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Proteins of the Cauda Epididymal Fluid Associated With Fertility of Mature Dairy Bulls

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Abstract

We evaluated the relationships between proteins in cauda epididymis fluid (CEF) and fertility scores of dairy bulls. Fertility was expressed as the percentage point deviation (PD) of bull nonreturn rate from the average fertility of all bulls at an artificial insemination center. The number of services for each bull ranged from 1074 to 52 820, and PD values ranged from +7.7% to -6.6%. CEF from 20 bulls was obtained from vasa deferentia cannulae and was separated from sperm by centrifugation immediately after collection. Samples were evaluated by 2-dimensional (2-D) sodium dodecyl sulfate polyacrylamide gel electrophoresis gels stained with Coomassie blue, and polypeptide maps were analyzed by PDQuest software. Protein quantities, defined as the total integrated optical density of the spots, were compared between groups of high-fertility sires (n = 12; PD ≥ 0) and low-fertility sires (n = 8; PD < 0) and were also used as independent variables in regression analysis. Proteins were identified by capillary liquid chromatography–nanoelectrospray ionization–tandem mass spectrometry. An average of 118 spots was detected in 2-D maps of the CEF, but we were unable to distinguish any protein that was expressed only in high-fertility or in low-fertility bulls. However, the amount of α-L-fucosidase 2 and cathepsin D was 2.3- and 2.4-fold greater ($P < .05$) in high-fertility than in low-fertility bulls, respectively. Conversely, the intensities of 3 isoforms (24–27 kd; pI

6.3–5.8) of prostaglandin D-synthase (PGDS) were from 3.2- to 2.2-fold greater in low-fertility sires ($P < .05$). An empirical regression model established that a significant proportion ($R^2 = 0.72$; $P < .0001$) of the variation in fertility scores (PD values) was explained by the intensities of cathepsin D and 1 isoform of PGDS (24 kd; pI 6.3). Thus, multiple proteins present in the CEF are potential biomarkers of fertility in high-use, mature Holstein bulls.

Key words: α-L-fucosidase, cathepsin D, epididymis, mass spectrometry, prostaglandin D-synthase, sperm

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Immature spermatozoa newly formed in the seminiferous tubules have a period of transit through the epididymis where they become motile and undergo a series of events that include changes in the composition of membrane lipids and proteins, ion exchange between the extra- and intracellular environments, and cytoskeleton rearrangement, among others (Olson et al, 2002; Gatti et al, 2004; Sullivan et al, 2005). Completion of epididymal transit and maturation leads to storage of sperm at a dormant state in the cauda region until ejaculation occurs. The epididymal epithelium secretes proteins that potentially affect not only sperm maturation (Dacheux and Dacheux, 2002) but also other aspects of sperm physiology while these cells are stored in the cauda compartment (Hinton et al, 1995). Those proteins may determine important attributes of the fertilizing capacity of spermatozoa. Factors isolated from epididymal fluid or epithelial cells in culture have been linked to sperm motility and protection of membranes against damage caused by cryopreservation (Reyes-Moreno et al, 2002), anticapacitation effects (Roberts et al, 2003), or sperm number (Gatti et al, 2004), but evidence linking epididymal proteins to male fertility indexes is limited.

Previously, we utilized catheterization of the vasa deferentia to recover secretions from accessory sex glands and cauda epididymis of mature Holstein bulls (Henault et al, 1995). These surgically altered bulls had documented fertility based on artificial insemination (AI) of large numbers of cows and therefore provide a unique resource to study molecular indicators of fertility of the normal male. Using such a model, we have recently shown that proteins of the accessory sex gland secretions identified as spermadhesin Z13, osteopontin, BSP 30 kd, and phospholipase A_2 were related to fertility indexes of sires (Moura et al, 2006). Thus, given the important events that take place in the epididymis and their potential effects on sperm function, we tested the hypothesis in the present study that proteins of the cauda epididymal fluid (CEF) are associated with fertility scores of mature dairy bulls.

