ABSTRACTS FOR POSTER PRESENTATION

Male Physiology

291 PRESENCE OF PLATELET-ACTIVATING FACTOR (PAF) RECEPTOR IN BULL SPERM AND POSITIVE CORRELATION OF SPERM PAF CONTENT WITH FERTILITY

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Male fertility involves the capacity to obtain viable pregnancy and offspring after insemination. Currently, the most common way to measure bull fertility is through non-return rates (NRR) calculated after insemination of many females. However, this method is time-consuming and expensive. A number of biochemical molecules in sperm have been proposed as potential predictors of male fertility, e.g. platelet-activating factor (PAF). Platelet-activating factor (1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphorylcholine) is a ubiquitous phospholipid that is implicated in the mediation of a wide variety of reproductive processes. The mechanism of PAF's action is a receptor-mediated event reported to affect intracellular calcium levels. Bull sperm contain PAF and its content has a positive relationship with motility. While the PAF-receptor has been reported in other species, it has not been demonstrated in bull sperm. Therefore, our objectives were to determine: 1. the relationship between PAF content in bull sperm and Estimated Relative Conception Rates (ERCR, a 3-year rolling average of NRR); and 2. the presence of the PAF-receptor in bull sperm. Sperm PAF content for bulls ($n = 8$) with different ERCR was determined by radioimmunoassay. PAF-receptor expression was determined as follows: total RNA was purified by acid phenol extraction and ethanol precipitation. Complementary DNAs were synthesized by reverse transcriptase with dNTPs and random primers at 37°C, 60 min; followed by 65°C, 5 min. Reverse transcription (cDNA) products were amplified with Taq polymerase, dNTP, and PAF receptor primer pair (upper, 5′-AATCCAGTCACCCTGGTTGTAG-3′; lower, 5′-TGGACTCAGAGTTCCGATACAC-3′) at 94°C, 1 min; 55°C, 1 min; 72°C, 1 min for 35 cycles followed by 72°C, 7 min. RT-PCR products were analyzed by 2% agarose gel electrophoresis. PAF-receptor protein was determined as follows: PBS-washed bull sperm was exposed to human PAF-receptor antibody at 4°C for 3 h, washed in PBS, then exposed to fluorescein isothiocyanate-conjugated anti-IgG for 90 min at 37°C, and again washed in PBS. Specimens were examined by epifluorescence microscopy at 400×. PAF content in bull sperm ranged from 1.39 ng/10^6 sperm cells to 13.68 ng/10^6 sperm cells. There was a positive correlation ($P < 0.05$) between PAF content and ERCR. Presence of PAF-receptors in bull sperm was confirmed by immunofluorescence. However, distribution of PAF-receptors in bull sperm was not uniform within or between specimens. A cDNA clone containing the coding region for PAF-receptor was isolated from bull sperm using a reverse transcription-polymerase chain reaction protocol. There is a positive correlation ($R = 0.40$; $P < 0.05$) between PAF content in sperm and in vivo fertility of individual bulls as determined by NRR. Molecular and immunofluorescence data confirm the presence of PAF-receptor (mRNA and protein) in bull sperm. Additional studies are warranted to elucidate the mechanism of PAF's action in sperm. Early selection for fertility in bulls represents a potentially valuable application to enhance efficiency in cattle breeding.

292 CHANGES IN MEMBRANE SULFHYDRYL STATUS OF BOAR SPERMATOZOA BY FREEZING

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The processes of cooling and freezing/thawing produce physical and chemical stresses on the sperm membrane that reduce the viability and fertilizing capacities. The cold shock and freezing of spermatozoa are associated with an oxidative stress, with reactive oxygen species (ROS) generation (Chatterjee *et al*., 2001, Mol. Reprod. Dev. 60, 498–506) and with a significant reduction of the GSH content (Gadea *et al*., in press). In the processes of capacitation, fertilization and freezing, qualitative and quantitative changes in protein membrane composition occurs, including changes in distribution of sulphydryl groups on the sperm membrane. The aim of this work was to evaluate the changes in the sulfhydryl groups of

