Animal Science Papers and Reports vol. 29 (2011) no. 1, 75-84
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Correlation between the proteins and protein profile(s) of different regions of epididymis and their contents in goat buck

Ranjna S. Cheema, Amrit K. Bansal*, G.S. Bilaspuri , V. K. Gandotra

Department of Veterinary Gynaecology and Obstetrics,
Guru Angad Dev Veterinary and Animal Sciences University,Ludhiana 141004, India

(Received September 17, 2009; accepted September 7, 2010)

Total proteins and their profile(s) in three regions of epididymis, its fluid and sperm membrane extract (SME) were analysed with an objective to determine the changes in spermatozoa during epididymal maturation in goat buck. The protein content from caput to cauda increased non-significantly ($P \geq 0.05$) while significantly ($P \leq 0.05$) in tissue homogenate and spermatozoa. SDS-PAGE analysis indicated the removal of >205, 205 and 95 kDa; 40 kDa proteins only in the caput and corpus spermatozoa, respectively. The proteins with molecular weight of 10, 18, 25, 35 and 20 kDa, detected only in cauda and corpus spermatozoa, respectively, seem to be associated in the maturation process of spermatozoa during epididymal transit. SDS-Page analysis of tissue and fluid indicated that the >205, 205, 195, 200, 45, 25, 18, 15 and 12 kDa proteins are structural as well as secretory proteins because of their presence both in the epididymal tissue and fluid. The nature of 100, 97, 75 and 70 kDa was found to be purely structural because of their presence in the tissue only, whereas 90, 35, 6.5 and 3.0 kDa proteins, mainly of secretory nature, due to their detection only in the fluid. Therefore, the presence of 35, 25, 18 kDa proteins both in the epididymal fluid and cauda sperm indicate that these proteins are associated with goat buck sperm maturation and fertility. Therefore, it can be concluded that the proteins of 35, 25, and 18 kDa, associated with goat sperm maturation are similar to the mentioned molecular markers in other species and can be worked out as fertility markers for goat buck semen.

KEY WORDS: bucks / detergents / epididymis / proteins / spermatozoa

*Corresponding author: bansal2amrit@yahoo.co.in

Mammalian spermatozoa acquire their fertilizing ability during the epididymal transit. Testicular spermatozoa are differentiated morphologically, but are still functionally immature as they cannot recognize, bind to, and penetrate the oocytes. Being transcriptionally and translationally silent, spermatozoa progressively acquire their fertilizing potential during epididymal transit, relying on the activities of the epididymal epithelium that creates an ever changing luminal environment along the duct [Chabory *et al*. 2009]. Caput and corpus regions of epididymis are responsible for sperm maturation whereas cauda region is involved in sperm storage ensuring that male gametes are available in sufficient number at ejaculation [Sullivan 2004].

Various investigations show extensive and sequential surface modifications characterized by large surface changes in the caput epididymis and acquisition of new components at the surface membrane as the sperm transit in the terminal part of the organ [Hammerstedt and Parks 1987]. Such changes may result either in removing, masking or un-masking pre-existing surface components [Bedford and Cooper 1978, Olson and Hamilton 1978, Voglmayr *et al.*1982] or the absorption and incorporation of epididymal fluid polypeptide [Olson and Orgebin-Crist 1982]. The importance of these membrane proteins on the physiological activities of sperm motility and fertilizing ability is still to be determined. A lot of work has been done in this aspect in different species, but the nature of these proteins in various species is still unclear. Facing the above, the aim of this study was to measure total proteins and their profile(s) in three regions of epididymis, its fluid and sperm membrane extract (SME) of goat buck.

Material and methods

Reagents

All chemicals were purchased from SISCO RESEARCH LABORATORIES (SRL) Private, Ltd., Mumbai, India.

Collection of homogenate, fluid, and spermatozoa of three regions of epididymis

Testes from five goat males (*Capra hircus*) aged 18-24 months were procured from the Department of Veterinary Clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. The caput, corpus and cauda parts of epididymes were separated, freed from blood and washed in phosphate buffer saline (PBS, 0.1M, pH7.4). Thereafter, each part was cut into pieces and epididymal fluid was collected by squeezing and repeatedly washing in PBS. Subsequently, epididymal fluid of each part was centrifuged at 3000 rpm to separate out spermatozoa from it. Then, each region was homogenized in PBS and centrifuged at 10,000 rpm to obtain the tissue homogenate.