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Experimental Design

This project was approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Samples of CEF from 20 Holstein sires were obtained by cannulation of the vas deferens and centrifuged (700 x *g*) immediately after collection (Henault et al, 1995; Moura et al, 2006). The sperm-free fluid was transferred to cryovials for storage in liquid nitrogen for later analysis. CEF proteins were subjected to 2-dimensional (2-D) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and gel images and analyzed by PDQuest software (Bio Rad, Rockville Centre, NY). Spots that showed significant associations with fertility indexes were identified by tandem mass spectrometry.

Information about nonreturn rates (NRRs) of Holstein bulls was provided by AI cooperatives in the northeastern United States and was based on the number of cows that did not return to service 60

days after the first insemination. Compensation for small variations among data sets obtained from different AI centers was achieved by expressing the fertility index of each bull as the percentage point deviation (PD) of its NRR from the average NRR of all bulls in a given AI unit (Killian et al, 1993). In this case, a bull with a PD of 0 had average fertility, relative to the average for the population it came from. The number of services using frozen semen from each bull ranged from 1074 to 52 820, and sires had PDs ranging from +7.7% to -6.6%. Bulls with PD values of 0 or higher were considered as high fertility (n = 12), and bulls with PD values lower than 0 were considered as low fertility (n = 8).

Eletroctrophoresis

Samples of CEF pulled from liquid nitrogen were thawed at room temperature and centrifuged at 10 000 x *g* (60 minutes at 5 °C). The supernatant was then assayed for protein content (Lowry et al, 1951) by using bovine serum albumin as standards and aliquots frozen at -80 °C. For electrophoresis, samples were thawed at room temperature and subjected to 2-D electrophoresis according to a previously described procedure (Killian et al, 1993). Briefly, isoelectric focusing was carried out in tube gels (Bio Rad) containing a mixture of ampholytes with pH ranging from 3 to 7 (0.4 mL) and 3 to 10 (0.1 mL; Serva, Heidelberg, Germany). Samples of CEF containing 500 µg of protein were brought to a volume of 100 µL with a solution of ß-mercaptoethanol, urea, and the same ampholytes used in the gels. Gels were then subjected to 200 V for 15 minutes, 300 V for 30 minutes, 400 V for 30 minutes, 375 V for 16 to 18 hours, and 800 V for 1 hour. After focusing, gels were removed from the tubes and placed on stacking gels that had been prepared on the top of gels containing a linear gradient of acrylamide (10%–17.5%). Standards from 66 to 14 kd were also used (Sigma Chemical Co, St Louis, Mo). Gels were stained with Coomassie brilliant blue R-250; destained in a solution of methanol, acetic acid, and deionized distilled water; and scanned with a GS-670 imaging densitometer (Bio Rad). Images saved as TIFII files were analyzed by PDQuest software (Bio Rad) as previously described (Moura et al, 2006). Briefly, for the set of 20 images of CEF gels, a single master gel was generated by the software representing the best pattern of spots in the samples. Additional spots consistently present in some gels were also added to the master so that they could be matched to all samples. Proteins in key regions of the gels were used as landmarks, and final matching of spots was achieved after several rounds of extensive comparisons. Control of spot matches was checked in each gel with the respective pattern in the master. Protein quantities were given as parts per million (ppm) of the total integrated optical density of the spots, according to PDQuest.

Protein Identification

Proteins separated by 2-D SDS-PAGE and selected by PDQuest were subjected to in-gel trypsin digestion (Koc et al, 2001). Excised gel pieces were washed 3 times with 100 µL of ammonium bicarbonate (25 mM) and dehydrated with 100 µL of acetonitrile (50%) and dried in a speed vacuum. They were then incubated overnight at 37 °C with trypsin (12.5 ng/µL in 25 mM ammonium bicarbonate). Peptides were then extracted twice with 25 µL of formic acid (5%) for 20 minutes. The extracts were dried in a speed vacuum again and resuspended in 10 µL of 5% acetonitrile with formic acid (0.1%). Tryptic digests were analyzed by capillary liquid chromatography–nanoelectrospray ionization–tandem mass spectrometry (CapLC-MS/MS). A Micromass Q-Tof API US mass spectrometer coupled with a Waters CapLC high-performance liquid chromatography (HPLC) unit (Waters Co, Milford, Mass) was used for the analysis (Abbas et al, 2005). The proteolytic digests (1–5 µL) were injected into solvent A (acetonitrile–water–formic acid mixture of 5:95:0.1) supplied by the auxiliary pump of the capillary HPLC unit and trapped in a Waters Symmetry 300 column (C-18, 5-µm film; 0.3 x 5 µm) for on-line desalting and preconcentration (Abbas et al, 2005). After washing for 3 minutes with solvent A at 20 µL/min, trapped peptides were then back flushed with the gradient solvent flow onto the analytical column—a Dionex PepMap fused silica capillary column (C-18 5 µm, 0.075 x 150 mm)—with a

