İstanbul Üniv. Vet. Fak. Derg.
35 (2), 1-12, 2009

J. Fac. Vet. Med. Istanbul Univ.
35 (2), 1-12, 2009

Araştırma Makalesi

GOAT SPERM SURFACE ALTERATION DURING EPIDIDYMAL MATURATION: AN EVALUATION BY MARINE BIOACTIVE COMPOUND FROM *TELESCOPIUM TELESCOPIUM*[*]

Manik Lal HEMBRAM [1], Uttam DATTA [2] and Raju DASGUPTA [3]

Geliş Tarihi : 09.02.2009
Kabul Tarihi : 20.04.2009

Epididimal Olgunlaşma Sırasında Keçi Sperma Yüzeyinde Meydana Gelen Değişimler: Deniz Bioaktif Bileşimi Olan *Teleskopium teleskopium'un* Değerlendirilmesi

Abstract: Bio-active compounds from marine flora and fauna contain various types of pharmacologically active novel biomolecules enriched with different types of lectins. Lectins, with high saccharide binding specificities have been considered important probe to monitor the changes in plasmamembrane during physiological maturation of spermatozoa. Cytosol fraction, isolated from the spermatheca and / or ovotestis gland of a marine mollusc *Telescopium telescopium*, specific for sialic acid, has been found responsible for "Head to Head" sperm agglutination, and the agglutinability varies in number ($p<0.01$) among the epididymal regions. Number of hypo-osmotic swelling-positive spermatozoa also exhibited region wise variation ($p<0.01$) where both the characters remained maximum in caudal region. The differences in the number of agglutinating spermatozoa and their pattern reflects modification of sperm surface carbohydrate moieties linked either with lipid or protein that occur during epididymal transit and heterogenic nature was due to regional variation in the carbohydrate domain of sperm plasmamembrane. Differences ($P<0.01$) in the number of hypo-osmotic swelling-positive spermatozoa between the epididymal regions indicated sperm plasmamembrane in the caput are less permeable and less functional than spermatozoa from corpus and caudal segment. These indicated maturation along with alteration of sperm plasmamembrane occurs gradually during epididymal passage. Present approach with this bio-active compound obtained from marine snail could be used as a biomarker to study the sperm maturation process, and heterogeneity and status of the plasmamembrane which might help to improve the diagnosis of reproductive problems in relation to distribution of lectin receptors on sperm cells of any species to predict status of the male gametes before use in artificial reproductive technologies.

Key Words: Sperm plasmamembrane, *Telescopium telescopium*, agglutination, lectin.

---

[*] M.V. Sc. Research work of the first author.

[1] Department of veterinary Gynaecology & Obstetrics, WBUAFS, Kolkata-700037.
[2] Senior Lecturer, Department of veterinary Gynaecology & Obstetrics, WBUAFS, Kolkata-700037.
[3] Department of Animal Genetics and Breeding, WBUAFS, Kolkata-700037.