Extraction of protein from tissue homogenate, fluid and spermatozoa from three parts of epididymis

Proteins were partially purified from tissue homogenate and fluid by precipitating with saturated ammonium sulphate. The precipitates were dissolved in PBS (pH 7.4).

Sperm membrane proteins were extracted from the spermatozoa by incubating 1.0×10^9 spermatozoa in 1.0 ml of 1% deoxycholate (DOC)/ sodium dodecyl sulphate (SDS) in 62.5 mM Tris-HCl buffer (pH 6.8) in boiling water bath (5 min). Sperm suspension was centrifuged at 10,000 rpm for 10 min. To get sperm membrane extract (SME), 5 % mercapto-ethanol was added to the supernatant, kept in boiling water bath for 5 min and again centrifuged at 10,000 rpm for 10 min.

Analysis / separation of tissue homogenate-, fluid- and sperm membrane-protein of three regions of epididymis

Total protein and SDS-PAGE. In all the samples total protein was estimated according to Lowry *et al.* [1951] while SDS-PAGE was performed according to Laemmli *et al*.[1970].

Proteins were fractionated in all the samples by SDS-PAGE using 10% acrylamide gel and Tris-glycine (pH 8.3) as tank buffer. Gels were run at a current of 3mA/sample till the dye reached the other end. Gels were fixed in 10% TCA and stained with 0.5 % commassiie brilliant blue stain for 1 h and de-stained with 7% acetic acid and 10 % methanol to remove the background stain. Molecular weights of unknown bands in the samples were calculated by comparing with those of standard (Banglore genei 3.0, 6.5, 14.3, 20.1, 29, 43, 66, 97.4 and 205 kDa) run along with the samples.

Statistical

The data were analysed by computerized software programme for analysis of randomized block design (RBD). P value of 0.05 was selected as a criterion for statistically significant differences.

Results and discussion

Total protein

The protein content of tissue homogenate (1.8-3.0 mg/g) and of spermatozoa (1.66-4.50 mg/10^7 sperms) shows an increase from caput to cauda. The protein content from caput to cauda increased non-significantly, while significantly in tissue homogenate ($P \leq 0.05$) and in spermatozoa ($P \leq 0.05$) - Table. 1. Protein content declined progressively in epididymal fluid (3.5 to 1.80 mg/ml) being significant ($P \leq 0.05$) from corpus to cauda (Tab.1).

Sperm membrane proteins extracted with SDS and DOC showed highest percentage of extraction in caput as compared to corpus and cauda (Tab. 2).

Table1. Means and standard errors for total protein in tissue, fluid and spermatozoa of three regions of epididymis of goat buck

Epididymis	Tissue-homogenate (mg/g tissue)	Fluid (mg/ml)	Spermatozoa ($mg/10^7$)
Caput	$1.80^{a}\pm0.40$	$3.50^{a}\pm0.44$	$1.66^{a}\pm0.07$
Corpus	$2.40^{a}\pm0.15$	$3.00^{a}\pm0.19$	$3.29^{b}\pm0.21$
Cauda	$3.00^{a}\pm0.32$	$1.80^{b}\pm0.11$	$4.50^{c}\pm0.13$

[abc]Any two means in a column bearing different superscripts are significantly different at P≤0.05.

Table 2. Means for protein content ($mg/10^7$ spermatozoa) in sperm membrane extracts of epididymal spermatozoa of goat buck

Epididymis	Deoxycholate (%)	Sodium-dodecyl sulphate (%)
Caput	1.00^{a} (60)	1.16^{a} (70)
Corpus	0.96^{a} (29)	1.08^{a} (33)
Cauda	1.17^{a} (26)	1.09^{a} (24)

[a]Any two means in a column bearing different superscripts are significantly different at P≤0.05.
Figures in parentheses represent per cent extraction of proteins from spermatozoa.

