

Effect of different storage temperatures on the metabolic activity of spermatozoa following liquid storage of boar semen*

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ABSTRACT

This study investigated the effects of different temperatures (5° and 16°C) on the metabolic activity of boar spermatozoa preserved in a standard semen extender, Kortowo 3 (K3), supplemented with or without egg yolk lipoprotein fractions (LPF) isolated from hen or ostrich egg yolk. Besides motility and plasma membrane integrity (PMI) assessments of spermatozoa, analysis of the metabolic activity of spermatozoa included mitochondrial energy status, oxygen uptake, ATP content and L-lactate production. Total motility and PMI of spermatozoa decreased over time. The metabolic activity of spermatozoa was significantly higher in the extenders containing LPF than in the K3 extender. Marked changes in the sperm metabolic activity observed during storage of semen samples at 5°C indicated that glycolysis (fructolysis) was the major metabolic pathway. It seemed likely that mitochondrial respiration was the predominant metabolic activity exhibited by spermatozoa during storage at 16°C. The findings of this study indicate that different storage temperatures can modulate the metabolic pathways of boar spermatozoa during liquid semen storage.

KEY WORDS: boar, semen, spermatozoa, storage temperatures, metabolic activity

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INTRODUCTION

Mitochondrial respiration and anaerobic glycolysis are considered to be the major source of energy production in spermatozoa; however, these metabolic pathways vary among animal species (Mann and Lutwak-Mann, 1981; Strzeżek, 1998). Sugars such as glucose and fructose, and to some extent, sorbitol and mannose, are metabolized to pyruvic acid or lactate acid during glycolysis (Marin et al., 2003). It has been reported that boar spermatozoa have a limited capacity to metabolize exogenous substrates because they possess relatively low hexokinase activity (Medrano et al., 2005). A decline in the energy production by spermatozoa is associated with impaired mitochondrial oxidative phosphorylation, resulting in reduced motility (Thomas et al., 1998; Huo et al., 2002).

A gradual reduction in the metabolic activity of boar spermatozoa during storage at cold shock temperature could limit the production of detrimental by-products, which might compromise sperm function (de Leeuw et al., 1990; Althouse et al., 1998). It has been postulated that the components of the semen extender protect the sperm structures against the damaging effect of cold shock (Demianowicz and Strzeżek, 1996). Several studies in our laboratory have demonstrated that egg yolk lipoprotein fractions (LPF) provide excellent protection for the plasma membrane and acrosome integrity of boar spermatozoa against cold shock-related injury (Demianowicz and Strzeżek, 1996; Strzeżek et al., 1999, 2004, 2005; Fraser et al., 2002). More recently, acceptable fertility results in terms of farrowing rate and litter size were obtained when sows were artificially inseminated with LPF-extended boar semen stored at 5°C (Strzeżek et al., 2006).

In a previous study we showed that the age of boars and seasons had a marked effect on sperm metabolic activity, characterized by oxygen uptake and ATP content, during liquid semen storage (Fraser et al., 2003). In this study, we investigated the effect of different storage temperatures (5° and 16°C) on the metabolic status of boar spermatozoa following storage of semen in a standard semen extender, Kortowo 3 (K3), supplemented with or without egg yolk lipoprotein fractions (LPF) isolated from hen egg yolk (K3-LPFh) or ostrich egg yolk (K3-LPFo). Besides motility and plasma membrane integrity assessments, parameters of the metabolic activity of spermatozoa included analysis of mitochondrial energy status, measurements of ATP content, oxygen uptake and L-lactate production.

MATERIAL AND METHODS

Animals and ejaculate collections

Whole ejaculates were collected from 6 Polish Large White boars, aged 1.5 to 3 years. The boars were kept under standard hygienic conditions, and fed a commercial ration. The gloved-hand technique was used to collect ejaculates once per week from each boar over a 4-month period, beginning from September through December. A total of 96 ejaculates were collected and analysed for sperm quality characteristics, unless otherwise stated. At collections the gel portion was removed using double gauze. Only semen samples that had more than 70% sperm motility and less than 15% abnormal spermatozoa were used in this study. Spermatozoa were assessed for morphology (Watson, 1975) and counted using a haemocytometer. Permission to conduct this study was granted by the Local Ethics Committee.

