Changes in activities of plasma membrane bound sperm enzymes during semen processing

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INTRODUCTION

The physico-chemical changes in spermatozoa that take place during semen processing and storage regulate the fertilising ability of spermatozoa. Spermatozoa1 motility, traditionally used as a marker of fertilising ability, does not indicate the true fertilising ability of spermatozoa (Smith et al., 1993), although motility is required for in *vivo* fertilisation. In this study we have monitored changes in activities of spermatozoa1 enzymes at various stages of semen processing. In future, these changes could be correlated to fertilising ability. It is also anticipated, that the information on these enzymes would improve our understanding of basic sperm physiology.

MATERIALS AND METHODS

Semen processing and incubations: Both ram and bull semen were used in this study. Semen from 3 different lots of 6 rams were combined in separate pools and ejaculates from 3 bulls were used individually The Semen from both ram and bull was diluted to 400 x 10^6 spermatozoa/ml in a Tris-egg yolk-glycerol diluent and equilibrated to 20°C. The enzymatic measurements were made at the following stages of semen processing: equilibrated to 20°C after cooling to 5°C after freezing in liquid N2 and thawing at 35°C, and during post-thaw incubations at 38°C at 4 and 24 hours after diluting the frozen-thawed semen in RSD-1 (Upreti et al., 1995) to 80 x 10^6 spermatozoa/ml.

Enzymatic assays: Aliquots of semen containing a total of 2400x 10^6 spermatozoa were obtained at each stage. Spermatozoa were separated from the diluent by pelleting the sperm through a dense sucrose medium (Ashworth *et al.* 1994). The pellet was resuspended in Tris buffer and the suspension was extracted with Tris buffer containing Triton X- 100, citrate and glycerol. The 10,000g supernatant of the extract was used for enzyme assays.

The enzymes Snucleotidase, Ca-Mg-ATPase and alkaline phosphatase were measured by following the release of inorganic phosphate as previously described (Upreti, 1996).

RESULTS

The level of 5'nucleotidase and alkaline phosphatase differed ($P<0.01$) between ram and bull spermatozoa, but the levels of Ca-Mg ATPase were similar in both species (Table 1) at 20°C. Cooling to 5°C did not influence the activities of these enzymes for either ram or bull spermatozoa.

The freeze-thaw process resulted in significant ($P<0.001$ to <0.01) increases of all three enzymes for ram spermatozoa while with bull spermatozoa, the enzyme activities were either similar or marginally reduced.

Incubations at 38°C resulted in a decline of enzyme activities for both species, with the exception of ram sperm Snucleotidase.

DISCUSSION AND CONCLUSIONS

This study has shown that sperm enzymes from both species differ in their levels as well as in changes to the levels of enzyme activities when semen is processed. 'Similar differences in levels of 5'nucleotidase in the seminal plasma of bull and man have also been reported (Minelli et al., 1990).

We suggest that the dramatic increases in activities of ram sperm enzymes during the freeze-thaw step may be associated with the relatively low fertilising ability of ram spermatozoa. We anticipate using these enzyme markers to optimize the freeze-thaw process for ram spermatozoa.

REFERENCES

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TABLE 1: Activities of plasma membrane bound enzymes (Units/ 10^{12} spermatozoa) at various stages of semen processing.

	5'Nucleotidase		Ca-Mg-ATPase		Alkaline phosphatase	
Stages	Ram	Bull	Ram	Bull	Ram	Bull
Prefreeze -20°C	9.5 ± 3.2	98 ± 28	179 ± 13	172 ± 26	350 ± 38	50 ± 16
Prefreeze -5°	9.1 ± 4.2	103 ± 27	176 ± 14	180 ± 84	34 ± 64	52 ± 14
Post thaw -0 h	16.6 ± 4.9	91 ± 19	310 ± 41	153 ± 13	496 ± 83	49 ± 14
Post thaw -4 h	17.1 ± 7.9	71 ± 15	263 ± 8	110 ± 3	358 ± 50	34 ± 8
Post thaw -24 h	20.9 ± 11 .0	87 ± 18	223 ± 21	95 ± 13	472 ± 78	30 ± 9

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