Characterization of sperm membrane, epididymal fluid and tissue proteins by SDS-PAGE in sperm membrane proteins

>205, 205, 97, 70, 65, 45, 30, 25 kDa; 97, 70, 65, 60, 45, 40, 25 kDa and 97, 65, 60, 45, 30, 25 kDa proteins were separated by SDS-PAGE in 97, 70, 65, 45, 20 kDa and 97, 30, 25, 18, 10 kDa proteins from the sperm surface of caput, corpus and cauda spermatozoa, respectively (Tab. 3). SDS-PAGE analysis indicated the removal of >205, 205, 95 kDa; 40 kDa proteins only in the caput and corpus spermatozoa, respectively. A 30 kDa protein of caput spermatozoa was missing in the corpus region, its reappearance in the cauda spermatozoa indicate its reorganization during epididymal transit. The presence of 10 and 18 and of 20 kDa proteins only in the cauda and corpus spermatozoa, respectively, indicates their association in the maturation process of spermatozoa during epididymal transit.

Epididymal fluid/ tissue proteins

The proteins with molecular weight of >205, 205, 200, 97, 75, 70, 65, kDa; >205, 205, 200, 100, 97, 70, 65, 45, kDa; >205, 205, 200, 97, 70, 65, 45, 25, 20, 18, 15 kDa

Table 3. Protein profile (molecular weight, kDa), of epididymal sperm membrane extracts of goat buck

Sample No.	DOC-SME			SDS-SME		
	Caput	Corpus	Cauda	Caput	Corpus	Cauda
1	>205	--	--	--	--	--
2	205	--	--	205	--	--
3	97	97	97	97	97	97
4	--	--	--	95	--	--
5	70	70	--	70	70	--
6	65	65	65	65	65	--
7	--	60	60	--	--	--
8	45	45	45	45	45	--
9	--	40	--	--	--	--
10	35	--	35	35	--	35
11	--	--	25	--	--	25
12	--	--	--	--	20	18
13	--	--	--	--	--	10

Table 4. Protein profile (molecular weight, kDa) of epididymal tissue and fluid proteins of goat buck

Sample No.	Tissue			Fluid		
	Caput	Corpus	Cauda	Caput	Corpus	Cauda
1	>205	>205	>205	---	>205	>205
2	205	205	205	205	205	205
3	200	200	200	195	195	195
4	---	100	---	---	---	---
5	97	97	97	---	---	---
6	---	---	---	90	90	---
7	75	---	---	---	---	---
8	70	70	70	---	---	---
9	65	65	65	65	65	65
10	---	45	45	45	45	45
11	---	---	---	35	35	35
12	---	---	25	25	---	---
13	---	---	20	20	20	20
14	---	---	18	18	18	18
15	---	---	15	15	15	15
16	---	---	---	12	---	12
17	---	---	---	6.5	---	6.5
18	---	---	---	3.0	---	---

were separated by SDS- PAGE in the epididymal tissue of caput; corpus and cauda spermatozoa respectively (Tab. 4). Whereas 205, 195, 90, 65, 45, 35, 25, 20, 18, 15, 12, 6.5, 3.0 kDa; >205, 205, 195, 90, 65, 45, 35, 20, 18, 15 kDa; >205, 205, 195, 65, 45, 35, 20, 18, 15, 12, 6.5 kDa proteins were detected by SDS-PAGE in caput; corpus

Table 5. Nature of epididymal proteins of goat buck

Molecular weight (kDa)	Detected in		Nature of protein
	tissue	fluid	
>205	caput, corpus, cauda	corpus, cauda	structural/secretory
205	--oo--	caput, corpus, cauda	structural/secretory
195-200	--do--	--do--	structural/secretory
100	corpus	----	structural
97	caput, corpus, cauda	----	structural
90	----	caput, corpus	secretory
75	caput	----	structural
70	caput, corpus, cauda	----	structural
45	corpus, cauda	caput, corpus, cauda	structural/secretory
35	----	--do--	secretory
25	cauda	caput	structural/secretory
18	cauda	caput, corpus, cauda	structural/secretary
15	cauda	--do--	structural/secretory
12	cauda	--do--	structural/secretory
6.5	----	caput,---------, cauda	secretory
3.0	----	caput	secretory

and cauda fluid respectively. SDS- Page analysis of tissue and fluid indicated that the >205, 205,65, 45, 25, 20, 18 and 15 kDa proteins are structural as well as secretary proteins because of their presence both in the epididymal tissue and fluid(Tab. 5). The nature of 200, 100, 97, 75 and 70 kDa was found to be purely structural because of their presence in the tissue only. Whereas, 90, 35, 12, 6.5, 3.0 kDa proteins mainly of secretary nature due to their detection only in the fluid (Tab. 5).