Processing and liquid storage of semen

Semen samples were diluted (30×10^6 spermatozoa/ml) in the following extenders: K3 extender (69.3 mM fructose, 64.6 mM sodium citrate, 8.0 mM $\text{Na}_2\text{-EDTA}$, 14.2 mM potassium acetate; pH 6.8, 250 mg gentamycin sulphate), K3 extender supplemented with 5% (v/v) LPFh (K3-LPFh), and K3 extender supplemented with 5% (w/v) LPFo (K3-LPFo). Lipoprotein fractions were isolated from hen and ostrich egg yolk, respectively, according to previously described methods (Demianowicz and Strzeżek, 1996; Strzeżek et al., 2005). Due to the large quantities of LPF extracts obtained from the ostrich egg yolk, we considered using lyophilization as a means of preserving these extracts for storage. Furthermore, research in our laboratory has shown that lyophilization does not have a significant effect on the biochemical composition of the LPF extracts (Strzeżek et al., 1999, 2005).

Diluted semen was equilibrated for 2 h at room temperature prior to cooling at 5°C (refrigerator) and 16°C (Thermobox, Minitüb GmbH, Tiefenbach, Germany). Besides motility and plasma membrane integrity (PMI) assessments of spermatozoa, analysis of the metabolic activity of spermatozoa included mitochondrial energy status, oxygen uptake, ATP content and L-lactate production. Semen samples were analysed before cooling (Day 0), and after 48 h (Day 2) and 96 h (Day 3) of storage at 5° or 16°C. The number of sperm samples assessed for PMI (n=35), mitochondrial function (n=29) and L-lactate production (n=45) was restricted due to the limited available supply of reagents. Furthermore, L-lactate

production was determined only in semen samples before cooling (Day 0) and after 48 h of storage at both 5° and 16°C.

Assessments of motility and plasma membrane integrity of spermatozoa

Total motility of spermatozoa was assessed visually by the same technician throughout the study. For the assessments, aliquots (6 µl) of diluted semen samples were placed on pre-warmed slide, covered with a glass cover slide and examined under a light microscope (200 × magnification) equipped with an attached heated stage (37°C).

Sperm plasma membrane integrity (PMI) was assessed using propidium iodide, PI (Sigma, St. Louis, MO, USA), as described in a previous study (Fraser et al., 2002). Membrane-damaged PI-stained spermatozoa were examined under an epifluorescence microscope (Olympus CH 30, Tokyo, Japan) and PMI was defined as the percentage of intact spermatozoa. Two slides were assessed per sample and 200 spermatozoa were evaluated per slide.

Mitochondrial energy status assessed by fluorescent microscopy

The mitochondrial energy status in spermatozoa was assessed using a fluorescent probe, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbo-cyanineiodide (JC-1) (Molecular Probes, Eugene, USA), according to a previously described method (Thomas et al., 1998), with some modifications. Briefly, semen samples (30 × 10⁶ spermatozoa/ml) were washed (600 g, 5 min at room temperature) and the sperm pellets were re-suspended in a Hepes saline medium (130 mM NaCl, 4 mM KCl, 14 mM fructose, 10 mM Hepes, 1 mM CaCl₂, 0.5 mM MgCl₂, 0.1% BSA). Three microliters of JC-1 solution (1 mg JC-1/ml anhydrous dimethyl sulphoxide) were added to aliquots (1 ml) of washed sperm samples and incubated for 15 min at 37°C. Following incubation, sperm samples were counterstained with PI (10 µl of PI solution in 0.5 mg/ml phosphate buffered solution) in order to allow identification of non-viable spermatozoa with compromised mitochondrial function. Aliquots (10 µl) of stained sperm samples were examined under an epifluorescence microscope. Viable spermatozoa with high mitochondrial energy status emitted orange-red fluorescence. Two slides were evaluated per sample and 200 spermatozoa were counted per slide.

Oxygen uptake

Oxygen uptake was measured polarographically in a Yellow Springs oxygen monitor (YSI 5300 Biological Oxygen Monitor, Co., Inc., Yellow Springs,

Ohio, USA) equipped with a Clarke's electrode, according to the manufacturer's instruction manual. Oxygen measurements were expressed as $\mu\text{l O}_2/10^8$ spermatozoa/1h/37°C (ZO_2).