Özet: Deniz florası ve faunasının bio-aktif yapıları farmakolojik olarak aktif ve değişik bimolekülleri içerirler ve bu moleküller çeşitli lektinlerle zenginleştirilmiştir. Lektinler, yüksek oranda sakkarit bağları ile özelleşmişlerdir ve spermanın olgunlaşması sırasında plazma membranında meydana gelen fizyolojik değişimlerin izlenmesi araştırılmalarında bu lektinler önemli oranda düşünülmelidir. Deniz yumuşakçalarından olan *teleskopium teleskopiumun* ovotestis bezinden ve/veya spermatekadan izole edilen sitoplazma fraksiyonu sialik asit açısından spesifiktir ve bu spermanın baş başa olan aglutinasyonundan sorumlu bulunmuştur ve aynı zamanda epididimal bölgeler arasında aglutine olabilirliği P<0.01 dir. Aynı zamanda, çok sayıda hipoosmotik şişme-positive spermatozoa mevcut bölgede geniş oranda bulunmuş P<0.01 ve her iki özellikte kaudal bölgede maksimum oranda saptanmıştır. Aglutine olan spermatozoonların sayısında ve şekillerindeki (aglutine olma şekli) değişiklikler spermatozoitlerin yüzeyindeki yağ ve proteinlere bağlı karbonhidratlardaki farklılıkları göstermekte ve bu farklılıkların epididimal geçiş sırasında ortaya çktığını ve bu değişikliklerin sperma plazma membranının ana karbonhidrat yapısını etkilediğini göstermektedir. Epididimal bölgeler arasında çok sayıdaki hipoosmotik şişme pozitif spermatozoa farkı P<0.01, kaput bölgesindeki sperma plazma membranının korpus ve kaudal bölgedeki spermadan daha az geçirgen ve daha az fonksiyonel olduğuna işaret eder. Sperma plazma membranındaki değişiklerle ilgili olan bu belirlenmiş olgulaşma kademe kademe epididaml bölgede meydana gelir. Mevcut bilgilerle, deniz yumuşakçalarından elde edilen bu bio-aktif bileşik spermanın olgunlaşması çalışmalarında biomarker olarak kullanılabilir ve ayrıca suni üreme teknolojilerinde kullanılmadan once sperm hücrelerinin üzerindeki lektin reseptörlerinin dağılımı ile ilişkili olan reprodüktif problemlerinin tanısını geliştirmede yardımcı olacak heterojenlikve plazma membran durumunu belirlemede kullanılabilir.

Anahtar Kelimeler: Sperm plazmamembranı, *Teleskopium teleskopium*, aglutinasyon, lektin

Introduction

Structural and functional asymmetry as well as surface heterogeneity of spermatozoa apparently indicates different activities in the different regions of the sperm plasmamembrane (SPM). Epididymides make their important contributions in the alteration of various properties of SPM including surface glycoconjugates during maturation phases of spermatozoa **(16)**. These changes systematically alter the SPM integrity required for the development of sperm functions. Thus, the distinct cell surface topography of sperm glycocalyx and other membrane components that acquired / modified during epididymal passage play a crucial role in temporal physiology of spermatozoa in its maturation in the epididymis **(16)** in capacitation and acrosome reaction **(34, 32)**, that are importantly necessary for the recognition of surface glycoconjugates during sperm-oocyte interaction **(13, 12, 28, 22)**.

Lectins, the carbohydrate binding proteins of plant, vertebrate, invertebrate origin possess specific binding affinities for particular terminal sugar residues of specific sugar sequences **(11)**. Cell surface glycoconjugates have fundamental roles in variety of cell functions including development, growth regulation and cellular locomotion **(10)**. As lectins have high specificity to the accessible carbohydrate side chains, therefore, they are widely used as specific probes to investigate the cellular stages of differentiation, maturation, microenvironments and their architectures and can reveal heterogeneity in the distribution of glycoconjugates. Moreover, lectin binding proteins exhibit changes in embryonic differentiation, cell maturation, aging,

metaplastic alterations, malignant transformations, blood typing, mitogenic stimulation **(29, 21)**.

Lectins have also been used extensively to localize the sugar residues of the SPM and to demonstrate changes that occur during their epididymal transport and after ejaculation **(37, 38)**. Sperm membrane integrity is one of the most carefully examined characteristics for Artificial insemination in human and in other mammals which can be evaluated with different type of lectins, such as, use of FITC labeled peanut agglutinin was found to be suitable for easy evaluation of pig SPM integrity **(35)**. Moreover, changes in the pattern of lectin binding of SPM also contribute to the understanding about the mechanism of cell surface changes during spermatozoal propagation in the epididymal segments. Hence, lectin binding may bear potential roles for biological in-vitro test to predict functional activity of spermatozoa **(25)**.

Therefore, evaluation of SPM is one of the important criteria which will reflect the nature of changing integrity and binding properties of SPM during epididymal passage through which spermatozoan status as well as epididymal environment could be predicted.