Therefore, the presence of 35, 25 and 18 kDa proteins both in the epididymal fluid and cauda sperm indicate that these proteins are associated with goat buck sperm maturation and fertility.

In this study the higher number of proteins in the caput epididymal fluid and lower in caput tissue homogenate as compared to corpus and cauda regions shows the secretory activity of caput region. Djakiew *et al*. [1984] suggested that most of the proteins present in caput epididymal fluid decrease gradually to corpus and cauda regions probably due to selective re-absorption by the lining epithelium as has been not clearly shown for transferring macro-globulins in rats. In the present study, significant increase in the protein content of the spermatozoa from caput to cauda region indicates the absorption of protein from the epididymal fluid.

Sperm membrane extracts

Higher percentage of extraction of surface protein with DOC and SDS from caput spermatozoa indicates the increase in the rigidity of sperm membrane during maturation which may be required for fertility of spermatozoa. Numerous studies reported that membranes of mature spermatozoa can be selectively extracted with

detergents, proteases and lipases [Ahuja *et al.*1984, 1985, Kinger *et al*.1989, Sundhey *et al.*1992].

Characterization of sperm membrane, epididymal fluid and tissue proteins with SDS-PAGE.

About 13 bands within the range of 10- >205 were identified by SDS-PAGE in DOC and SDS-SME of caput-, corpus- and cauda- spermatozoa. Haden *et al.* [2000] resolved at least 14 different swine sperm membrane proteins by 2-d electrophoresis, 13 of which possessed acidic PIS range 4.2-4.8. Ten and 18 kDa proteins were detected mainly in the cauda spermatozoa of goat buck. Vierula and Rajaneimi [1981] identified 42-47 kDa proteins as predominant of bull cauda spermatozoa. Major 17-36 kDa proteins were also detected in the cauda spermatozoa of rat and sheep [Zaheb and Orr 1984].

SDS-PAGE analysis also indicated that in male goat >205, 205, 95, 40, 30 kDa proteins were removed from the sperm surface, whereas 10, 18 and 20 kDa proteins were deposited to the spermatozoa during epididymal transit. Several studies on mammalian sperm suggested that 25 kDa [Daucheux *et al*. 1989], 45, 26 kDa [Hegde *et al*. 1991] and 16, 22.5, 26, 37, 60 kDa [Robitaille *et al.* 1991] proteins disappeared or accumulated in the spermatozoa during epididymal transit.

A series of events including changes in the composition of membrane lipids and proteins, ion exchange between the extra and intracellular environment of spermatozoa [Olson *et al*.2002, Gatti *et al*.2004, Sullivan *et al*. 2005] occurs during the epididymal transit. The epidiymal epithelium secretes proteins that potentially affect not only sperm maturation [Dacheux and Dacheux, 2002], but also other aspects of sperm physiology while these are stored in the cauda [Hinton *et al,* 1995]. These proteins may determine important attributes of the fertilizing capacity of spermatozoa. Electrophoretic analysis of epididymal fluid proteins has been performed in various mammals, mainly rodents. A few major secretory proteins have been identified biochemically, some of them appearing to represent sperm surface components. In the present study the proteins with molecular weight of >205, 205, 200, 195, 45, 25, 18, 15, 12 kDa; 100, 97, 75, 70 kDa and 90, 35, 6.5, 3.0 kDa were recognized as mainly of secretory / structural, structural or secretory nature, respectively. About 32 [Deepanee *et al*. 2007], 17 [Alumot *et al.* 1971] and 16 [Koskimies *et al*. 1975] protein bands were also identified by SDS-PAGE in the epididymal fluid of goat, ram and rat, respectively.