10-port switching valve. The analytical column was run with a gradient (5%–42% solvent B, acetonitrile–water–formic acid mixture of 95:5:0.2, for 44 minutes). The mass spectrometry was calibrated with Glu-Fib product ion fragments as needed to maintain mass accuracy within 10 ppm. The Q-Tof mass spectrometer was operated to acquire MS/MS of tryptic peptides in data-dependent acquisition mode for precursor ion selection by using charge-state recognition and intensity threshold as selection criteria with MassLynx 4.0 SP1. To carry out the tandem mass spectrometric data acquisition, a survey scan (2 seconds) over the *m/z* of 400 to 1500 was performed. From each survey scan, up to 4 most intense precursor ions based on the selection criteria were selected for tandem mass spectrometry to obtain the production spectra resulting from collision-induced dissociation in the presence of argon. The product ion spectra (6–8 seconds) collected were processed with Protein Lynx Global Server 2.1 and were converted to peak list text files for database searching. To identify the proteins, MS/MS ion searches were performed on the processed spectra against a locally maintained copy of the National Center for Biotechnology Information nonredundant (NCBI NR) database by using MASCOT Daemon and search engine (Matrix Science Inc, Boston, Mass). The searches were made with the assumption that there was 1 maximum missed trypsin cleavage and that peptides were monoisotropic and oxidized at methionine residues (variable modifications) and carbamidomethylated at cysteine residues (fixed modifications). Peptide mass tolerance and fragment mass tolerance were initially set to 1.2 and 0.6 daltons, respectively, for MS/MS ion searching; however, peptide mass values were ensured to be within 0.1 dalton (typically less than 0.05 dalton) when manually reviewing MASCOT search results.

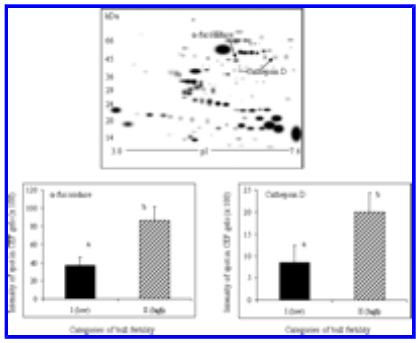

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Figure 1. Two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis of cauda epididymal fluid (CEF) and intensity of spots (±SE) identified as α-L-fucosidase 2 and cathepsin D in bulls of high and low fertility. Gel image was generated by PDQuest software, and proteins were identified by tandem mass spectrometry (CapLC-MS/MS). Fertility of bulls was based on the percentage point deviation of their nonreturn rates (PD values): group I (PD < 0, n = 8); group II (PD ≥ 0; n = 12). a, b: $P < .05$.

Statistical Analysis

Differences in protein expression between 2-D CEF maps from high-fertility bulls (n = 12; PD ≥ 0) and low-fertility bulls (n = 8; PD < 0) were evaluated by *t* test (Statistical Analysis Systems, 2003). Protein quantities that significantly differed among these bulls were used as independent variables to determine the extent by which the percentage PD of bull NRRs (PD values) was explained by regression equations. Criteria used to evaluate the regression models were R^2, Mallow's C(p) value, and multicollinearity (Statistical Analysis Systems, 2003).