In the present investigation we performed in-vitro evaluation of spermatozoa to observe the changes between epididymal zones that occur in the SPM specially in its integrity, nature of agglutinability and heterogeneity during maturation phases with a sialic acid specific crude cytosol fraction obtained from the marine gastropod *Telescopium telescopium* **(30, 6)**, and to correlate the changeable membrane integrity with hypo-osmotic swelling test (HOST) which perhaps synthesize a plausible picture of the changeable nature of sperm surface during propagation in the epididymal segments.

Materials and methods

Isolation of the spermatheca / ovotestis gland and preparation of cytosol fraction:

The cone snail *Telescopium telescopium,* under phylum-mollusca, class-gastropoda, were collected from the estuaries of inter tidal zone during low tide at the Bay of Bengal near Sagar Island (22° 19′ N; 80° 03′ E) in West Bengal, India. Fifty adult snails were collected randomly during the month of May to mid June, 2007.

Crude cytosol fraction from the spermatheca / ovotestis glands of the snails were isolated as per the method described by DATTA **(6)**. Briefly, each spermatheca / ovotestis gland was dissected out from the body, after breaking the shell and minced carefully in a Petri dish then homogenized with 4 volume of 0.5 M phosphate buffer saline (PBS), p^{H}-7.2 at 4 °C. The grinded tissue fragmented further by sonicator at 0 °C, set at 100 % output for four 15 -s brusts at ≥30 -s interval for a total period of 1 h. The sonicated material was spun at 15,000 g for 30 min at 4 °C to discard the cellular debri.

Cytosol fraction was collected from each centrifuge tube, passed through membrane filter and was lyophilized and kept at -20 °C in sealed sterile polystyrene containers. The lyophilized cytosol fraction obtained from the mollusc was named as marine bioactive compound (MBC).

Estimation of total protein and pH of MBC: Total protein concentration of the MBC (1 mg/ml) was estimated **(24)** against a standard solution of bovine serum albumin (BSA; globulin free, Sigma chemicals, USA; 1 mg/ ml in double distilled water). The readings were recorded by a spectrophotometer at 280 nm. The pH value of the MBC solution (1 mg/ml) was also estimated by a p^H meter.

Isolation of goat epididymal spermatozoa: Testes from six Black Bengal bucks were collected from local abattoir immediately after slaughter and were brought to laboratory in thermos flask containing normal saline solution (NSS) at 37 °C. Tunica albuginea were removed from the testes and washed thoroughly with NSS. Fat-pad, blood vessels, adipose and connective tissues were removed carefully. As per anatomical position ligatures were placed unilaterally at proximal end of the vas deferens and cauda epididymis separately, and distal to the caput epididymis and vas efferentia respectively. After making ligations, whole epididymides were dissected out from each testis. Each individual ligated segments i.e. caput, corpus and cauda were cut again and were kept separately into different polystyrene Petri dishes containing 5 ml of phosphate buffer saline (PBS), p^H 7.4. Individual portions taken together from caput, corpus and cauda epididymis respectively were minced carefully and allowed to suspend in the medium for 10 min. To obtain sperm, gentle pressure on excised tissues were given by separate clean glass rods. Each resultant suspension in the medium was filtered individually through double glass wool columns to free the cellular debri. Each filtrate from individual epididymal segments were collected into separate clean glass test tubes, centrifuged at 500 g for 10 min and the supernatants were discarded. Finally, 500 μl of 0.15 M PBS, pH 7.4 was added to each sperm pellet and vortexed for 3 s. The concentration of spermatozoa from each segment were adjusted to 4×10^6 /ml using a Neubauer haemocytometer. Above procedures were performed at room temperature (35 ± 2 °C) and was completed within 2 h after slaughter.

All the luminal contents collected and processed from the three epididymal regions of six bucks were kept separately into individual sterile polystyrene test tubes at 37 °C into an incubator in humid condition containing 5 % CO_2 in air before the experiment.

Evaluation of epididymal spermatozoal plasma membrane: To observe the nature of changes, heterogeneity as well as integrity of the SPM of maturing goat spermatozoa, two different tests were performed.

