and that antibody incubated with UTF decreased embryo development *in vitro*. This study was conducted to determine whether IVF of bovine oocytes would be affected by pretreating either the sperm or oocytes, or both, with L-PGDS antibody. *In vitro*-matured bovine oocytes were incubated for 1 h in IVF TALP medium supplemented with penicillamine, hypotaurine, epinephrine, and heparin containing (a) no antibody, or (b) a rabbit polyclonal antibody against recombinant bovine L-PGDS (α L-PGDS; 1:2000). Frozen–thawed spermatozoa were washed by a 45:90% layered Percoll gradient centrifugation and incubated for 1 h in IVF TALP with (a) no antibody, or (b) α L-PGDS. For this study we had 4 different treatments: (1) no antibody (control), (2) α L-PGDS at fertilization time, (3) α L-PGDS-treated oocytes, or (4) α L-PGDS-treated sperm. Oocytes were inseminated with 10×10^4 washed spermatozoa in 4-well culture dishes. After 18 h (39°C, 5% CO_2 in air), oocytes were vortexed to remove cumulus cells and accessory spermatozoa, and fixed in 3.7% paraformaldehyde and 10% Triton X–100 for 1 h. Oocytes were washed and transferred to a solution with PBS, 0.3% BSA, and 1% Triton for 1 h, stained with Hoechst 33342, and observed in the presence of 2 pronuclei in the cytoplasm. There were 4 replicates of 200 to 250 oocytes for fertilization assays. Data were analyzed by SAS. Addition of α L-PGDS with sperm, oocytes, or both significantly decreased fertilization ($P < 0.05$) compared with the control: (1) $89.2 \pm 2.0\%$; (2) $19.4 \pm 2.0\%$; (3) $27.2 \pm 3.1\%$; or (4) $14.1 \pm 3.4\%$. These studies demonstrated that a rabbit polyclonal antibody against recombinant bovine L-PGDS reacts with both oocytes and spermatozoa, resulting in inhibition of fertilization *in vitro*, and has a possible role in bovine fertilization.

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204 AMINO-REACTIVE CROSSLINKER BIS(SULFOSUCCINIMIDYL)SUBERATE INDUCES ZONA PELLUCIDA HARDENING AND REDUCES PENETRATION IN PIG *IN VITRO* FERTILIZATION

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Zona pellucida (ZP) hardening is considered to be the final step in the prevention of polyspermy during fertilization in mammals. However, unfertilized pig oviductal oocytes show a resistance of hours or days to pronase digestion (Broermann *et al*. 1989 J. Anim. Sci. **67**, 1324–1329). We previously demonstrated that the amino-reactive crosslinker DSP is effective in inducing ZP hardening and improves the monospermy levels at pig IVF (Coy *et al*. 2007, Reprod. Dom. Anim., in press). In this study, a different chemical crosslinker, BS^3 [bis(sulfosuccinimidyl) suberate], which also forms stable amide bonds among proteins, was used to evaluate its effect on ZP digestion time, penetration, male pronuclear formation and monospermy percentages, and the mean number of sperm per oocyte. In experiment 1, porcine *in vitro*-matured oocytes ($n = 300$) were incubated for 30 min at 0, 0.06, 0.30, or 0.60 mg mL^{-1} of BS^3 in TALP medium and assessed for ZP digestion time in 0.5% pronase solution. The results (analyzed by ANOVA in all the experiments) showed a significant ($P \leq 0.01$) dose-dependent increase in ZP hardening, from 69.0 s in the control to 426.3, 2028.3, and 2979.2 s, respectively, for the different BS^3 concentrations. In experiment 2, oocytes ($n = 473$) were fertilized *in vitro* after no treatment or treatment with BS^3 at 0.06, 0.30, and 0.60 mg mL^{-1}. Fresh ejaculated spermatozoa were selected by Percoll® gradient 45:90. Oocytes were inseminated with 10^5 sperm mL^{-1}, which resulted in high penetration and polyspermy percentages in the control group (83.1 and 89.9%, respectively). However, for the BS^3-treated oocytes, significant differences compared with the control group ($P \leq 0.001$) were observed in all 3 groups, showing penetration percentages of 22.2, 18.1, and 21.5%, respectively, and monospermy percentages of 100, 88.2, and 95.0%, respectively. The mean numbers of sperm per oocyte were 1.0, 1.1, and 1.05 for the BS^3 groups, which were significantly different from 5.0 for the control group. In conclusion, BS^3 can be used to induce ZP hardening in the pig and regulate polyspermy in IVF systems, although additional experiments are necessary to find the optimal concentration to improve the penetration percentages with high levels of monospermy.

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205 CAPACITATION OF STALLION SPERMATOZOA EVALUATED BY FERTILIZATION OF BOVINE OOCYTES

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In vitro fertilization in the horse does not work reliably. Several methods of capacitating sperm in other species fail in the horse. The goal of this experiment was to develop a method to capacitate equine spermatozoa using calcium ionophore A23187 or phosphatidylcholine 12 (PC12). We also studied effects of maturing bovine oocytes for 24 or 28 h on fertilizability by capacitated equine sperm, hypothesizing that longer maturation would yield oocytes more easily fertilized by equine spermatozoa. Two sets of bovine oocytes were aspirated from 3 to 8 mm follicles of abattoir ovaries 4 h apart, but fertilized at the same time. On the day of fertilization, semen from 1 of 3 stallions was collected, evaluated, and centrifuged through 33% Percoll to remove seminal plasma. The resultant pellet was extended to 5×10^7 cells mL^{-1} in M199 containing 0.6% BSA, 2 mM caffeine, and 5 mM $CaCl_2$. Sperm were treated with A23187 (1 or 3 μM) or PC12 (40 or 70 μM) or both A23187 and PC12 (1 μM/40 μM) in 500-μL aliquots. Sperm were incubated at 39°C for 10 min (for A23187 and combination treatments) or 15 min (for PC12 treatments), and then diluted 1:20 for fertilization. Oocytes from each maturation time were fertilized using the same semen preparation for each treatment. Oocytes and sperm were incubated together for 18 h in FCDM in 5% CO_2 at 39°C (De La Torre-Sanchez *et al.* 2006 Reprod. Fertil. Devel. **18**, 585–596). Presumptive zygotes were cultured for 30 h in CDM-1, vortexed to remove cumulus cells, and evaluated for cleavage. Oocytes were also co-incubated with killed sperm to determine the level of parthenogenesis. Cleaved embryos were stained with orcein to ensure that each cell had a nucleus. Number of cell divisions were recorded as 0 for a 1-cell, 1 for a 2-cell, 1.5 for a 3-cell, etc. More oocytes cleaved after 28 h (18%) than 24 h (14%) maturation ($P < 0.01$). Sperm of Stallion