Results

An average of 118 spots was detected in 2-D maps of the CEF, and 31 of them were present in all 20 gels of the PDQuest match set (protein map of CEF is

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shown in both Figures 1 and 2). We were unable to distinguish any protein that was unique to the groups of bulls with high or low fertility scores. However, there were significant differences between these 2 groups with respect to the amounts of α-L-fucosidase 2, cathpesin D, and isoforms of prostaglandin D-synthase (PGDS) in the CEF. The intensity of α-L-fucosidase and cathepsin D was 2.3- and 2.4-fold greater in high-fertility bulls than in low-fertility bulls, respectively ($P < .05$; Figure 1; Table). Conversely, the average intensity of 3 isoforms of PGDS was from 3.2-fold (spot 1) to 2.2-fold (spot 2) greater in low-fertility bulls (P, .05; Figure 2; Table). Spot "A" of the PGDS train was not associated with fertility indexes. Analysis of tryptic peptides from that particular spot generated 2 matches with significant scores, though with different magnitudes: PGDS, with score 53, and apolipoprotein A-I, with score 318 (Table).

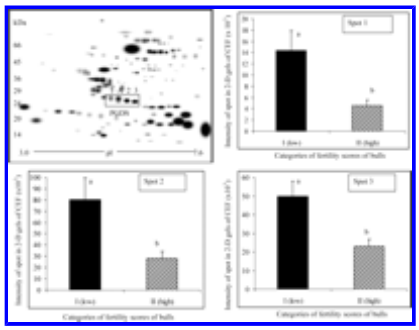

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Figure 2. Two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis of cauda epididymal fluid (CEF) and intensity (±SE) of prostaglandin D-synthase (PGDS; spots 1, 2, and 3) in bulls of high and low fertility. Two proteins were identified in spot "A"—apolipoprotein A-I and PGDS—and the intensity of this particular spot was not associated with fertility indexes. Gel image was generated by PDQuest software, and proteins were identified by tandem mass spectrometry (CapLC-MS/MS). Fertility of bulls was based on the percentage point deviation of their nonreturn rates (PD values): group I (PD < 0, n = 8).

A regression model including cathepsin D and isoform # 3 of PGDS (24 kd; pI 6.3) as independent variables generated the equation PD = 44.79 + 0.0016 x (cathepsin D) - 4.6 x log(PGDS_spot 3), where $R^2 = 0.72$ and $P < .0001$. PD represents the percentage PD from the average NRR of bulls, and variables in parentheses are the integrated optical density of the respective spots in the CEF gels, as calculated by PDQuest.

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We determined that proteins from CEF identified by tandem mass spectrometry as α-L-fucosidase 2, cathepsin D, and PGDS were expressed at different levels in bulls of high and low fertility. Moreover, an empirical regression model established that a significant proportion ($R^2 = 0.72$) of the variation in fertility scores was explained by cathepsin D and 1 isoform of PGDS. Holstein bulls of this study represent a population of tested, mature sires that have been extensively used for AI with frozen semen. Inseminations are performed with similar sperm numbers, and differences in fertility scores among bulls were not correlated with results of routine semen analysis conducted at AI centers. Animals were reproductively normal, and although selected primarily for their ability to transmit genetic traits linked to milk yield, these sires have also been screened for fertility merit. To our knowledge, this is the first study to report that certain cauda epididymal proteins are related to fertility of bulls with these characteristics.

We have previously reported the existence of associations between bull fertility and accessory sex gland components (Moura et al, 2006). This previous report and the findings of the present study suggest that molecular markers of male fertility are associated with both epididymal sperm physiology and postejaculation events regulated by accessory sex gland components. Studies have reported correlations between NRRs of bulls and seminal plasma proteins that are originally synthesized by the accessory sex glands and epididymis (Killian et al, 1993; Gerena et al, 1998; McCauley et al, 2001). However, fluid produced by the epididymis is diluted about 8- to 10-fold when mixed with accessory sex gland secretions at ejaculation (Gerena et al, 1998). This makes it difficult to accurately identify epididymal proteins in the seminal plasma milieu, particularly those secreted in low abundance or if they are also secreted by other organs, such as the accessory sex glands. Unpublished results from our laboratory show that most of the minor spots seen in the CEF 2-D maps are greatly diminished or undetectable in Coomassie-stained seminal plasma gels or when the CEF is mixed 1:8 with the accessory sex gland fluid from the same bulls.