ATP content

The ATP content in spermatozoa was assessed using a bioluminescence kit (ATP Bioluminescence Assay Kit CLSII; Roche Molecular Biochemical). Prior to assay, 100 μl aliquots of sperm suspension (30×10^6 spermatozoa/1 ml) were added to TRIS buffer (900 μl) containing 4 mM EDTA (pH 7.75) and then boiled for 5 min. After boiling and cooling, 100 μl sperm extract were added to 100 μl assay kit. Measurements of ATP content were performed using a Junior Bioluminometer (Berthold Technologies, GmbH and Co. KG, Germany), according to the assay kit protocol. The ATP content in spermatozoa was calculated from an ATP standard curve and expressed as nmol ATP/ 10^8 spermatozoa.

L-lactate production as a marker for the rate of fructolysis

The rate of L-lactate production in spermatozoa was determined, as described in a previous study (Rodriguez-Gil and Rigau, 1995), with some modifications. Extended semen samples were washed twice (600 g, 5 min at room temperature) in K3 extender in order to remove exogenous substrates. Sperm pellets (100×10^6 spermatozoa/ml) were re-suspended in a buffer containing 2.94% (w/v) sodium citrate and 5.4% (w/v) fructose (pH 7.4) and incubated for 1 h at 37°C. Following incubation, the mixture was centrifuged, as mentioned above. L-lactate production by spermatozoa was measured in the supernatant using a reagent kit (Pointe Scientific Inc., Canton, Michigan, USA). Absorbances was measured spectrophotometrically (Beckman DU®- 62) at a wave length of 550 nm. The L-lactate production was expressed as nmol/ $10^8/1\text{h}/37^\circ\text{C}$.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure from the Statistica software package, version 8 (StatSoft Incorporation, Tulsa OK, USA). The statistical model used to investigate the effects of extender type, storage temperature, storage time and their interactions on sperm motility, plasma membrane integrity mitochondrial energy status, oxygen uptake and ATP content included a mixed factorial design ($3 \times 2 \times 2$). The effects of extender type and storage temperature and their interactions on L-lactate production were investigated with a 3×2 factorial design. All results are

expressed as the mean \pm standard error of the mean (SEM), and were considered significant at $P < 0.05$.

RESULTS

ANOVA results regarding sperm quality characteristics in liquid-stored semen are shown in Table 1. The main effects of extender type, storage temperature and storage time significantly affected ($P < 0.001$) all of the analysed sperm quality characteristics. In addition, it was observed that only the interaction between extender type \times storage temperature exerted a significant effect on sperm quality characteristics.

Table 1. ANOVA sources of variations in sperm quality characteristics following liquid storage of boar semen

| Source | df | Motility | | Plasma membrane integrity | | Mitochondrial energy status | |
|--|----|---------------|---------|---------------------------|---------|-----------------------------|---------|
| | | F-test | P-value | F-test | P-value | F-test | P-value |
| Extender type | 2 | 729.16 | 0.001 | 216.09 | 0.001 | 259.72 | 0.001 |
| Storage temperature | 1 | 17.52 | 0.001 | 48.24 | 0.001 | 103.23 | 0.001 |
| Storage time | 1 | 274.36 | 0.001 | 48.11 | 0.001 | 21.10 | 0.001 |
| Extender type \times storage temperature | 2 | 3.89 | 0.021 | 20.24 | 0.001 | 19.55 | 0.001 |
| Source | df | Oxygen uptake | | ATP content | | | |
| | | F-test | P-value | F-test | P-value | | |
| Extender type | 2 | 77.93 | 0.001 | 41.86 | 0.001 | | |
| Storage temperature | 1 | 152.02 | 0.001 | 103.47 | 0.001 | | |
| Storage time | 1 | 20.70 | 0.001 | 80.71 | 0.001 | | |
| Extender type \times storage temperature | 2 | 5.27 | 0.005 | 3.73 | 0.005 | | |

