Sertoli cells are the only somatic cells present in the seminiferous tubules. Their function is to guarantee proper sperm formation and maturation. Each Sertoli cell is responsible for nursing a finite number of spermatogonia. At puberty, Sertoli cell maturation and lumen formation have occurred within the seminiferous tubules and germ cells have proliferated rapidly followed by the onset of spermatogenesis. At least two hormones are known to play a role in Sertoli cell proliferation and maturation: follicle-stimulating hormone (FSH) and thyroid hormone. FSH secretion has been assumed to be the stimulus for proliferation. The thyroid hormone is responsible for normal postnatal growth and development. Alterations in thyroid activity have frequently been associated with changes in male reproductive functions, since hypothyroidism, induced with 6-N-propyl-2-thiouracil (PTU) soon after birth, is associated with a marked delay in sexual maturation and development. The goal of this study was to report the effect of FSH and PTU on the stages of sperm cell development of young pigs. Six piglets of 1, 7, 14, 25, and 55 days of age were castrated and their testes were sectioned to grafts of 5 mm^3. The grafts were then transplanted subcutaneously into the dorsum of 12 castrated nude mice per age group. Two days post-surgery mice were randomly assigned to one of four treatment groups: control, FSH (5 IU rFSH), PTU (0.015% solution), and FSH+PTU. Following 14 days of treatment, testicular tissue pieces were allowed to grow for 2 additional weeks. Tissues were then harvested, immersion-fixed in neutral buffered formalin, and embedded in paraffin. Five-micron-thick sections were stained using hematoxylin and eosin. Slides were evaluated under light microscopy and the oldest germ cell type present in each section was recorded. Germ cell types were recorded as spermatogonium, spermatocyte, early spermatid, and late spermatid. Statistical differences between all groups were detected using paired Student *t*-tests. There were no differences noted between control groups and those treated with PTU or FSH alone. No effect concerning age of castration on grafts development was observed. There was a slightly significant increase ($P = 0.05$) in the number of spermatocytes observed in the groups treated with FSH+PTU. These data suggest that there is a potential synergistic effect of FSH and PTU on sperm cell development. Based on these results, further studies need to be performed to completely understand the effect of these two hormones on Sertoli cells.

221 PREPUBERTAL MOUSE BIOASSAY FOR OVULATION-INDUCING FACTOR IN SEMINAL PLASMA

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A substance in the seminal plasma of llamas and alpacas has been discovered that induces ovulation and growth of the corpus luteum (CL) in the female of the same species. The ovarian effects of the ovulation-inducing factor (OIF) are associated with a surge release of LH into circulation. Ultrasonographic detection of ovulation and CL development is currently the only method available for testing the bioactive effects of OIF. The purpose of this study was to determine if a superstimulatory prepubertal mouse model could be developed as an *in vivo* bioassay for OIF. Prepubertal female CD1 mice ($n = 144$), 20 days of age and weighing 20–25 g, were housed at 24°C with lights on from 0500 to 1900 h and free access to food and water. An intramuscular dose of 5 IU of eCG (Novormon, Bioniche Animal Health, Belleville, ON, Canada) was given (Day 0) for ovarian superstimulation. On Day 2, mice were assigned randomly to 4 groups ($n = 36$ per group) and given a single 0.1 mL intraperitoneal dose of (1) 5 IU of hCG (Chorulon, Intervet Canada, Ltd., Whitby, ON, Canada), (2) 5 µg GnRH (gonadotropin-releasing hormone: Cystorelin, Merial, Ltd., Iselin, NJ, USA), (3) llama seminal plasma, or (4) phosphate-buffered saline (negative control). On Day 3, females were euthanized by an overdose of inhaled halothane. Oviducts were collected and oocytes were counted using trans-illumination stereomicroscopy. The proportion of mice that ovulated did not differ among groups treated with hCG, GnRH, and seminal plasma (31/36, 31/36, 28/36, respectively); however, the proportion of mice that ovulated in each treatment group was greater than that in the saline-treated group (9/36) ($P < 0.001$). The number of oocytes counted (mean $\pm$ SEM) was also similar among groups treated with hCG (25.8 ± 2.9), GnRH (27.4 ± 2.7), and seminal plasma (19.2 ± 2.8), all of which were greater ($P < 0.01$) than in the saline-treated group (6.2 ± 2.1). We conclude that the superstimulated prepubertal CD1 mouse model is effective as an *in vivo* bioassay for OIF in seminal plasma. Whether the bioassay may be used for quantitative estimates of OIF activity will require dose-response trials using serial dilutions of seminal plasma.

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222 SPECIES SPECIFICITY OF PORCINE SPERM MOTILITY REDUCTION BY A HIGH MOLECULAR WEIGHT FRACTION OF OVIDUCTAL FLUID