Hypo-osmotic swelling test (HOST): HOS test was performed as described by JEYENDRAN *et al.* **(15)** with prepared sperm suspension each from caput, corpus and

cauda epididymis. The test tubes containing sperm-HOS mixture were incubated for 60 min at 37 °C in a water bath. After incubation, 20 μl of incubated sperm suspension from each test tube was placed on clean microscopic glass slides separately by different micropipettes. Sperm smears were prepared very gently and carefully and allowed to air dry. The smears were fixed in formal-saline solution for 10 min. All the smears were stained with 3 % Rose Bengal stain for 10 min at 37 °C in humid condition. After staining smears were washed in double distilled water by three dips each for 5 s and allowed to air dry. The stained slides were then observed under light microscope (x 200; x 450). A total of 200 spermatozoa from 10 different microscopic fields that showed coiled tail were counted randomly.

Agglutination test with MBC: To evaluate the changing nature and heterogeneity of SPM, prepared MBC solution, 25 μg/ ml in 0.15 M PBS, pH 7.4, 20 μl each, were taken into three sterile test tubes and 100 μl of prepared sperm suspension from three different epididymal regions was placed separately into each tube and mixed gently by separate clean glass rods and incubated for 30 min at 37 °C into an incubator in humid condition. After incubation, 10 μl of each sperm -MBC mixture was placed individually on different microscopic glass slides by micropipettes and smeared very gently. Smears were air dried at 37 °C and stained with 3 % Rose Bengal stain. Observations were made under the light microscope (x 450; x 1000).

For the evaluations, observations were made in triplicate and their mean results were recorded.

Statistical analysis

All the data were analyzed (SPSS 10, Statistical Package for Social Sciences; **8**) in general linear model (univariate data) with Duncan Multiple Range Test used for multiple comparison and the correlations were evaluated by Pearson correlation method.

Results

The total protein content of MBC was 9.6 mg/ ml and the pH value was 7.6.

During observation sperm samples from caput, corpus and cauda epididymis were assessed for pattern and degree of MBC induced agglutination as well as integrity of SPM by HOS test.

Table 1 depict that epididymal spermatozoa from the three regions exhibited "Head to Head" type of agglutination (Fig.1) after application of MBC, and number of agglutinated spermatozoa between the regions varied significantly ($p<0.01$). MBC which was responsible to agglutinate spermatozoa targeting acrosomal region did not alter their agglutinating pattern even in caudal region. The HOS positive (coiled tail)

spermatozoa also varied significantly ($p<0.01$) between the regions and both the characters were found highest in caudal region.

Table 1: Spermatozoa (4×10^6/ ml) showing (a) number of "Head to Head" agglutinated spermatozoa (%) treated with MBC (25μg/ml) and (b) coiled tailed spermatozoa (% HOS positive) from three epididymal regions.

Tablo 1: Epididimisin üç bölgesinden alınan spermatozoa örnekleri ($4x10^6$/ ml) (a) MBC ile (25μg/ml) muamele edilmiş ve "Baş Başa" aglutine olmuş spermatozoidler (%) (b) üç epididimal bölgeden alınan kıvrık kuyruklu spermatozoalar (% HOS pozitif)

		Caput	Corpus	Cauda	P value
(a)	Agglutination (%)	35.66 ± 2.37^a	50.5 ± 1.91^b	78 ± 0.89^c	P<0.001
(b)	HOST (%)	35 ± 2.47^a	$47.66 \pm 2.\ 1^b$	74.3 ± 1.35^c	P<0.001
	Significant level	P<0.001	P<0.001	P<0.001	P<0.001

n = 6; Values expressed as Mean ± S. E.
Mean within a parameter bearing common superscripts (a, b, c) were significant at 1 % level (Duncan multiple range test).

Figure 1. Microphotograph showing less number of agglutinated spermatozoa from caput epididymis treated with MBC (Rose Bengal; x 450).