ANOVA was used to analyse the interactions of the main effects: extender type (K3; K3-LPFh; K3-LPFo), storage temperature (5°C; 16°C) and storage time (Day 2; Day 3); df - degree of freedom; F - Fisher test; K3 - Kortowo extender; LPFh - Kortowo 3 (K3) extender supplemented with lipoprotein fractions isolated from hen egg yolk; LPFo - Kortowo 3 (K3) extender supplemented with lipoprotein fractions isolated from ostrich egg yolk

Means \pm SEM for motility and PMI of spermatozoa during liquid semen storage are shown in Table 2. Total motility of spermatozoa decreased continuously over time ($P < 0.05$) during storage, being less marked in both K3-LPFh and K3-LPFo extenders. There was a gradual deterioration in PMI of spermatozoa during storage in K3 extender. Furthermore, marked changes in PMI were more evident

Table 2. Total motility (%) and plasma membrane integrity (%) of spermatozoa following liquid storage of boar semen in different extenders at 5° and 16°C. Values represent the means (± SEM) for motility analysis (n=96) and plasma membrane integrity (PMI) assessment (n=35). Within rows values with different superscripts (a-f) are significantly differed at P<0.05 (Neuman-Keuls *post hoc* test)

| Sperm variables | Day 0 | | | Day 2 | | | Day 3 | | | | | | | | |
|-----------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | 5°C | | | 16°C | | | 5°C | | | 16°C | | | | | |
| | K3 | LPFh | LPFo | | | |
| Motility | 61.6 ± 1.4 ^a | 75.2 ± 0.5 ^b | 74.4 ± 0.6 ^b | 31.5 ± 2.0 ^c | 65.4 ± 1.3 ^a | 63.9 ± 1.4 ^a | 39.2 ± 2.0 ^d | 66.5 ± 1.2 ^a | 63.9 ± 1.3 ^a | 18.3 ± 1.3 ^e | 51.5 ± 1.8 ^f | 49.8 ± 1.9 ^f | 24.3 ± 1.5 ^g | 52.2 ± 1.8 ^f | 50.1 ± 1.7 ^f |
| PMI | 86.9 ± 1.8 ^a | 91.6 ± 0.6 ^a | 90.9 ± 0.7 ^a | 55.8 ± 2.7 ^b | 87.8 ± 1.1 ^a | 86.5 ± 4.5 ^a | 73.8 ± 2.2 ^c | 89.6 ± 0.8 ^a | 87.7 ± 1.2 ^a | 47.1 ± 2.8 ^d | 79.6 ± 2.3 ^e | 78.6 ± 3.3 ^e | 63.7 ± 2.5 ^e | 81.2 ± 1.3 ^e | 80.8 ± 1.6 ^e |

explanation see Table 1

Table 3. Mitochondrial energy status (%), oxygen uptake ($\mu\text{O}_2/10^8$ spz./1h /37°C) and ATP content (nmol ATP/ 10^8 spz./ 10^8) in spermatozoa following liquid storage of boar semen in different extenders at 5° and 16°C. Values represent the means (± SEM) for mitochondrial function assessments (n=29), and measurements of oxygen uptake and ATP content (n=96, respectively). Within rows values with different superscripts (a-g) are significantly differed at P<0.05 (Neuman-Keuls *post hoc* test)

| Sperm variables | Day 0 | | | Day 2 | | | Day 3 | | | | | | | | |
|------------------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| | 5°C | | | 16°C | | | 5°C | | | 16°C | | | | | |
| | K3 | LPFh | LPFo | K3 | LPFh | LPFo | K3 | LPFh | LPFo | K3 | LPFh | LPFo | | | |
| Mitochondrial energy status (JC-1) | 89.0 ± 1.7 ^{ac} | 93.6 ± 0.6 ^a | 92.8 ± 0.7 ^a | 55.4 ± 2.7 ^b | 85.6 ± 1.3 ^{ce} | 83.8 ± 1.9 ^{ce} | 73.5 ± 2.2 ^d | 91.5 ± 0.7 ^a | 88.7 ± 0.9 ^{ac} | 50.4 ± 2.7 ^b | 80.9 ± 1.4 ^e | 79.4 ± 2.0 ^{de} | 68.8 ± 2.3 ^g | 88.0 ± 1.0 ^e | 84.9 ± 1.2 ^c |
| Oxygen uptake | 27.0 ± 1.2 ^a | 28.4 ± 1.4 ^a | 28.7 ± 1.4 ^a | 12.3 ± 0.9 ^b | 20.8 ± 1.1 ^c | 19.7 ± 1.1 ^c | 17.8 ± 1.2 ^c | 28.3 ± 1.8 ^a | 28.2 ± 1.6 ^a | 9.1 ± 0.7 ^d | 16.1 ± 0.9 ^{ce} | 14.7 ± 1.1 ^e | 14.2 ± 0.8 ^e | 24.7 ± 1.5 ^a | 26.3 ± 1.9 ^a |
| ATP content | 12.9 ± 0.4 ^{ac} | 12.6 ± 0.3 ^{ac} | 12.4 ± 0.3 ^{ac} | 9.2 ± 0.4 ^b | 12.3 ± 1.1 ^{ac} | 11.0 ± 0.4 ^c | 12.4 ± 0.9 ^{ac} | 13.3 ± 0.9 ^a | 13.1 ± 0.9 ^a | 6.9 ± 0.3 ^d | 10.6 ± 0.4 ^b | 9.3 ± 0.3 ^b | 9.6 ± 0.9 ^b | 11.7 ± 0.4 ^e | 11.4 ± 0.3 ^c |