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proteins from the sperm surface after cooling and freezing procedures as a marker of membrane changes. Ejaculate-rich fractions from three mature Pietrain boars were diluted in Beltsville Thaw Solution (BTS) extender and ooled to 15°C over 2 h (control). Thereafter sperm were centrifuged and diluted in lactose/egg-yolk extender cooled to 5°C over 2 h and later frozen with glycerol and equex by classic methodology (Westendorf *et al*., 1975, Dtsch. Tierärztl. Wschr. 82, 261–267). Sperm parameters were measured in extended semen (control) at 0, 1 and 2 h after cold shock at 5°C and after freezing-thawing. The structure of the sperm membrane was evaluated with carboxyfluorescein diacetate/propidium iodide (DCF) (Harrison and Vickers, 1990, J. Reprod. Fert. 88, 343–352), and the sulfhydryl status of proteins from spermatozoa surface are evaluated with fluorescent-staining 5-iodoacetamidofluoresceine (5-IAF) and by acrosome integrity (normal apical ridge, NAR). Some seminal parameters to evaluate functionality such as motility (MOT), forward progressive motility (FPM, 0–5), and mitochondria activity with Rhodamine 123(MIT) were also evaluated. Data from 11 freezing batches were analyzed by one-way ANOVA. When ANOVA revealed a significant effect, values were compared by the Tukey test. The freezing process significantly affected all the sperm parameters studied. Motility was negatively affected from the onset of cooling to 5°C. However, DCF, NAR and 5-IAF were only affected after freezing process. Mitochondria activity decreased in the last period of the cooling procedure (2 h) and it was lower after freezing. An inverse significant relation was found between 5-IAF and motility, viability, NAR and mitochondria functionality ($P < 0.01$). These results show that freezing damage produces an alteration in the structure of the sperm membranes (DCF, NAR, 5-IAF) and sperm functionality (motility and mitochondrial). However, only motility (MOT and FPM) was affected by cold shock when lactose/egg-yolk extender was used. Previous studies of cold shock with no cryo-protective medium (BTS) showed a marked effect on sulphydryl membrane characteristics (Marco and Gadea, 2003). These preliminary results in the use of 5-IAF in boar semen showed that freezing produces an alteration in the structure of the sperm membranes, which could be detected by simple fluorescent staining. This research was supported by grant AGL 2000-0485-C02-01.

	MOT	FPM	DCF	NAR	5-IAF	MIT
Control	80.91 ± 1.67^{a}	3.32 ± 0.11^{a}	87.59 ± 0.90^{a}	97.45 ± 0.26^{a}	21.64 ± 2.03^{a}	$72.00 \pm 3.45^{a,b}$
0 h 5°C	68.85 ± 1.66^{b}	2.69 ± 0.09^{b}	81.13 ± 1.19^{a}	95.79 ± 0.27^{a}	17.07 ± 0.66^{a}	$76.74 \pm 1.83^{a,b}$
1 h 5°C	57.50 ± 1.37^{c}	2.36 ± 0.08^{c}	83.11 ± 1.04^{a}	94.87 ± 0.34^{a}	18.02 ± 0.95^{a}	78.54 ± 1.73^{a}
2 h 5°C	50.96 ± 1.14^{c}	2.11 ± 0.09^{d}	83.23 ± 1.08^{a}	94.73 ± 0.29^{a}	18.92 ± 0.84^{a}	70.80 ± 2.05^{b}
Frozen-thawed	23.25 ± 2.11^{d}	1.80 ± 0.13^{d}	47.76 ± 1.91^{b}	55.95 ± 1.38^{b}	50.03 ± 1.34^{b}	43.58 ± 1.43^{c}
ANOVA	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Indices without common superscripts (^{a,b,c,d}) differ, $P < 0.001$.

293 QUANTITATIVE TESTICULAR HISTOLOGY IN THE COMMON WOMBAT (*VOMBATUS URSINUS*)

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The seminiferous epithelium cycle of the male Common Wombat (*Vombatus ursinus*) is documented here for the first time. Testicular material was obtained from 10 common wombats from the Southern Highlands of NSW in June ($n = 5$) and November ($n = 5$), fixed in Bouins solution and prepared for standard histological processing. Eight stages of the seminiferous cycle where identified based upon relative cellular associations and development of the spermatid during spermiogenesis. Stage 1 was further subdivided into 1A and 1B based on changes in shape of the spermatid nucleus. The relative frequency of each stage was also calculated using observations from 500 seminiferous tubule cross-sections as was the proportion of the various testicular tissue types. The relative proportions of the various stages of the seminiferous epithelial cycle in the common wombat testis were: Stage 1A, 4.0 ± 0.5; Stage 1B, 4.2 ± 0.4; Stage 2, 21.3 ± 1.9; Stage 3, 15.4 ± 1.2; Stage 4, 16.8 ± 1.2; Stage 5, 11.1 ± 1.5; Stage 6, 13.7 ± 1.5; Stage 7, 7.3 ± 0.6; Stage 8, 6.1 ± 0.6. Relative proportions of the various tissue types observed in testis included: seminiferous tubules ($41.5\% \pm 4.1$); seminiferous tubule lumen ($33.3 \pm 3.4\%$); leydig cells ($14.6 \pm 1.1\%$); connective tissue ($10.4 \pm 0.9\%$) and blood vessels ($0.2 \pm 0.03\%$).