Şekil 1. MBC uygulanmış kaput epididimisten alınan spermatozoalarda daha az aglutinasyon gösteren mikrofograf (Rose Bengal; x 450).

Correlation co-efficient (Table 2) between the epididymal regions exhibited that number of agglutinated spermatozoa (%) in cauda was indirectly varied with caput (r = -0.675) and corpus (r = -0.507) segments, and HOS positive spermatozoa (%) in cauda was positively related with caput (r = 0.119) and corpus (r = 0.171) respectively. However, any of the values were not significant at 5 % level.

Table 2: Correlation matrix of agglutination (%) and HOST (%) reactive spermatozoa from different epididymal regions (Pearson correlation).
Tablo 2: Epididimisin değişik bölgelerinde aglutinasyon (%) ve HOST (%) reaktif spermatozoalar arasındaki korelasyon

Agglutination (%)				HOST (%)			
Epididymal regions	**Caput**	**Corpus**	**Cauda**	**Epididymal Regions**	**Caput**	**Corpus**	**Cauda**
Caput	1			**Caput**	1		
Corpus	0.345	1		**Corpus**	-0.415	1	
Cauda	-0.675	-0.507	1	**Cauda**	0.119	0.171	1

Values were non-significant at 5% level.

Correlationship of agglutinability and HOS positive spermatozoa between the same epididymal region (Table 3) showed that in caput, percentage of agglutinated spermatozoa was positively related with HOS positive (%) spermatozoa (r = 0.691). However, in corpus and caudal regions coiled tiled spermatozoa (%) related negatively with number of agglutinated spermatozoa (r = -0.695; r = -0.137 respectively), and all the values were not significant at 5 % level.

Table 3: Correlation of agglutinating and HOS-positive spermatozoa between the same epididymal regions (Pearson correlation).
Tablo 3: Aynı epididimal bölgeler arasında aglutinasyon ve HOS-pozitif spermatozoalardaki korelasyon

	'r' values between epididymal parameters
Caput vs. caput	0.691
Corpus vs. corpus	-0.695
Cauda vs. cauda	-0.137

Discussion

Spermatozoa undergo many biochemical and morphological changes during their passage through the epididymal microenvironments which accompanied by distinct changes in protein and lipid compositions **(27)**, various types of glycoproteins **(2, 4)**, energy metabolism and surface characters **(2)**. These alterations of the SPM are influenced by various absorptive and secretory activities from the different segments of

the epididymal epithelium **(20, 14)**. Since different types of glycoproteins are synthesized in the different regions of the epididymis **(17, 5)** it is likely that these changes are essential for reorganization of sperm molecular architectures required for maturation and survival of the male gametes **(14)** which culminate in the conformant of fertilizing capacity of the spermatozoa.

Modifications of glycoproteins at the sperm surface have been described in different species during formation, maturation, capacitation and acrosome reaction of spermatozoa **(34)**. Membrane bound carbohydrate linked to protein and lipids are involved in different cell surface associated phenomena including cell differentiation, cell recognition and cell adhesion **(9)**, thus cell surface carbohydrate specific reaction might be due to lectins, which are able to recognize sugar residues on the cell surface receptors, of others **(26)**. Lectins as a probe are useful tools for examining these modifications in distributions of cellular glycoconjugates / cell surface receptors and the changes that occur in glycoconjugates during cell differentiation and maturation **(39)**.