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The significance of sperm motility with respect to fertilization is widely recognized and used as a criterion to assess the quality of ejaculates. Observations of sperm behavior in the oviductal isthmus of several species have shown that their motility is suppressed in this physiological environment because the spermatozoa bind to the oviductal epithelial cells, forming a sperm reservoir prior to ovulation (Hunter 1981 J. Reprod. Fertil. **63**, 109–117; Hunter and Wilmut 1984 Reprod. Nutr. Dev. **24**, 597–608). Once the spermatozoa are released from the reservoir, they progress toward the ampullar region to reach the oocyte, and an increase in motility at this point could, potentially, be crucial. It has been demonstrated that a soluble fraction of oviductal epithelial cell apical plasma membrane proteins (sAPM) suppresses sperm motility and enhances sperm survival (Holt *et al.* 2005 Reprod. Fertil. Dev. **17**, 683–692; Satake *et al.* 2006 J. Exp. Biol. **209**, 1560–1572). However, few studies to date have investigated the

influence of oviductal fluid (OF), the natural medium into which spermatozoa are released from the reservoir, on sperm motility. Consequently, this study aimed to determine the effects of different soluble fractions of OF on sperm motility and the species specificity of such effects. OF from pigs and cows was collected and selectively filtered to obtain two different fractions with molecular weights higher or lower than 100 kD. Diluted semen samples from 14 different boars were exposed to bicarbonate/CO_2 (to stimulate maximum motility) in the presence or absence of OF fractions. Sperm trajectories were measured using a Hobson Sperm Tracker (Hobson Tracker, Ltd., Sheffield, UK) and analyzed by PATN analysis as described previously to identify subpopulations of high and low motility spermatozoa (Abaigar *et al.* 1999 Biol. Reprod. **60**, 32–41; Satake *et al.* 2006). The results showed that neither of the bovine OF fractions affected the proportions of the fast linear boar sperm subpopulation in the samples, which was similar to that of the control. However, when the high molecular weight fraction of porcine OF was used, a significant suppression of the fast linear sperm subpopulation was observed ($P \leq 0.05$). These data support the hypothesis that species-specific, high molecular weight components in OF are involved in the suppression of sperm motility. Further studies are required to confirm the significance of this finding, although it may not be unreasonable to speculate that the OF, in addition to other sperm selection mechanisms, acts to protect oocytes against fertilization by poor quality spermatozoa (Okada *et al.* 1986 J. Submicrosc. Cytol. **18**, 233–247). In fact, similar results demonstrating that oviductal fluid decreases sperm motility have been obtained in cow (Grippo *et al.* 1995 J. Reprod. Fertil. **105**, 57–64) and rabbit (Overstreet and Cooper 1979 J. Reprod. Fertil. **55**, 53–59).

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223 ENZYMATIC ACTIVITY LEVEL OF DIFFERENT GLYCOSIDASES IN INTACT AND ACROSOME-REACTED PORCINE SPERM

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The sperm–egg interactions are species-specific forms of cell recognition and the binding event which are a necessary prerequisite for fertilization (Park *et al*. 2002 Anim. Reprod. Sci. **72**, 83–94). Glycosidase enzymes that remove carbohydrates could play an important role in the reproductive tract, modulating decisive physiological events mediated by carbohydrates, which play a key role in sperm–oocyte recognition. The aim of this study was to analyze the presence of the glycosidases α-D-mannosidase, α-L-fucosidase, β-D-glucosaminidase, and β-D-galactosaminidase in intact and acrosome-reacted sperm from fertile matured boars. Sperm were washed three times in PBS by centrifugation at 800*g* for 10 min. The pelleted sperm were resuspended in the same buffer to obtain a final concentration of 250×10^6 spermatozoa mL^{-1}. The acrosome reaction was induced by incubation of the sperm with 10 µM of calcium ionophore A23187 at 37°C for 30 min. Different enzymes were detected by incubating 8 µL (for α-D-mannosidase) or 80 µL (for the rest of the enzymes) of sperm sample with the corresponding substrate conjugated to 4-methylumbelliferil for 2 h at 37°C in PBS at pH 7.3. Fluorescences were read on a Fluostar Galaxy fluorimeter (BMG LabTech GmbH, Offenburg, Germany), using wavelengths of 340 and 450 nm for excitation and emission, respectively, and were corrected by subtracting tissue and substrate blanks. The results were analyzed using a one way ANOVA. An average of fluorescence units of 9685.86 ± 1081.75, 7394.63 ± 874.29, 3154.17 ± 514.10, and 1666.40 ± 117.86 was detected in the intact sperm sample for the α-D-mannosidase, α-L-fucosidase, β-D-glucosaminidase, and β-D-galactosaminidase, respectively. For the acrosome-reacted sperm sample (60–65% acrosome-reacted sperm in the samples measured by fluorescence microscope), an average of 9756.14 ± 1011.45, 7026.93 ± 771.48, 1185.70 ± 277.51, and 1111.60 ± 176.70 for α-D-mannosidase, α-L-fucosidase, β-D-glucosaminidase, and β-D-galactosaminidase, respectively. Statistically significant differences ($P < 0.05$) between intact and acrosome-reacted sperm were detected only for the β-D-glucosaminidase and β-D-galactosaminidase. These results suggest that the four different enzymes detected are mainly present in the sperm plasma membrane. Under the conditions used in this study, α-D-mannosidase is the main enzyme activity present in the sperm. Importantly, β-D-glucosaminidase and β-D-galactosaminidase activity detected in the intact sperm is decreased after the induction of the acrosome reaction.

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224 A COMPARATIVE STUDY BETWEEN WOOD AND PLAINS BISON

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In Canada, brucellosis and tuberculosis threaten an estimated 4500 wood bison (*Bison bison athabascae*), a species considered at risk by the Committee on the Status of Endangered Wildlife In Canada (COSEWIC). To help rescue this species, our Wood Bison Reproductive Research group proposes to employ advanced reproductive technologies. Unfortunately, little is known about the reproductive physiology of the wood bison, which hinders the application of these reproductive technologies. In order to modify advanced reproductive techniques developed in cattle for use in wood bison, the large amounts of semen, embryos, and oocytes from wood bison required are not available. The purpose of this study was to compare semen collected from the more abundant and closely related plains bison (*Bison bison bison*) with that of wood bison. Semen from 3 wood and