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Sperm motility and lactate production at different sperm concentrations

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1. Introduction

Low-volume, deep-horn insemination with highly concentrated semen has become popular recently. However, few data are available about conservation of highly concentrated equine semen. Moreover, equine sperm metabolism remains poorly documented. Carbohydrates are actively incorporated within the cell. Unlike what has been reported in most species, it seems that equine sperm lack the fructose transport pathway, thus spermatzoa mainly depend upon glucose for metabolism [1]. However, to our knowledge, there are no reports about equine sperm glucose requirements and consumption. With a molecule of glucose, glycolysis produces two lactate molecules under anaerobic conditions, but respective contributions of aerobiosis and anaerobiosis to sperm motility are not known. The aim of this study was to determine lactate production in equine semen stored at different concentrations and to correlate lactate production with total or progressive motility.

2. Material and methods

Five stallions were collected four times. Raw semen concentration was determined with a Nucleocounter™ (Chemometec®, Allerod, Denmark). Total and progressive motility (TM and PM, respectively) were determined with Computer Assisted Semen Analysis (CASA, Hamilton-Thorn, Beverly, MA, USA) with Leja™ cells using semen diluted to 20x10^{6}sperm/mL. The computer adjustments were: temperature of 37°C, 60 frames per second (60Hz), sperm cell size between 4 and 6 pixels. Motility was defined when the Average Path Velocity (VAP) was above 15μm/s, progressive motility was defined when the VAP was above 30μm/s, and straightness was defined above 50%. Analyses of sperm motility were performed 10 times on 10 different frames (10 replicates for each sample). Volumes of semen containing respectively 110 (C1), 440 (C4) and 880 (C8) x10^6 spz were extended with INRA96™ (IMV, L'Aigle, France) (1:4v/v). Cushion medium (1mL, Ioxidanol, Maxifreeze™, IMV, L'Aigle, France) was added in the bottom of the 15mL Falcon™ tube, and samples were centrifuged (20 min, 1000xg). After centrifugation, cushion medium and supernatant were aspirated. The sperm-rich pellet was then re-suspended in 1mL of autologous supernatant (containing 25% seminal plasma). Semen was then stored in a dark box maintained at 20°C. After 8 and 24h of storage, concentration, TM and PM were determined by CASA. As TM and PM in raw semen were stallion dependent, preservation of total motility (PTM) was calculated as TM at the time of sampling divided by raw semen initial TM (TM 8 or 24 / TM initial). The same ratio was similarly calculated for progressive (PPM) motility (PM 8 or 24 / PM initial). Lactate concentrations were assayed on the supernatant of first centrifugation (T0) and on the supernatant of centrifuged (20 min, 1000xg) semen stored during 24 hr (T24). Lactate was measured by ^{1}H Nuclear Magnetic Resonance (NMR) using a Bruker Avance 500 MHz equipped with a cryoprobe. Samples (600μL) were added with deuterated phosphate buffer (100μL) and with a maleic acid solution (100μL) used as reference compound. The samples were analysed using a Carr-Purcell-Meiboom-Gill sequence with presaturation (32 transients). The methyl signal of lactate at 1.3 ppm was integrated and compared with the signal of the reference in order to give a concentration value. Non-parametric Kruskall-Wallis or Friedman test was used to analyse differences between groups with Dunn's post-test. Correlations were studied with Spearman's test for non-normally distributed data. Statistical significance was established at $p<0.05$.

3. Results

Mean storage concentrations for groups C1, C4 and C8 were, respectively: 70±30, 434±120 and 879±241

x10^6sperm/mL. Sperm recovery rate after centrifugation was lower in low concentration samples ($p<0.001$). Mean lactate concentration at T0 was the same in all three groups and directly correlated with raw semen volume ($r=0.5032$; $p=0.0103$). Lactate concentrations at T0 and T24 were not correlated. Compared to T0, lactate concentration was increased at T24 when groups were pooled, in C1, C4 and C8 groups separately, and lactate concentration was directly correlated with sperm concentration ($r=0.6080$; $p=0.0002$). At T24, lactate concentration was lower in C1 samples ($p<0.001$) when compared to lactate concentrations observed in higher sperm concentrations. In highly concentrated samples (C8), PTM was decreased after 8 hr but not PPM ($p<0.01$). After 24 hr, low concentration samples showed higher PTM and PPM ($p<0.01$). PTM and PPM after 24 hr were negatively correlated to lactate concentrations at T0 ($r=-0.4568$; $p=0.0217$ and $r=-0.4684$; $p=0.0182$). Concentrations of TM spermatozoa after 8 and 24 hr were correlated to lactate concentration at T24 (respectively, $r=0.5373$; $p=0.0015$ and $r=0.5320$ and $p=0.0017$). The same correlation was observed for the concentrations of PM spermatozoa after 8 and 24 hr ($r=0.5738$; $p=0.0006$ and $r=0.5579$ and $p=0.0009$, respectively). Concentration of NPM after 24 hr of storage was highly correlated to lactate concentration at T24 ($r=0.6767$; $p<0.0001$).

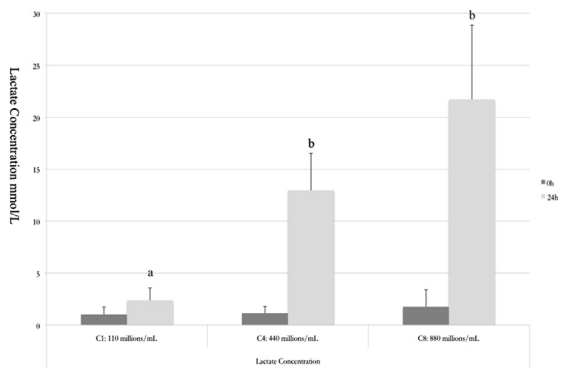

Fig. 1. Lactate concentration (mmol/L) according sperm concentration in samples and storage. Different letters in superscript indicate statistically different values.

4. Discussion

Raw semen parameters are not associated with lactate concentration at T0, except the initial ejaculate volume. These data show that lactate concentration at T0 depends on collection conditions. It also shows that the negative effect of lactate on semen parameters is not observed immediately but during storage. In the present study, semen storage with high sperm concentration is rapidly deleterious for total motility, whereas progressive motility decreases between 8 and 24 hr. This delayed effect of high storage concentrations on progressive motility suggests that insemination with highly concentrated semen should not be performed later than 8 hr. Volume of the raw ejaculate is associated with lactate concentrations at T0 while raw semen concentration is not. Lactate concentration at T0 was negatively associated with progressive and total motility after 24 hr. This suggests that production and composition of seminal plasma could interfere with lactate concentration in raw semen and with its long-term conservation. Seminal plasma components should be studied to determine changes in metabolic products. A direct association between lactate concentration after 24 hr and total sperm concentration was observed, reflecting anaerobic metabolism by spermatozoa. Concentration of NPM spermatozoa was strongly correlated to lactate concentration after 24 hr of storage, suggesting an increased use of anaerobic glycolysis by the subpopulation of non-progressively motile spermatozoa. To conclude, lactate production is associated with total sperm concentration. It negatively affects preservation of total and progressive motility, showing an effect of by-products of anaerobic metabolism on long-term sperm storage. Moreover, our data show that non-progressively motile spermatozoa are highly associated with lactate concentration, and thus, anaerobic glycolysis. More studies are required to determine relative contributions of aerobiosis and anaerobiosis to sperm motility under different storage conditions.

Reference

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