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*Proteins in the CEF of mature Holstein bulls identified by 2-dimensional SDS-PAGE and CapLC-MS/MS**

From 118 spots detected in the CEF maps, 26.3% were present in all gels. Despite the relatively small number of spots present in all members of the match set constructed by PDQuest, there was no protein expressed either in only high- or low-fertility bulls. These results for CEF are similar to those obtained with accessory sex gland fluid 2-D maps (Moura et al, 2006), when it was shown that no proteins were expressed solely in bulls with the highest fertility scores and absent in those with the lowest scores, or vice-versa.

Because most proteins from the rete testis are not present in the milieu of the epididymis lumen (Olson and Hinton, 1985; Dacheux et al, 1989), there is a general assumption that proteins of the epididymal fluid are mainly the product of the epididymis itself. Numerous proteins have been detected in the epididymal milieu of mammalian species (Cornwall et al, 2002; Dacheux and Dacheux, 2002) but the exact roles of most of them in sperm maturation are yet to be determined (Gatti et al, 2004). The cases of fertility-related proteins identified in the present study are not exceptions; therefore, the explanation for the links between their expression and fertility is still a matter of hypothesis.

As mentioned, both α-L-fucosidase and cathepsin D were more predominant in high-fertility bulls. α-L-fucosidase catalyzes the hydrolysis of α-L-fucose, which is part of oligosaccharide moieties of glycoproteins, glycolipids, and glycosaminoglycans. The enzyme detected in the CEF by tandem mass spectrometry (54.4 kd; pI 6.6) appears similar to a soluble form that has been found in seminal plasma (56 kd) of humans (Alhadeff et al, 1999; Khunsook et al, 2002) and epididymal fluid of bulls (Jauhiainen and Vanha-Perttula, 1986). α-Fucosidase secreted in the epididymal fluid may participate in the modification of carbohydrate moieties of sperm membrane proteins during epididymal transit. This protein has been found suppressed in the seminal plasma of bulls with elevated percentage of abnormal sperm (Jauhiainen and Vanha-Perttula, 1986), and dogs with insufficiency of α-fucosidase also have impaired sperm maturation (Veeramachaneni et al, 1998).

Cathepsin D is a cysteine peptidase that degrades proteins commonly found as part of the extracellular matrix and is involved in tissue growth and remodeling (Dickinson, 2002). A study has

reported that seminal plasma of oligo- and azoospermic men has less cathepsin D than their normal counterpart (Dandekar and Harikumar, 1997). In the stallion, cathepsin D is synthesized mainly in caput and corpus epididymis (Fouchécourt et al, 2000), and in this regard it may participate in proteolytic remodeling of membrane components of sperm during epididymal transit. Such modifications, like those suggested to be mediated by α-fucosidase, may contribute to the fertilizing capacity of epididymal sperm (Chapman and Killian, 1984; Cuasnicú et al, 2002; Sullivan et al, 2005).

In contrast to what was found with α-L-fucosidase and cathepsin D, 3 isoforms of PGDS were significantly higher in bulls with low fertility scores. An additional isoform of PGDS apparently comigrated with another protein identified as apolipoprotein A-I, and, coincidentally, the intensity of that particular spot did not show any association with bull fertility. Apolipoprotein A-I is a component of high-density lipoproteins (Sparrow et al, 1992) and is suggested to be involved in sperm cholesterol efflux and capacitation (Manjunath et al, 1989; Thérien et al, 1997). Previous studies have reported that PGDS detected in the seminal plasma was positively related to sperm quality in men (Olsson, 1975; Diamandis et al, 1999; Leone et al, 2001) and field fertility of dairy bulls (Killian et al, 1993; Gerena et al, 1998; Fouchécourt et al, 2002). The apparent contrast between the results presented here (with CEF) and those previously reported by our own laboratory and other authors (with seminal plasma) are interesting but not necessarily in conflict. PGDS appears in the CEF as a series of spots with 24 to 27 kd and pI from 6.3 to 5.8, based on the analysis from tandem mass spectrometry and Western blots (data not shown). These results are similar to what was detected in the rete testis fluid of bulls (Gerena et al, 1998) and epididymis of rams and stallions (Fouchécourt et al, 1999). Concentration of PGDS is much lower (eightfold) in the seminal plasma than in the CEF (Gerena et al, 1998), and the protein that was originally related to fertility scores of bulls by Killian et al (1993) was detected as a single 26-kd (pI 6.2) spot in the seminal plasma 2-D maps. These facts undoubtedly emphasize that PGDS spots from seminal plasma and CEF gels are different variables and that their relative importance in explaining variations in fertility scores may be different as well. Changes occur in both molecular weight and pI of PGDS isoforms during epididymal transit in the ram and stallion, and such changes may alter the attributes of this protein (Fouchécourt et al, 1999). It is also possible that modifications occur in epididymal PGDS when it is mixed with accessory sex gland secretions at ejaculation.