explanation see Table 1

on Day 3 of storage in the extenders containing LPF, regardless of the storage temperature. Within respective storage time, there were no significant changes ($P>0.05$) in total motility or PMI of spermatozoa between semen samples stored at 5° and 16°C in either K3-LPFh or K3-LPFo extender.

Means \pm SEM for mitochondrial energy status, oxygen uptake and ATP content in spermatozoa during liquid semen storage are shown in Table 3. Compared with K3 extender spermatozoa stored in either K3-LPFh or K3-LPFo extender exhibited higher mitochondrial energy status, as assessed by the JC-1 fluorescent probe. Moreover, marked changes in the mitochondrial energy status of spermatozoa were more evident during storage at 5°C, particularly in K3 extender. It was observed that spermatozoa stored at 5°C exhibited a marked decline ($P<0.05$) in oxygen uptake and ATP content compared with those stored at 16°C. Furthermore, oxygen uptake by spermatozoa did not differ over time ($P>0.05$) during storage in the extenders containing LPF at 16°C. There were no marked differences in ATP content in spermatozoa prior to cooling (Day 0) or on Day 3 of storage in both K3-LPFh and K3-LPFo extenders.

ANOVA results showed that the rate of L-lactate production was significantly affected by the extender type (d.f.=2, $F=21.56$, $P<0.001$) and storage temperature (d.f.=1, $F=150.13$, $P<0.001$). Compared with K3 extender, spermatozoa stored in either K3-LPFh or K3-LPFo extender exhibited a greater ($P<0.05$) rate of fructolysis, as indicated by the higher levels of L-lactate production (Table 4). Furthermore, the rate of fructolysis was markedly suppressed ($P<0.05$) in spermatozoa stored at 5°C compared with those stored at 16°C, irrespective of the extender type.

Table 4. Lactate production (nmol/10⁸/1h/37°C) by spermatozoa during liquid storage of boar semen in different extenders at 5° i 16°C. Values represent the means (\pm SEM) (n=45). Within rows values with different superscripts (a-f) are significantly differed at $P<0.05$ (Neuman-Keuls *post hoc* test)

| Day 0 | | | Day 3 | | | | | |
|--------------|--------------|--------------|--------------|-----------------|--------------|-----------------|--------------|--------------|
| | | | 5°C | | | 16°C | | |
| K3 | LPFh | LPFo | K3 | LPFh | LPFo | K3 | LPFh | LPFo |
| 653.1 | 635.5 | 608.5 | 229.4 | 340.2 | 265.7 | 369.1 | 502.4 | 490.0 |
| $\pm 30.8^a$ | $\pm 31.2^a$ | $\pm 33.3^a$ | $\pm 18.4^b$ | $\pm 23.8^{ef}$ | $\pm 18.2^e$ | $\pm 26.4^{df}$ | $\pm 25.7^e$ | $\pm 20.5^e$ |

explanation see Table 1

DISCUSSION

Liquid boar semen is usually stored at 15° to 20°C due to sperm susceptibility to cold shock damage (Pursel et al., 1972; Parks and Lynch, 1992). Sperm motility

is commonly used to evaluate the quality of liquid-stored semen at artificial insemination (AI) stations because it is one of parameters that is most seriously affected during storage. There were no marked changes in motility and PMI of spermatozoa during storage in the LPF-extended semen on days 2 and 3 at 5°C compared with those stored at 16°C, indicating the protective action of egg yolk lipoprotein fractions against cold shock-induced damage.

