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Does Seminal Plasma PSP-I/PSP-II Spermadhesin Modulate the Ability of Boar Spermatozoa to Penetrate Homologous Oocytes In Vitro?

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Low concentration (0.15 mg per million of spermatozoa) of seminal plasma–derived PSP-I/PSP-II spermadhesin heterodimer is able to preserve the viability of highly extended boar spermatozoa. Whether spermatozoa also keep their fertilizing capacity is not yet known. The present study evaluated the effect of exposing freshly extended and frozen-thawed boar spermatozoa (10 million/mL) to PSP-I/PSP-II (1.5 mg/mL) for 30 or 120 minutes on sperm characteristics and the outcome of in vitro penetration of immature (IM) and in vitro matured (IVM) homologous oocytes, aiming to identify this spermadhesin as a suitable modulator for sperm-handling protocols. Although exposure to the heterodimer improved sperm viability and motility without increasing the levels of sperm acrosome exocytosis in both freshly extended and frozen-thawed spermatozoa, this pretreatment did not affect sperm penetration rates or sperm numbers per oocyte when pretreated fresh spermatozoa were coincubated with IM or IVM oocytes compared with controls. When cryopreserved spermatozoa were tested, however, on IVM oocytes, already a 30-minute preincubation exposure to PSP-I/PSP-II showed a significant blocking effect on penetration rate (from 90% to 32%, $P < .05$) and on mean sperm numbers per oocyte (2.9 to 1.6, $P < .05$). To disclose the nature of this paradox, frozen-thawed spermatozoa were cleansed (by centrifugation in saline bovine serum albumin or through Percoll density gradient separation) and the procedure repeated. Oocyte penetration (but not number of spermatozoa per oocyte) increased ($P < .05$) when spermatozoa were cleansed with Percoll compared with either washed or unwashed controls (53% vs 13% vs 31%, respectively). In addition, the percentages of polyspermic oocytes remained lower than control (38.5% vs 68.7%, respectively; $P < .05$). In conclusion, the results confirm that exposure of fresh or frozen-thawed boar spermatozoa to a low dose of seminal PSP-I/PSP-II spermadhesin preserves sperm viability and motility in vitro. Although there was no obvious influence of the heterodimer on the capability of freshly extended boar spermatozoa to penetrate homologous oocytes (either IM or IVM), PSP-I/PSP-II

exerted a deleterious effect when frozen-thawed spermatozoa were used to penetrate IVM oocytes. Such an effect of cryopreservation seems to a certain extent reversible, since cleansing of the sperm surface decreased, at least partially, this blocking effect, increasing both penetration and the monospermic rates.

Key words: Seminal plasma, preservation, sperm viability, IVF, pig

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