The presence of 35, 25, 18 kDa proteins in goat buck sperm extracts and fluid seems to indicate that these compounds are specific to the sperm and are related to its maturation during the epididymal transit.. Olson and Hinton [1985] were also of the opinion that major luminal fluid proteins may be associated with cauda spermatozoa. Similarly, a rat epididymal glycoprotein of 37 kDa (DE) is synthesized by the epithelium of the proximal segments of the epididymis and associates with the sperm surface during epididymal transit [Kohane *et al.* 1980]. Guo *et al.* [2007] indicated that ERP-29 (a rat precursor) is related to secretory protein synthesis and absorbed

by spermatozoa, may play a role in sperm maturation during the epididymal transit, particularly in the sperm/ organelle membrane. Moura *et al.* [2006] also presented empirical evidence that certain cauda epididymal proteins are significant molecular indicators of bull fertility.

The involvement of proteins of 14, 18, 20 kDa has been shown in *zona pellucida* binding [Hanguing *et al.* 1991, Naz 1992]. It is known that fertility-associated antigen (FAA), a heparin binding protein from seminal vesicles and prostate glands, binds to spermatozoa membrane and modulates heparin-sperm interactions that are indicative of fertility [Killian *et al.*1993, Ax *et al.* 1999, McCauley *et al*. 1999). Two seminal plasma proteins, prostaglandin-D-synthetase and osteoponin are more abundant in the semen of high fertility bulls when compared to low fertility bulls [Cancel *et al*. 1997, Gerena *et al.* 1998]. The role of another protein of sperm i.e. P34H involves in *zona pellucida* binding in humans ovum is well documented [Boue *et al.* 1996]. Using the different molecular tools and antibodies developed against human p34H epididymal sperm protein, a 25 kDa protein has been characterized in bovine sperm, which is added to the sperm during epididymal transit and anchored to glycophosphatidylinositol [Parent *et al.* 1999]. The ability of p25b to serve as a marker for sperm damages that can occur during freezing-thawing processes has been documented. Differential expression of 125 proteins was significant between high- and low-fertility bull spermatozoa and these proteins are potential biomarkers for bovine male fertility [Peddinti *et al*. 2008]. Therefore, it can be concluded that the proteins of 35, 25, 18 kDa, related to goat sperm maturation are similar to the above mentioned molecular markers and can be worked out as fertility markers for goat buck semen.

RFERENCES

1. AHUJA S.P., HJORT T., POULSEN F., JACOBSON S.R., 1984 – Detection and partial characterization of auto antigens from human sperm membrane. ***Journal of Nuclear Agricultural Biology*** 13, 130-136.
2. AHUJA S.P., HJORT T., POULSEN F., JACOBSON S.R., 1985 – Release and partial characterization of auto antigens from human sperm membrane. ***Annals of Biology*** 2, 1-8.
3. ALUMOT E., LENSKY Y.,SCHINDLER H.,1971 – Separation of proteins in the epididymal fluid of the ram. ***Journal of Reproduction and Fertility*** 25, 349-353.
4. AX R.L., BELLIN M.E., ZHANG H.M., 1999 – New approach to estimating bull fertility, Proceedings of "The range beef cow symposium" XVI Dec., 14,15 & 16 Greeley Colorado.
5. BEDFORD J.M., COOPER G.W., 1978 – Membrane fusion events in the fertilization of vertebrate egg. In: Membrane Fusion (G. Poste, G.L. Nicholson, Eds.), pp.65-125. Amsterdam: Elsevier /North-Holland Biomedical Press.
6. BOUE F., SULLIVAN R., 1996 – Cases of human infertility are associated with the absence of P34 H, an epididymal sperm antigen. ***Biology of Reproduction*** 54, 1018-1024.
7. CANCEL A.M., CHAPMAN D.A., KILLIAN G.J., 1997 – Oteopontin is the S-S kilodalton fertility associated protein in Holstein bull seminal plasma. ***Biology of Reproduction*** 57, 1293-1301.
8. CHABORY E., DAMON C., LENOIR A., KAUSEL-MANN G., KERN H., ZEVNIK B., GARREL C., SAEZ F., CADET R., HENERY-BERGER J., SCHOOR M., GOTTWALD U., HABENICHT U., DREVET J.R., VERNET P., 2009 – Epididymis seleno-independent glutathione peroxidase 5 maintains sperm DNA integrity in mice. ***The Journal of Clinical Investigation*** 119, 2074-2085.