PGDS acts as a lipophilic ligand-binding protein after secretion (Urade and Hayaishi, 2000). It has the ability to bind molecules such as testosterone, thyroid hormones, and retinoids (Urade and Hayaishi, 2000; Leone et al, 2002), but its functions in the epididymis are unclear. As we have previously suggested (Gerena et al, 2000), if one considers PGDS as a retinoid-carrying protein, it is important to mention that retinoids can be detrimental to phospholipid membranes and their permeability to ions (Stillwell and Wassall, 1990). Also, PGDS binds to docosahexanoic acid, a major polyunsaturated fatty acid of human sperm (Alvarez and Storey, 1995) that regulates membrane fluidity and permeability (Stillwell and Wassall, 2003). The net concentration of docosahexanoic acid in sperm during epididymal transit is important because its loss prevents peroxidative damage to sperm, but a minimal amount is also required to maintain membrane fluidity and sperm mobility (Ollero et al, 2000). Thus, PGDS could influence male fertility by mediating the action of hydrophobic molecules on sperm during epididymal transit or cauda epididymal storage.

In conclusion, we have presented empirical evidence that certain cauda epididymal proteins are significant molecular indicators of bull fertility. It is known that secretions of the epididymis contain biologically active molecules (Dacheux and Dacheux, 2002; Gatti et al, 2004), and the present study confirms that only a select group of those molecules is linked to a fertility phenotype of proven, high-use dairy bulls. The distinct expression of these proteins may have been

favored by the type of selection applied to dairy sires.

Footnotes

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Abbas A, Koc H, Liu F, Tien M. Fungal degradation of wood: initial proteomic analysis of extracellular proteins of *Phanerochaete chrysosporium* grown on oak substrate. *Curr Genet*. 2005; 47: 49 –56.[CrossRef][Medline]

Alhadeff JA, Khunsook S, Choowongkomon K, Baney T, Heredia V, Tweedie A, Bean B. Characterization of human semen α-L-fucosidase. *Mol Hum Reprod*. 1999; 5: 809 –815.[Abstract/Free Full Text]

Alvarez JG, Storey BT. Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. *Mol Reprod Dev*. 1995; 42: 334 –346.[CrossRef][Medline]

Chapman DA, Killian GJ. Glycosidase activities in principal cells, basal cells, fibroblasts and spermatozoa isolated from the rat epididymis. *Biol Reprod*. 1984; 31: 627 –636.[Abstract]

Cornwall GA, Lareyere J-J, Matusik RJ, Hinton BT, Orgebin-Crist M-C. Gene expression and epididymal function. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*. New York, NY: Kluwer Academic/Plenum Publishers; 2002 : 169–199.

Cuasnicú PS, Chen DJ, Ellerman DA, Busso D, Da Ros VG, Morgenfeld MM. Changes in sperm proteins during epididymal maturation. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*. New York, NY: Kluwer Academic/Plenum Publishers; 2002: 389 –404.

Dacheux JL, Dacheux F. Protein secretion in the epididymis. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*. New York, NY: Kluwer Academic/Plenum Publishers; 2002: 151 –168.

