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Isolation and identification of sperm maturation proteins (ER-α, CK-19, AQP-1, and SLPI) in epididymis of bull (*Bos taurus*)

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ABSTRACT

Epididymal sperm maturation is considered as an integral part of the process that permits mammalian gamete fusion and formation of the zygote. The present studies aimed at the identification of sperm maturation proteins namely, ER-α, CK-19, AQP-1, and SLPI in the different region (cauda, corpus, caput) of the normal and castrated bull epididymis by employing RT-PCR and western blot analysis. The study also aimed at understanding the intrinsic role of androgens in regulation of epididymal functions. The study revealed the presence of ER in all the three regions of the epididymis. However, castration caused a significant decrease in expression of ER-α in caput region and complete down-regulation on corpus and cauda region of epididymis. The expression of CK-19 was found to be more or less equally expressed in all the three regions of the normal bull epididymis. AQP-1 expression was observed only in the caput region of normal bull epididymis and there was complete absence of its expression in castration bull epididymis. Likewise, SLPI expression was also observed in the regions of normal bull epididymis. However, it was completely down-regulated upon castration. Thus, the presence of ER-α, CK-19, AQP-1 and SLPI in the epididymis of the bull suggests their functional role in sperm maturation. Further, the presence of ER-α and CK-19 only in a particular region and complete absence of AQP-1 and SLPI in all the three regions of the epididymis of the castrated bull was suggestive of dependence of epididymal function on androgens. Identification of specific markers for these proteins may pave way for diagnosis and treatment of infertility in domestic species as well as potential targets in the design of specific contraceptive medicines.

KEYWORDS

Bull;
Castrated;
Intact;
Epididymis;
Sperm;
Proteins.

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INTRODUCTION

In cattle breeding, semen production is a matter of utmost importance since value of the bull is more than half of the herd. Hence, bulls should possess superior seminal characteristics with negligible sperm abnormalities for optimum fertility. At present, most breeders employ breeding soundness exams (BSE) as a test for assessing male fertility status, although it has been opined that the intact sperm motility and its morphology do not indicate that the sperm has the complete ability to fertilize the ova[15]. Further, it has also been put forth that the testicular sperms lack the ability to move and to fertilize an ovum, postulating that they acquire these properties during their passage through the epididymis[1]. Thus, indicating the importance of epididymis in sperm maturation and its fertilizing capacity.

As a part of the sperm maturation process, a host of epididymal proteins are involved in modifying and tailoring the sperm membrane and its proteins, besides maintaining a favorable environment for the residing sperms[12]. Metamorphosis of an immature and immotile sperm into a mature sperm capable of progressive motility and fertility may be a result of highly regulated and complex sequential events in the epididymis[6]. Furthermore, spermatozoa of scrotal mammals possess limited biosynthetic activities[7] and display a crucial dependence on the activity of the epididymal epithelium for their maturation and survival[12]. The biochemical composition of the epididymal fluid microenvironment exhibits regional variation along the length of the epididymal duct. This variation results from the differential absorptive and secretory activities of the epididymal epithelium [12].

Increasing our knowledge on spermatozoa maturation in epididymis would provide specific molecular markers in order to develop new criteria that are more accurate and objective in predicting and improving male fertility. Hence, the present study was carried out with the objective of identifying the genes namely, Estrogen receptor-α (ERα) Cytokeratin-19 (CK19), Aquaporin-1 (AQP1) and Secretory leucocyte protease inhibitor (SLPI) expressed in three different regions of epididymis as well as western blot analysis of the protein products identified for the genes detected. To study the hormonal control of epididymis function, we have employed intact and castrated bulls. This approach was selected due to the problems in carrying out experiments with large animals especially monitoring the effects of administration or deprival of hormones. We have made an attempt to circumvent this problem by comparing the epididymal gene expression of intact bull and castrated bulls since they are easily accessible in the slaughter house. Analysis of the transcripts from two groups, hitherto provide valuable information on the androgen regulated genes in the epididymis.

MATERIALS AND METHODS

Six proven bulls, *Bos taurus* (three intact and three castrated) of 5-6 year old from the local slaughterhouse were employed for the present study. The epididymal samples were surgically obtained under aseptic conditions from the testis of each of these animals just before slaughter under anesthesia. Briefly, the epididymis was divided into caput, corpus and cauda segments. Each segment was cut into small fragments, squeezed with forceps and washed twice with 1X PBS to release the contents of the ducts.

RNA isolation

Total RNA was extracted from the tissue using TRIzol reagent (Sigma, St. Louis, MO, USA) which employs a convenient single-step liquid phase separation for isolation of RNA, DNA and protein[5]. All steps during isolation were performed according to manufacturer's instructions. All RNA used for PCR experiments were treated with RNase-free DNase to remove any contaminating DNA.

