

EFFECT OF CRYOPRESERVATION AND EXTENDERS ON THE EXPRESSION AND LOCALIZATION OF SPERM PROTEIN AT 22 KDA (SP22) ON EQUINE SPERMATOZOA

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The presence of sperm protein at 22 kDa (SP22) on spermatozoa has been correlated with fertility in rats. We showed that cryopreservation of equine semen changed the localization pattern of SP22 significantly compared to pre-freezing samples; we hypothesized that the localization pattern of SP22 vary among cryopreservation protocols. The objective of this report was to compare the expression and localization of SP22 on equine spermatozoa following cryopreservation of semen in two different extenders with different sugar and buffer base. Eighteen semen samples from three fertile stallions were collected and divided into three treatment groups: (a) fresh semen, (b) cryopreserved semen in freezing extender A, and (c) cryopreserved in freezing extender B. Semen undergoing cryopreservation was subjected to a standard cryopreservation and thawing protocol, with the extenders as the only variable. After thawing, all samples were stained immunocytochemically using a primary SP22-antibody and FITC-conjugated secondary antibody. SP22 localization was determined using fluorescence microscopy. Specific staining patterns were determined on 100 sperm from each ejaculate. Data were expressed as means $\pm$ SEM. SP22 was detected by immunocytochemistry in all samples. Four different ($p < 0.01$) localization patterns of SP22 were observed in fresh semen: (1) overlying the acrosome and equatorial region (AER; $69\% \pm 3$), (2) overlying the acrosome (A; $13\% \pm 2$), (3) equatorial region (ER; $13\% \pm 3$), and (4) neck (N; $5\% \pm 1$). There was a significant difference in the localization pattern of SP22 between fresh and frozen/thawed semen ($p < 0.01$), and between freezing extender A and freezing extender B ($p < 0.01$). Staining for SP22 over the equatorial region (ER) was found in $42\% \pm 3$ of spermatozoa frozen in extender A, but only in $12\% \pm 1$ of spermatozoa frozen in extender B. Staining for SP22 overlying the acrosome was found in $4\% \pm 1$ of spermatozoa frozen in extender A, and $42\% \pm 1$ of spermatozoa frozen in extender B. No significant differences were found in staining overlying the acrosome and the equatorial region (AER; A: $13\% \pm 3$ and B: $4\% \pm 1$), and the neck (N; A: $20\% \pm 2$ and B: $23\% \pm 2$), between spermatozoa frozen in the two extenders. Scattered staining of SP22 over the head (S) was not detected in fresh semen, but was observed in $21\% \pm 3$ of spermatozoa frozen in extender A, and in $18\% \pm 2$ of spermatozoa frozen in extender B. The altered localization of SP22 on frozen/thawed spermatozoa suggests that the spermatozoa's plasma membrane has been altered or that SP22 has been redistributed during the freeze/thaw process. The effect of freezing extenders on the localization of SP22 on cryopreserved semen warrant further investigations to determine if this protein may be a potential marker for sperm plasma membrane stability of cryopreserved sperm, as well as for fertility of equine spermatozoa.

Keywords: SP22; Equine; Spermatozoa; Cryopreservation; Immunohistochemistry