Dacheux JL, Dacheux F, Paquignon M. Changes in sperm surface membrane and luminal protein fluid content during epididymal transit in the boar. *Biol Reprod*. 1989; 40: 635 –651.[Abstract]

Dandekar SP, Harikumar P. Seminal profiles of lysosomal enzymes in normal and infertile men. *J Postgrad Med*. 1997; 43: 33 –37.[Medline]

Diamandis EP, Arnett WP, Foussias G, Pappas H, Ghandi S, Melegos DN, Mullen B, Yu H, Srigley J, Jarvi K. Seminal plasma biochemical markers and their association with semen analysis findings. *Urology*. 1999;53: 596 –603.[CrossRef][Medline]

Dickinson DP. Cysteine peptidases of mammals: their biological roles and potential effects in the oral cavity and other tissues in health and disease. *Crit Rev Oral Biol Med*. 2002; 13: 238 –275.[Abstract/Free Full Text]

Fouchécourt S, Charpigny G, Reinaud P, Dumontand P, Dacheux JL. Mammalian lipocalin-type prostaglandin D2 synthase in the fluids of the male genital tract: putative biochemical and physiological functions. *Biol Reprod*. 2002; 66: 458 –467.[Abstract/Free Full Text]

Fouchécourt S, Dacheux F, Dacheux JL. Glutathione-independent prostaglandin D2 synthase in ram and stallion epididymal fluids: origin and regulation. *Biol Reprod*. 1999; 60: 558 –566. [Abstract/Free Full Text]

Fouchécourt S, Méayer S, Locatelli A, Dacheux F, Dacheux JL. Stallion epididymal fluid proteome: qualitative and quantitative characterization; secretion and dynamic changes of major proteins. *Biol Reprod*. 2000; 62: 1790 –1803.[Abstract/Free Full Text]

Gatti J-L, Castella F, Dacheux F, Ecroyd H, Métayer S, Thimon V, Dacheux J-L. Post-testicular sperm environment and fertility. *Anim Reprod Sci*. 2004; 82/83: 321 –339.

Gerena RL, Irikura D, Eguchi N, Urade Y, Killian GJ. Immunocy-tochemical localization of lipocalin-type prostaglandin D synthase in the bull testis and epididymis and on ejaculated sperm. *Biol Reprod*. 2000;62: 547 –556.[Abstract/Free Full Text]

Gerena RL, Irikura D, Urade Y, Eguchi N, Chapman DA, Killian GJ. Identification of a fertility-associated protein in bull seminal plasma as lipocalin-type prostaglandin D synthase. *Biol Reprod*. 1998; 58: 826 –833.[Abstract/Free Full Text]

Henault MA, Killian GJ, Kavanaugh JF, Griel LC Jr. Effect of accessory sex gland fluid from bulls of differing fertilities on the ability of cauda epididymal sperm to penetrate zona-free bovine oocytes. *Biol Reprod*. 1995; 52: 390 –397.[Abstract]

Hinton BT, Palladino MA, Rudolph D, Labus JC. The epididymis as protector of maturing spermatozoa. *Reprod Fertil Dev*. 1995; 7: 731 –745.[CrossRef][Medline]

Jauhiainen A, Vanha-Perttula T. Comparison of glycosidase levels in bovine seminal plasma. *Int J Androl*. 1986; 10: 489 –494.

Khunsook S, Alhadeff JA, Bean B. Purification and characterization of human seminal plasma α-L-fucosidase. *Mol Hum Reprod*. 2002;8: 221 –227.[Abstract/Free Full Text]

Killian GJ, Chapman DA, Rogowski LA. Fertility-associated proteins in bull seminal plasma. *Biol Reprod*. 1993; 49: 1202 –1207.[Abstract]

Koc EC, Burkhart W, Blackburn K, Moyer MB, Schlatzer DM, Moseley A, Spremulli LL. The large subunit of the mammalian mitochondrial ribosome—analysis of the complement of ribosomal proteins present. *J Biol Chem*. 2001; 276: 43958 –43969.[Abstract/Free Full Text]

Leone MG, Abdel HH, Gennaro G, Amici S, Conte D, Romanelli F, Latini M, Isidori A, Saso L, Silvestrini B. Changes in lipocalin type prostaglandin D-synthase in the seminal plasma in the subfertile man. *Res Commun Mol Pharmacol*. 2001; 110: 17 –25.