9. DACHEUX J.L., DACHEUX F.,2002 – Protein secretion in the epididymis . In : Robaire B, Hinton BT, eds.The epidiymis: From Molecules to Clinical Practice : A Comprehensive Survey of the Efferent Ducts.The Epididymis and the Vas Deferens. New York, NY: Kluwer Academice /Plenum Publishers.151-168.
10. DAUCHEX J.L., DAUCHEX F., PAQUIGNON M. 1989 – Changes in sperm surface membrane and luminal protein fluid content during epididymal transit in the boar. ***Biology of Reproduction*** 40, 635-651.
11. DEPANEE J.K.A., SUBRAMANIUM R., GUNAWARDANA V.K., ARIYARANTNE H.B.S., RAJAPAKARA R.P.V.J, PRERA N., GUNAVARDANA D.G.A., MUNASINGHE D.M.S. 2007 – A preliminary study on the proteins found in the epididymal fluid of goat. Proceedings of the Peradeniyaa University Research Sessions, Sri Lanka Part I, 12, 170-171.
12. DJAKIEW D., BYERS S.W., LEWIS D.M., DYM M., 1984 – Receptor-mediated endocytosis of alpha 2 macroglobulin and transferring in rat caput epididymal epithelial cells in vitro**.** ***Biology of Reproduction*** 31, 1073-1085.
13. GATTI J.L., CASTELLA F., DACHEUX F., ECROYD H., METAYER S., THIMON V., DAUCHEX J.L., 2004 – Post testicular sperm environment and fertility. ***Animal Reproduction Science*** 62, 547-556.
14. GERENA R.L., IRIKURA D., URADE Y., EGUCHI N., CHAPMAN D.A., KILLIAN G.J., 1998 – Identification of a fertility associated protein in bull seminal plasma as lipocalin type prostaglandin D synthase**.** ***Biology of Reproduction*** 58, 826-833.
15. GUO W., QU F, XIA L., GUO Q., YING X.,DING Z., 2007 – Identification and characterization of ERp29 in rat spermatozoa during epididymal transit**.** ***Reproduction*** 133, 575-584.
16. HADEN N.P., HICKOX J.R., WHISHANT C.S., HARDY D.M., 2000 – Systematic characterization of sperm specific membrane proteins in swine. ***Biology of Reproduction*** 63, 1839-1847.
17. HAMMERSTEDT R.H., PARKS J.E., 1987 – Changes in sperm surface associated with epididymal transit. ***Journal of Reproduction and Fertility***. Supplement 34, 113-149.
18. HANGUING M.O., YING Y.T., WEN S.Z., 1991 – Isolation, characterization and location of the zona-pellucida binding proteins of boar sperm. ***Molecular Reproduction and Development*** 28, 124-130.
19. HEGDE V.C.,VRINDA K., SUDHA P.C., 1991 – Immuno-chemical localization and characterization of proteins from mouse cauda epididymis using human sperm specific monoclonal antibody. ***Human Reproduction*** 6, 259-262.
20. HINTON B.T., PALLAINO M.A., RUDOLPH D., CABUS J.C., 1995 – The epididymis as protector of maturing spermatozoa. ***Reproduction, Fertility and Development*** 7, 731-745.
21. KILLIAN G.J., CHAPMAN D.A., ROGOWSKI L.A., 1993 – Fertility associated proteins in Holstein bull seminal plasma. ***Biology of Reproduction*** 49, 1202-1207.
22. KINGER S., ANUJA S.P., KINGER A.K. 1985 – Purification and characterization of sperm membrane autoantigens by chromatography of their enzyme/detergent extracts. ***Annals of Biology*** 5, 87-102.
23. KOHANE A.C., GONZALEZ E., CHEVERRIA F., PINEIRO L., BLAQUIER J.A., 1980 –Interaction of proteins of epididymal origin with spermatozoa. ***Biology of Reproduction*** 23, 737-742.
24. KOSKIMIES L.A., KORMANO M., 1975 – Proteins in fluids from different segments of the rat epididymis. ***Journal of Reproduction and Fertility*** 43, 345-348.
25. LAEMELLI U.K.,1970 – Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. ***Nature*** 220, 680-685.
26. LOWRY O.H., ROSENBROUGH N.J., FARR A.L., RANDALL R.J., 1951 – Protein measurement with the Folin phenol reagent. ***Journal of Biological Chemistry*** 193, 265-267.

