality. Furthermore, the subsequent cryopreservation procedure additionally has a negative effect on spermatozoa that were exposed to considerable stress during sorting. With this in mind, it can be stated that sexed sperm comprise a highly selected population of spermatozoa and their characteristics will differ from those of unsexed sperm. This is supported by Holden et al. (2017) and Carvalho et al. (2010), who investigated the effect of sorting using spermatozoa from the same ejaculates. The ejaculates were separated: one part was sexed and frozen while the other was frozen without sexing. Sexing caused decreased motility and an increased percentage of sperm with damaged acrosome and cell membrane. It is believed that this negative effect of sexing on cell membranes may result from mechanical tension (mechanical stress), because previous studies revealed that lower hydrostatic pressure during sorting increased sperm survival as well as fertility (Suh et al., 2005) and pregnancy parameters (Schenk et al., 2009).

A study with stallion semen showed that sex sorting induces kinematic and plasmalemmal changes in spermatozoa, and these changes are related to decreased energy status of the cell (ATP content) and the initial steps of the triggering of an apoptotic-like mechanism (Yo-Pro-1 assay) (Balao da Silva, 2013). The authors suggested that semen sexing causes the loss of ATP through specific channels that are opened due to the high hydrostatic pressure. Our study with sexed bull semen showed that the ATP content and the marker of apoptosis (Yo-Pro-1 assay) are not correlated with fertility.

The sperm chromatin structure assay has been performed with semen of many different species, including bull, and a relationship between male fertility and SCSA parameters has been reported (Ballachey et al., 1987; Sailer et al., 1996; Bochenek et al., 2001). The effect of various semen handling procedures, including sexing, on sperm DNA integrity has not been adequately studied. In our earlier study we noted a significant difference between sexed and unsexed semen in terms of sperm DNA integrity measured by the SCSA (Gogol and Trzcińska, 2020). Sexed spermatozoa were characterized by much lower DFI when compared to unsexed sperm. This dem-

onstrates that there was less DNA damage in sorted than unsorted sperm. In our present study, the degree of chromatin damage in sexed sperm was relatively low (0.3 to 9.7%) and showed no correlation with fertility. Although the existing data show that changes in chromatin packaging may play a significant role in infertility (Sailer et al., 1996), it is suggested that DFI levels lower than 2% should have no substantial effect on fertility after insemination. In practice, pregnancy rates have been observed to decrease by around 10% when DNA damage exceeded 20% (Rybak et al., 2004).

In conclusion, the present study identified markers of sexed bull sperm function that were related to the fertility of inseminated heifers. More research on more animals and ejaculates is needed to further define the relationship between sexed semen quality and its fertilizing ability.

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