In the present study, sperm mitochondrial energy status was monitored with a mitochondria-selective fluorescent probe, JC-1, which enabled measuring membrane potentials measurements. Furthermore, the relatively high ATP content in spermatozoa observed in the LPF-extended semen might suggest that impairment of sperm motility was not clearly related to mitochondrial function. Using a bioluminescence assay, a previous study also reported that there were no significant changes in ATP content in boar spermatozoa preserved in Beltsville Thawing Solution (BTS) at 17°C (Long and Guthrie, 2006). It is noteworthy that ATP production, a sensitive metabolic function of spermatozoa, is directly linked to mitochondrial oxidative phosphorylation (Strzeżek, 1998). We suggested that the addition of egg yolk lipoprotein fractions to the semen extender helped to reinforce the stability of the plasma membrane of spermatozoa, thereby rendering them less susceptible to damage incurred during liquid storage, particularly at 5°C. It seemed likely that differences in the mitochondrial energy status of spermatozoa between semen samples stored at 5° and 16°C were not related to changes in sperm motility and plasma membrane integrity. Furthermore, the results of the current study indicate that cooled-storage of semen in K3 extender compromised sperm metabolic function.

It has been hypothesized that the ATP concentrations required for sperm motility are provided by mitochondrial respiration and glycolysis (Mukai and Okuno, 2004). Moreover, it is still under debate whether the ATP produced by the mitochondria can be delivered in sufficient quantities to supply the entire sperm flagellum (Westhoff and Kamp, 1997). It is important to emphasize that glycolytic ATP production in the principal tail piece of spermatozoa may compensate for the insufficient transport system of ATP to the distal dynein ATP-ases (Halang et al., 1985; Kamp et al., 1996). It has been postulated that boar spermatozoa are almost totally reliant on the mitochondrial respiration pathway due to the low energy output contributed by glycolysis/fructolysis (Strzeżek, 1998). This assertion is reinforced by the fact that the rate of fructose utilization by boar spermatozoa is significantly lower than in other animal species, probably due to the relatively low hexokinase activity (Medrano et al., 2005). Besides mitochondrial respiration and glycolysis, boar spermatozoa can utilize non-hexose substrates through other metabolic pathways to maintain the optimal energy level required for their basic functions (Medrano et al., 2005).

Boar spermatozoa are able to produce L-lactate during glycolysis and under aerobic conditions (Jones, 1997). During storage in the LPF-extended semen at 5°C, the reduction in L-lactate production by spermatozoa, accompanied by a decline in oxygen uptake and ATP content, indicates that glycolysis (fructolysis) was the primary source of the sperm energy pathway. In contrast, spermatozoa stored in the LPF-extended semen at 16°C relied more on mitochondrial respiration as their main energy pathway. The differences in energy metabolism observed during storage of semen at 5° and 16°C indicated that spermatozoa could directly modulate the consumption of energy substrates in response to varying environmental conditions. The findings of the current study suggest that spermatozoa stored at 5°C were able to conserve their metabolic activity, which is needed during cooling of semen prior to cryopreservation. Furthermore, the reliance of liquid semen during storage at 5°C on the glycolytic pathway as the principal source of energy reaffirmed that spermatozoa can modulate their energy metabolism to adapt to the extracellular environment when under found in stressful conditions. Such a feature is of particular importance during semen cryopreservation.

CONCLUSIONS

In conclusion, these findings reinforce and support the view that the metabolic activity of boar spermatozoa preserved in extenders containing egg yolk lipoproteins is affected by the different storage temperatures. Furthermore, the findings of this study indicate that both glycolysis and mitochondrial respiration are important metabolic pathways of boar spermatozoa that should be considered in improving semen preservation. Finally, further studies on the fundamental energy metabolism of spermatozoa are needed to develop optimal liquid storage and cryopreservation conditions for boar semen.

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