Effect of semen extender and centrifugation through Percoll on binding of stallion sperm to bovine zonae pellucidae

Marco A. Coutinho da Silva, E.M. Carnevale, G.E. Seidel Jr. and E.L. Squires
ARBL, Colorado State University, Fort Collins, Colorado 80523

During equine intraoviductal inseminations, sperm often are centrifuged through Percoll, diluted in extender, and deposited into the oviduct. Sperm centrifugation though Percoll and presence of semen extender in the oviduct could interfere with some aspects of fertilization. Objectives were to determine effects of semen extenders and centrifugation through Percoll on binding of stallion sperm to bovine zonae pellucidae (ZP). Denuded immature bovine oocytes were stored at 5 ºC in salt solution (1.5 M $MgCl_2$, 40 mM Hepes, 0.1% PVP). Experiment I used 6 ejaculates from 3 stallions. Semen was centrifuged at 300 x g for 6 min, and sperm pellets were resuspended in 1 ml of each of 4 extenders [TALP, EZ-Mixin® "CST" (EZ), EmCare® (EMC), and Lactose-EDTA (LAC)]. Sperm were stained with Hoechst 33342 (20 μg/ml) and incubated for 15 min at 37 ºC. Sperm were then centrifuged at 300 x g for 6 min and resuspended with the same extender to $2x10^6$ sperm/ml. Oocytes were washed twice and incubated in TALP for 1 h at 38.5 ºC before placement into 4 droplets of 45 μL of TALP (5 to 10 oocytes/Trt). Extended sperm (5 μL) were added to oocytes, resulting in a final concentration of $2x10^5$ sperm/ml, and incubated for 2 h at 38.5 ºC. After incubation, oocytes were pipetted in TALP to remove loosely attached sperm. Oocytes were observed with a fluorescence microscopy (400x) to determine number of sperm bound to ZP (Table 1). Experiment II used 4 ejaculates from 2 stallions. After initial centrifugation, sperm were resuspended in 1 ml of TALP or EZ and stained with Hoechst 33342. Sperm were centrifuged at 300 x g for 10 min through 90/45 % Percoll (TALP+P, EZ+P) or not (TALP, EZ, EZ+TALP). All samples were resuspended to 2×10^6 sperm/ml with TALP, except that EZ was resuspended in EZ. Oocytes were inseminated, cultured and evaluated as described for Experiment I (Table 2). Data were Log transformed and analyzed by ANOVA and Tukey's hsd with treatments and stallions in the model. There were significant stallion effects ($P<0.01$). Untransformed data are presented.

Table 1: No. of sperm bound to ZP, Expt. I

Treatment	N	Mean	SEM
LAC	53	28.9[a]	2.7
TALP	56	32.1[a]	2.9
EMC	59	38.3[a]	2.5
EZ	60	95.6[b]	4.6

[a, b] Values with different superscripts differ ($P<0.01$).

Table 2: No. of sperm bound to ZP, Expt. II

Treatment	N	Mean	SEM
TALP	38	20.1[a]	2.2
TALP + P	35	25.8[a]	1.9
EZ	39	140.4[b]	7.9
EZ + P	38	31.7[a]	5.0
EZ + TALP	37	29.2[a]	3.0

Centrifugation of sperm through Percoll did not affect sperm binding to the ZP. Binding of stallion sperm to bovine ZP increased markedly when sperm diluted in EZ were added to oocytes, resulting in 10% EZ. The effect was negated when sperm diluted in EZ were washed prior to insemination. Thus, components of EZ enhanced sperm binding to ZP. Further studies using equine ZP are needed to determine if components of EZ affect stallion sperm binding to homologous ZP. These studies may increase our understanding of fertilization in the horse.

Keywords: horse, zona pellucida, sperm, extender, Percoll