Table 1. Cell types and cellular associations of the eight stages of the wombat seminiferous epithelial cycle

Cell Types		IA	IB	II	III	IV	V	VI	VII	VIII
A spermatogonia		■	■	■	■	■	■	■	■	■
Intermediate spermatogonia						■	?	?		
B spermatogonia								■	■	■
1' spermatocytes	Preleptotene	■	■							
1' spermatocytes	Leptotene			■	■					
1' spermatocytes	Zygotene					■	■			
1' spermatocytes	Pachytene	■	■	■	■			■	■	■
1' spermatocytes	Diplotene					↓				
Meiosis										
2' spermatocytes										
Spermatids	Round	■					■	■	■	■
Spermatids	Rod shaped		■							
Spermatids	Condensed			■						
Spermatids	Elongated				■	■				
Spermatozoa							■	■	■	
Acrosome vacuole		■							■	■
Sperm head straight							■			
Sperm head curved								■	■	
Residual bodies										■
Sperm in bundles								■		

?-Intermediate spermatogonia not yet identified in these stages.

294 EFFECTS OF LONG DAYLIGHT ON CAPRINE SCROTAL CIRCUMFERENCE AND SEMEN QUALITY

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Seasonal variation of the reproductive activity of small ruminants in the southern USA is controlled by photoperiod. We tested the hypothesis that artificial manipulation of photoperiod has an effect on caprine testicular activity and semen quality. The objective of this study was thus to determine the effects of photoperiod on caprine scrotal circumference and semen parameters during the nonbreeding season. Ten Saanen and Alpine bucks, two to three years old, raised at the Small Ruminant Research and Extension Center of Fort Valley State University (latitude 32°37′ N, longitude 83°39′ W), were used in the study. Throughout the study, the bucks were fed a concentrate diet containing 16% crude protein, and 2.7 Mcal/Kg of digestible energy. Two groups of bucks randomly selected were exposed to long daylight (16L : 8D; $n = 5$) or ambient daylight (12L : 12D, control; $n = 5$) for a period of three weeks during the month of March. Scrotal circumference measurements and semen collection were conducted on each buck twice a week for a total of five trials. Semen samples were collected using an artificial vagina and an electroejaculator. Samples were immediately transferred to the laboratory for further evaluation. Data were analyzed as a completely randomized design with repeated measures using SAS program (SAS, 1990). When means were significant by ANOVA ($P < 0.05$), they were separated by least significant difference. Results are presented as the least square means ± SEM. Long daylight did not affect semen volume (mL), % motility, sperm concentration (billions/mL), % viability, or % abnormality($P > 0.05$). However, semen motility, sperm concentration, and abnormality were influenced by treatment in trials 3, 1, and 5, respectively ($P < 0.05$). The least square means ± SEM values for these parameters were 35 ± 15, 0.58 ± 0.1, and 20.0 ± 3.7 for long daylight-treated bucks v. 87 ± 15, 0.13 ± 0.1, and 7.8 ± 3.7 for the control, respectively. Further, long daylight affected scrotal circumference ($P < 0.05$). Scrotal circumferences were decreased in trials 1, 2, and 5 ($P < 0.05$). Long daylight decreased scrotal circumference without affecting semen quality in this study.

Daylight	Scrotal circumferences (cm)				
	Trials				
	1	2	3	4	5
Long	23.6 ± 0.8	23.8^{a} ± 0.7	24.6 ± 0.9	24.0 ± 1.0	23.8^{a} ± 0.9
Ambient	26.2 ± 0.8	26.9^{b} ± 0.7	27.3 ± 0.9	26.7 ± 1.0	26.8^{b} ± 0.9

^{a,b}Means within columns with different superscripts differ significantly ($P < 0.05$).