In this experiment plausible explanation of sperm agglutination could well be the formation of glycoconjugates with the sialic acid moieties contained in the membrane component of spermatozoa **(31)** after addition of MBC, which is found to be specific for sialic acid **(30)**, i.e. sugar specific receptors (lectin) which are present on the SPM can interact with sugars (cell surface carbohydrates) on apposing cells resulting in the adhesion of two cells via carbohydrates and specific cell surface receptor **(9)**. Moreover, this finding indicated the presence of a sialic acid recognition protein remain in SPM. The "Head to Head" type agglutination of spermatozoa as observed, also clearly indicated the heterogenic nature of sperm head plasmamembrane and their biochemical nature that differ from other region of the sperm cells. It has been noted in various species that the sperm maturation involves the addition, subtraction, and / or alteration of SPM glycoprotein **(3, 23, 40)**, and the changing in lectin binding properties have also been observed when compared sperm cells from the testes, caput and caudal epididymis **(18, 33)**. Moreover, the general similarity in binding pattern of spermatozoa with MBC from the three epididymal regions might reflect that the nature of carbohydrate moieties remains specific in the spermatozoal regions during their transformation and that of sugar components (s) in the acrosomal glycoprotein remain unchanged. It is reported that surface alterations by the specific lectins were selective and not due to changes in non-specific properties **(36)**. The number of lectin binding sites and their distribution on spermatozoa is highly restricted to certain domains of the cell surface. These domains are often related to specific underlying morphological entities such as the acrosome, post acrosomal region and mitochondria associated with the middle piece **(18)**. ARYA and VANHA-PERTTLULA, **(1)** reported that the bovine spermatozoa expressed notable changes in their affinity to lectins along the epididymal duct not only in the number of binding sites but also in their pattern of distribution. Thus lectins with identical monosaccharide(s) specificity can identify differences in structural complexity of the cell surface receptors **(19)**.

It was also found that the agglutinability of sperm cells from different epididymal regions varied significantly ($p<0.01$) and was higher in number in caudal region followed by corpus and caput region. This finding also simulate with SARKAR *et al.*, **(33)** where mature sperm exhibited higher efficacy than the immature caput epididymal sperm for binding to concanavalin A (ConA) and wheat germ agglutinin (WGA). Furthermore, it could be that sialic acid concentration in the cauda epididymal epithelium is more than in other regions of the male reproductive tract in Black Bengal buck, thus SPM plausibly coated with more sialic acid in this region. However, agglutination of spermatozoa with MBC is related to their higher capacity for lectin binding with specific sialic acid receptor of sperm.

The ability of the spermatozoan tail to swell and / or coil in presence of HOS solution, demonstrates that the influx of water across the membrane occurs normally **(15)**. Sperm cell do swell in hypo-osmotic environment and that over a wide range of tonicities behave as perfect osmometer i.e. swelling is proportional to the degree of hypotonicity **(7)**. This phenomenon indicates the normal and functional integrity of the SPM. Moreover, the percentage of swollen cells within a sperm subpopulations has been suggested to be an indicative parameter for the membrane biochemical activity and fertility of male gametes **(15)**. Present observations revealed that there is significant variation in coiled tailed spermatozoa among the regions which indicated the changeable nature of SPM. SPM in caput are less permeable and less functional than in other epididymal regions.

The increasing trend in spermatozoan agglutinability and HOS positivity indicated the steady and gradual changes in the biochemical and physical nature of the SPM during their propagation through the epididymis and reflected more mature, stable and functional, thereby fertile spermatozoa remain in the caudal segment than caput and corpus regions.

Conclusion

Present experiment revealed bioactive compound isolated from the marine gastropod *Telescopium telescopium* could be considered as a biomarker to study the nature of SPM as well as its heterogeneity that undergo changes during their maturation phases. Moreover, the binding pattern of spermatozoa, could be used to identify the carbohydrate composition of the cell, because carbohydrate composition on the cell surface is region specific which can be utilized to analyze the variation in the distribution and density of exposed saccharides of any cell.

Present approach might be beneficial to improve the diagnosis of reproductive problems arising in relation to distribution of lectin receptors on male gametes of any species to be used for Artificial Reproductive Technology. Moreover, as MBC are able

to agglutinate spermatozoa, a suitable contraceptive medium could be thought off. However, purification of MBC and its application require more scientific approach.