27. MCCAULEY T.C., ZHANG H.M., BELLIN M.E., AX R.L., 1999 – Purification and characterization of fertility associated antigen (FAA) in bovine seminal fluid. ***Molecular Reproduction and Development*** 54, 145-153.
28. MOURA A.A., CHAPMAN D.A., KOC H., KILLIAN G.J., 2006 – Proteins of the cauda epididymal fluid associated with fertility of mature dairy bulls. ***Journal of Andrology*** 27, 534-541.
29. NAZ R.K., 1992 – Effects of antisperm antibodies on early cleavage of fertilized ova. ***Biology of Reproduction*** 46, 130-139.
30. OLSON G.E., ORGEBIN-CRIST M.C., 1982 – Sperm surface changes during epididymal maturation. ***Annals of the New York Academy of Sciences*** 383, 372-392.
31. OLSON G.E., HAMILTON D.W., 1978 – Characterization of the surface glycoproteins of rat spermatozoa. ***Biology of Reproduction*** 19, 26-35.
32. OLSON G.E., HINTON B.T., 1985 – Regional differences in luminal fluid polypeptides of the rat testis and epididymis related two-dimensional gel electrophoresis. ***Journal of Andrology*** 6, 20-34.
33. OLSON G.E., NAGDAS S.K., WINFREY V.P., 2002 – Structural differentiation of spermatozoa during post-testicular maturation. In: B. Robaire and B.T. Hinton, 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, 371-387.
34. PARENT S., LEFIEVRE L., BRINDLE Y., SULLIVAN R., 1999 – Bull subfertility is associated with low level of a sperm membrane antigen. ***Molecular Reproduction and Development*** 52, 57-65.
35. PEDDINTI D., NANDURI B., KAYA A., FEUGANG J.M., BURGESS S.C., MEMLI E., 2008- Comprehensive proteomic analysis of bovine spermatozoa of varying fertility rates and identification of biomarkers associated with fertility**.** ***BMC System Biology*** 2, 1-13.
36. ROBITAILLE G., ROBERT S., BLEAU G., 1991 – Identification of epididymal proteins associated with hamster sperm. ***Journal of Experimental Zoology*** 258, 69-74.
37. SULLIVAN R., SAEZ L., GIROUARD J., FRENETTE G., 2005 – Role of exosome in sperm maturation during the transit along the male reproductive tract. ***Blood Cells Molecular Discovery*** 35, 1-10.
38. SULLIVAN R., 2004 – Male infertility markers, myth or reality. ***Animal Reproduction Science*** 82-83, 341-347.
39. SUNDHEY R., AHUJA S.P., SINGH B., 1992 – Changes in the composition of membranes of buck (Capra hircus) spermatozoa during epididymal maturation. ***Small Ruminant Research*** 7, 135-149.
40. VIERULA M., RAJANEIMI H., 1987 – Radio-iodinization of surface proteins by sodium dodecyl sulphate – polyacrylamide gel electrophoresis. ***Journal of Reproduction and Fertility*** 58, 483-489.
41. VOGLMAYR J.K., FAIRBANKS G., VESPA D.B., COLLELA J.R., 1982 – Studies on the surface modifications in the ram spermatozoa during the final stages of differentiation. ***Biology of Reproduction*** 26, 483-500.
42. ZAHEB R., ORR G.A., 1984 – Characterization of a maturation-associated glycoprotein on the plasma membrane of rat caudal epididymal sperm. ***Journal of Biological Chemistry*** 259, 839-848.