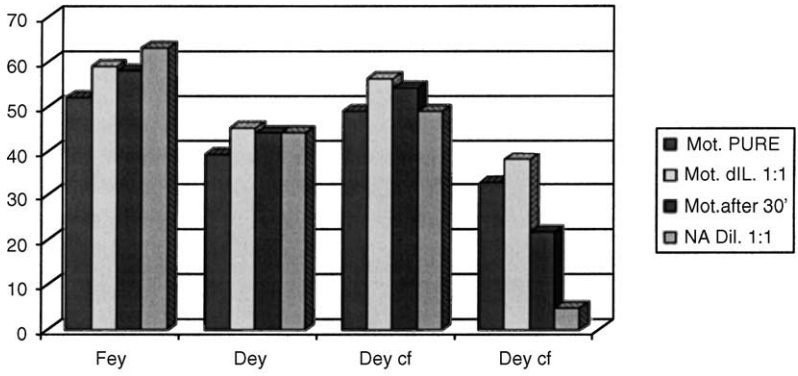

Fig. 1

One inconvenience of the use of egg yolk is the field nastiness when samples have to be microscopically evaluated. To avoid this, both egg yolks were centrifuged and filtered (Fey cf; Dey cf) before their study. Our results indicate that, despite the similar biochemical composition, the freezing results are better by using fresh egg yolk, in comparison with dehydrated egg yolk. However, further studies on the protective effects of the different egg yolk components on the spermatic outer membrane would help us to select from them those presenting the highest preservation ability in order to be isolated and utilized as freezing extenders in boar semen freezing.

2.7
EVALUATION OF MEMBRANE SULFHYDRYL STATUS OF BOAR SPERMATOZOA AFTER COLD SHOCK

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The process of cooling and freezing/thawing is associated with a marked effect on the sulfhydryl groups of proteins from the sperm surface. In this study, these groups were evaluated with a fluorescent staining 5-iodoacetamidofluoresceine (5-IAF) and some classical seminal parameters like motility (MOT), forward progressive motility (FPM), viability measured with carboxyfluorescein diacetate/propidiurn iodide (DCF) and acrosome (NAR) in diluted boar semen samples were assessed. In a second experience the effect of cooling at 5 °C for 30, 60 and 120 min was evaluated.

In 76 semen samples evaluated, 19.71 ± 1.08% of the spermatozoa were stained with 5-IAF. Most of them showed a homogeneous staining pattern over the whole sperm (74.93%), while only 13.33% showed only a post-acrosomal staining and 11.14% only the mid-piece. No effect of the time of conservation at refrigeration conditions (15 °C) of the samples was detected with percentage of 5-IAF staining at 0, 24 and 48 h (20.16 versus

18.53 versus 20.39, $P = 0.7515$). An inverse significant relation was found between 5-IAF and motility, viability and NAR ($P < 0.05$).

When boar spermatozoa were stored at 5 °C a significant decrease of NAR and 5-IAF was detected since 30 min, and a decrease of viability at 2 h of storing. However no effect was found in motility.

	MOT	FPM	NAR	DCF	5-IAF
Control	75.58 ± 1.51	2.91 ± 0.03	92.71 ± 0.79[a]	77.43 ± 2.18[a]	15.92 ± 1.45[a]
30 min, 5 °C	72.64 ± 2.71	2.85 ± 0.06	88.93 ± 0.86[b]	70.11 ± 2.94[ab]	30.51 ± 2.34[b]
60 min, 5 °C	73.46 ± 2.05	2.90 ± 0.04	89.08 ± 0.59[b]	68.04 ± 2.24[ab]	30.87 ± 3.17[b]
120 min, 5 °C	70.25 ± 2.22	2.87 ± 0.05	87.94 ± 0.84[b]	61.97 ± 3.56[b]	35.85 ± 7.84[b]
Probability	0.2965	0.7741	0.0001	0.0013	0.0047

These preliminary results in the use of 5-IAF in boar semen show as the cold shock produces an alteration in the structure of the sperm membranes that could be detected by simple fluorescent staining. It suggests 5-IAF could be a good tool to help in studding the generation of reactive oxygen species and changes in the proteins in the sperm membranes than take place during the cooling and freezing/thawing.

Acknowledgement

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2.8

EFFECT OF FREEZING RATE, THAWING TIME AND THAWING TEMPERATURE ON NORMAL ACROSOME AND MOTILITY OF FROZEN-THAWED BOAR SPERM

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This study was carried out to obtain information regarding the effects of freezing rate, thawing time and thawing temperature in the lactose–egg yolk and *N*-acetyl-D-glucosamine (LEN) diluent on acrosome morphology and motility of frozen-thawed boar sperm. Semen was collected from three Yorkshire boars one time per week. The sperm-rich fractions of ejaculates with greater than 90% motile sperm and normal acrosome were used. Experiment 1 was designed to determine the effect of aluminum rack distance from liquid nitrogen (LN) storage on post-thaw sperm motility and normal acrosome in the LEN diluent of 5 ml maxi-straw. Aluminum rack distances were 5, 11 and 17 cm from LN. Experiment 2 was carried out to investigate the effect of submersion time and sample temperature in 52 °C water bath on post-thaw sperm motility and normal acrosome in the LEN diluent of 5 ml maxi-straw. Submersion times were 30, 35, 40, 45 and 50 s at 52 °C.