References

1. **Arya, M., Vanha-Perttula, T.:** Lectin binding pattern of bull testis and epididymis. J. Androl., (1985a); 6: 230-242.
2. **Bedford, J. M.:** Maturation, transport & fate of spermatozoa in the epididymis. Handbook of physiology, Vol. V., Section-6, (ed.) Hamilton, D.W. & Green, R. O. Am. Phy. Soc., Washington D.C., (1975); 307-317.
3. **Bernal, A., Torres, J., Reyes, A., Rosado, A.:** Presence and regional distribution of sialyl transferase in the epididymis of the rat. Biol. Reprod., (1980); 23: 290-293.
4. **Briz, M. D., Bond, S., Pinart, B., Egozcue, J., Camps, R.:** Comparative study of boar sperm coming from the caput, corpus and cauda regions of the epididymis. J. Androl., (1995); 16 (2): 175-188.
5. **Brooks, D. E.:** Secretion of proteins and glycoproteins by the rat epididymis: regional differences, androgen dependence, and effects of protease inhibitors, procaine, and tunicamycin. Biol. Reprod., (1981); 25: 1091-1097.
6. **Datta, U.:** Bioactive substance(s) from a marine mollusc Telescopium telescopium and its role on mammalian reproduction. Ph.D. Thesis. Jadavpur University, Kolkata, India. 2003.
7. **Drevius, L. O.:** Bull spermatozoa as osmometers. J. Reprod. Fertility., (1972); 28: 29-39.
8. **Duncan, D. B.:** Multiple range and multiple F test, Biometrics., (1955); 11: 1-42.
9. **Frazier, W., Glaser, L.:** Surface components and cell recognition. Annu. Rev. Biochem., (1979); 48: 491-496.
10. **Gabius, H-J.:** Vertebrate lectins and their possible role in fertilization, development and tumor biology. In-vitro., (1987); 1: 75-84.
11. **Goldstein, I. J., Hayes, C. E.:** The lectins: carbohydrate binding proteins of plants and animals. Adr. Carhydr. Chem. Biochem., (1978); 35: 127-340.
12. **Green, D. P.:** Mammalian fertilization as a biological machine: a working model for adhesion and fusion of sperm and oocyte. Human Reprod., (1993); 8: 91-96.
13. **Gwatkin, R., Williams, D.:** Receptor activity of the solubilized hampster and mouse zona pellucida before and after the zona reaction. J. Reprod. Fetil., (1976); 19: 55-59.
14. **Hinton, B. T., Palladino, M. A.:** Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment. Micros. Res. Tech., (1995); 30: 67-81.
15. **Jeyendran, R. S., Vandervent, H. H., Perez-Paleac, Z. M., Crabo, B. G., Zanevld, L. J. D.:** Development of an assay to assess the functional integrity of human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil., (1984); 70: pp. 219-225.
16. **Jones, R.:** Membrane remodeling during sperm maturation in the epididymis. Oxford Reviews of Reproductive Biology, (1989);11: 285-337.

17. **Jones, R., Brown, C. R., Von Gios, K. I., Parker, M. G.:** Hormonal regulation of protein synthesis in the rat epididymis. Characterization of androgen-dependent and testicular fluid-dependent proteins. Biochem. J., (1980); 188: 667-676.

18. **Koehler, J. K.:** Lectins as probes of the spermatozoan surface. Arch. Androl., (1981); 6: 197-217.

19. **Kunz, A., Brown, D., Orci, L.:** Appearance of helix pomatia lectin binding sites on podocyte plasma membrane during glomerular differentiation. A quantitive analysis using the lectin-gold technique. Lab. Invest., (1984); 51 (3): 317-324.

20. **Levine, N., Marsh, D. J.:** Micro puncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. J. Physiol., (1971); 213 (3): 557-570.

21. **Lis, H., Sharon, N.:** Lectins as molecules and as tools. Ann. Rev. Biochem., (1986); 55: pp. 35-67.

22. **Liu, H. W., Lin, Y. C., Chao, C. F., Chang, S. Y., Sun, G. H.:** GP-83 and GP-39, two glycoprotein secreted by human epididymis are conjugated to spermatozoa during maturation. Mol Human Reprod., (2000); 6 (5): 422-428.