Leone MG, Haq HA, So L. Lipocalin-type prostaglandin D-synthase: which role in male fertility. *Contraception*. 2002; 65: 293 –295.[CrossRef][Medline]

Lowry OH, Rosebrough WJ, Farr AL, Randall RL. Protein measurement with Folin phenol reagent. *J Biol Chem*. 1951; 193: 265 –275.[Free Full Text]

Manjunath P, Marcel YL, Uma J, Seidah NG, Chretien M, Chapdelaine A. Apolipoprotein A-I binds to a family of bovine seminal plasma proteins. *J Biol Chem*. 1989; 264: 16853 –16857. [Abstract/Free Full Text]

McCauley TC, Zhang HM, Bellin ME, Ax RL. Identification of a heperin-binding protein in bovine seminal fluid as tissue inhibitor of metalloproteinases-2. *Mol Reprod Dev*. 2001; 58: 336 –341. [CrossRef][Medline]

Moura AA, Koc H, Chapman DA, Killian GJ. Expression of proteins of the accessory sex gland fluid as related to fertility indexes of dairy bulls: a proteomic approach. *J Androl*. 2006; 27: 201 –211. [Abstract/Free Full Text]

Ollero M, Powers RD, Alvarez JG. Variation of docosahexaenoic acid content in subsets of human spermatozoa at different stages of maturation: implications for sperm lipoperoxidative damage. *Mol Reprod Dev*. 2000;55: 326 –334.[CrossRef][Medline]

Olson GE, Hinton BT. Regional differences in luminal fluid polypeptides of the rete testis and epididymis revealed by twodimensional gel electrophoresis. *J Androl*.1985:6: 20 –34. [Abstract/Free Full Text]

Olson GE, NagDas SK, Winfrey VP. Structural differentiation of spermatozoa during post-testicular maturation. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice: A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens*. New York, NY: Kluwer Academic/Plenum Publishers; 2002 : 371–387.

Olsson JE. Correlation between the concentration of b-trace protein and the number of spermatozoa in human semen. *J Reprod Fertil*. 1975;42: 149 –151.[CrossRef][Medline]

Reyes-Moreno C, Boilard M, Sullivan R, Sirard MA. Characterization of secretory proteins from cultured cauda epididymal cells that significantly sustain bovine sperm motility in vitro. *Mol Reprod Dev*. 2002;63: 500 –509.[CrossRef][Medline]

Roberts KP, Wamstad JA, Ensurd KM, Hamilton DW. Inhibition of capacitation-associated tyrosine phosphorylation signaling in rat sperm by epididymal protein Crisp-1. *Biol Reprod*. 2003; 69: 572 –581.[Abstract/Free Full Text]

Sparrow DA, Lee BR, Laplaud PM, Auboiron S, Bauchart D, Chapman MJ, Gotto AM Jr, Yang CY, Sparrow JT. Plasma lipid transport in the preruminant calf, *Bos spp*: primary structure of bovine apolipoprotein A-I. *Biochim Biophys Acta*. 1992; 1123: 145 –150.[Medline]

Statistical Analysis Systems, *SAS User's Guide*. Cary, NC: Statistical Analysis Systems Institute Inc; 2003 .

Stillwell W, Wassall SR. Interactions of retinoids with phospholipids membranes: optical spectroscopy. *Methods Enzymol*. 1990;189: 373 –382.[Medline]

Stillwell W, Wassall SR. Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem Phys Lipids*. 2003; 126: 1 –27.[CrossRef][Medline]

Sullivan R, Saez1 F, Girouard J, Frenette G. Role of exosomes in sperm maturation during the transit along the male reproductive tract. *Blood Cells Mol Dis*. 2005; 35: 1 –10.[CrossRef][Medline]

Thérien I, Soubeyrand S, Manjunath P. Major proteins of bovine seminal plasma modulate sperm capacitation by high-density lipoprotein. *Biol Reprod*. 1997; 57: 1080 –1088.[Abstract]

Urade Y, Hayaishi O. Biochemical, structural, genetic, physiological and pathophysiological features of lipocalin-type prostaglandin D synthase. *Biochim Biophys Acta*. 2000; 1482: 259 –271.[CrossRef][Medline]

Veeramachaneni DN, Smith MO, Ellinwood NM. Deficiency of fucosidase results in acrosomal dysgenesis and impaired sperm maturation. *J Androl*. 1998;19: 444 –449.[Abstract/Free Full Text]

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