23. **Lopez, M. L., deSouza, W., Bustos-Obregon, E.:** Cytochemical analysis of the anionic sites on the membrane of the stallion spermatozoa during the epididymal transit. Gamete Res., (1989); 18: pp.319-332.

24. **Lowry, O. H., Rosebrough, N. J., Farr, A. l., Randall, R. J.:** Protein measurement with the folin-phenol reagent. J. Biochem., (1951); 193, 265.

25. **Mladenovic, I., Hajducovic, L., Genbacev, O., Cuperlovic, M., Movsesijan, M.:** Lectin binding as a biological test in vitro for the prediction of functional activity of human spermatozoa. Gamete Res., (1993); 24: 403-413.

26. **Monsigny, M., Roche, A. C., Kirnda, C., Midoux, P., Obernovitch, A.:** Characterization and biological implications of membrane lectins in tumors, lyphoid and myeloid cells. Biochem., (1988); 70: 1633-1649.

27. **Moore, H. D. M.:** Contribution of epididymal factors to sperm maturation and storage. Andrologia., (1998); 30: 233-239.

28. **Myles, D. G.:** Molecular mechanisms of sperm-egg membrane binding and fusion in mammals. Dev. Biol., (1993); 158: 35-45.

29. **Nicolson, G. L.:** The interactions of lectins with animal cell surfaces. Int. Rev. Cytol., (1974); 39:90-190.

30. **Pakrashi, A., Datta, U.:** In-vitro sperm agglutination and spermicidal activity of protein isolated from a marine mollusc *Telescopium telescopium* (Gastropod). Indian J. Marine Sci., (2001); 30: 93-97.

31. **Peleg, B. A., Ian Consscu, M.:** Sperm agglutination and sperm absorption due to mixoviruses, *Nature.*, (1966); 211: 1211-1212.

32. **Sanz, I., Calvete, J. J., Mann, K., Gabius, H. J., Topfer-petersen, E.:** Isolation and biochemical characterization of heparin binding protein from boar seminal plasma a dual role for sperm adhesion in fertilization. Mol. Reprod. Dev., (1993); 34: 37-43.

33. **Sarkar, M., Mazumdar, G. C., Chatterjee, T.:** Goat sperm membrane: Lectin binding sites of sperm surface and lectin affinity chromatography of the mature sperm membrane antigens. Biochion. Biophys. Acta., (1991); 1070 (1): 198-204.

34. **Schwarz, M. A., Koehler, J. K.:** Alteration in lectin binding to guineapig spermatozoa accompanying in vitro capacitation and acrosome reaction. Biol. Reproduction, (1979); 21:1295-1299.

35. **Serrano, H., Diaz-Esparza, L., Garcia-Suarez, D.:** Pig sperm membrane integrity evaluated by lectin labeling. Arch. Androl., (2001); 47 (1): 59-65.

36. **Signer, S. L., Lambert, H., Cross, N. L., Overshreet, J. W.:** Alteration of human sperm surface during in-vitro capacitation as assessed by lectin induced agglutination. Gamete Res., (1985); 12: 291-299.

37. **Sinowatz, F., Friess, A. E.**: Localisation of lectin receptors on bovine epididymal spermatozoa using a colloidal gold techniques. Histochemistry, (1983); 79: 335-344.

38. **Sinowatz, F., Volgmayer, J. K., Gabius, H. J., Friess, A. E.:** Cytochemical analysis of mammalian sperm membranes. Prog. Histoch. Cytoch., (1989); 19 (4): 1-74.

39. **Spicer, S. S.**: Advantages of histochemistry for the study of cell biology. Histochemical Journal., (1993); 25: 531–547.

40. **Tulsiani, D. R. P., Nagdas, S. K., Skudlarek, M. D., Orgebin-Crist, M-C.:** Rat sperm plasma membrane mannosidase: Localisation and evidence for proteolytic processing during epididymal maturation. Dev. Biol., (1995); 167: 584-595.