The primers for PCR amplification were designed for ERα, AQP1, CK19 and SLPI using the program Primer Express, Applied Biosystems, USA. Primers were designed ensuring that the percentage of GC content of the primers ranged between 40-60% with the Tm ranging from 52-65°C, in most cases. The primers were also subjected to analysis for formation of secondary structures, which decrease primer annealing efficiency. In addition, an extensive BLAST analysis was performed to ensure that the primers did not generate mismatch with other gene sequences. The

primer sequence for ERα, AQP1, CK19, SLPI and cyclophilin is mentioned in TABLE 1.

First strand cDNA synthesis and PCR amplification

First strand cDNA synthesis was performed in a cocktail containing 1.5 μM random hexamers (Roche Molecular Biochemicals, Germany), 100 units of Moloney murine leukemia virus reverse transcriptase (MMLV-RT) obtained from Promega Corporation, Wisconsin, Madison, 20 units of RNase inhibitor, 1x reverse transcriptase buffer and 400 μM dNTPs in a 20 μl reaction mixture. Following incubation at 37°C for one hour, the enzyme was inactivated by heating at 95°C for five minutes. The cDNA formed by the RT reaction was used for PCR amplification to analyze the expression of ERα, AQP1, CK19 and SLPI using gene specific primers. Simultaneously, a control without reverse transcriptase was included to verify absence of non-specific amplification resulting from genomic DNA contamination.

One-tenth volume of the RT reaction was employed for PCR amplification using specific primers. Primers used for RT-PCR analyses were synthesized by Bangalore Genei, India. The PCR reaction mixture contained 0.4 μM of the forward and reverse primers, 200 μM of the dNTPs in 10 mM Tris, 1mM Magnesium chloride buffer (Bangalore Genie, India) and 1 unit of Taq Polymerase (Bangalore Genie, India). The cDNA amplifications employed an initial heating at 94°C for three minutes, followed by different cycles of 94°C for 45-60 seconds, annealing temperature for 45-60 seconds and 72°C for 45-60 seconds. The PCR reactions were performed within the linear range of amplification for each amplicon (35 cycles) to facilitate quantitation. A 12 μl aliquot of the PCR product was electrophoresed on a 1.5% (w/v) agarose gel containing 0.5 μg/ml ethidium bromide in a buffer containing 45 mM Tris-borate-1 mM EDTA (IX TBE). The difference in intensities of the products following electrophoresis was analyzed using EDAS 120 Kodak Gel documentation system. The expression level of specific gene products was inferred upon intactizing their signal intensities to the expression of the internal control (cyclophilin). The RT-PCR details are compiled in TABLE 1. Primers used for RT-PCR analyses were synthesized by Bangalore Genei, India.

Preparation of protein lysates

Tissue samples were washed extensively with PBS (pH 7.4) and homogenized with a polytron homogenizer in the presence of ice-cold buffer (50 mM Tris pH 8.0, 150 mM Sodium chloride, 0.02% Sodium azide) containing 1X protease inhibitor cocktail (Roche Molecular Biochemicals, Germany). Protein lysates were clarified by centrifugation at 400 g for 20 minutes at 4°C to remove cellular debris. The supernatant thus obtained, was subjected to brief sonication for 5-7 seconds (25-30 Hz) and then centrifuged at 21400 g for 30 minutes. The supernatant containing cellular proteins were supplemented with 10% glycerol and stored at -20°C. The pellet containing the membrane fraction (and cytoskeletal proteins) was extracted in 1% Nonidet-P40 and 0.5% sodium-deoxycholate for 1-2 hours on ice and centrifuged at 21400 g. The supernatant containing membrane proteins was supplemented with 10% glycerol and stored at -20°C. An aliquot of these fractions was employed for protein estimation by the method of Lowry[14]. All the chemicals used for lysis were purchased from Sigma Chemical Co., St. Louis, MO, USA.

TABLE 1 : List of primers employed for PCR in bull

Primer	Sequence	Annealing temp (°C)	Product size (bp)
Cyclophilin	FP 5' CCGCGTCTCTTTTGAGCTGTT 3' RP 5' TCTTGCTGGTCTTGCCATTCC 3'	58	411
ER-α	FP 5' GGCATGGTGGAGATCTTTGAC 3' RP 5' TGGCTCTGATTCACGTCCTCT 3'	59	443
CK19	FP 5' TGACTTCCGCACCAAGTTTGA 3' RP 5' GGCTTTCATGCTGAGCTGAGA 3'	61	466
AQP1	FP 5' GTACATCATTGCCCAGTGCGT 3' RP 5' TCATCTCCACCCTGGAGTTGA 3'	62	512
SLPI	FP 5' TCTTAACAAGTGGACCGCCAG 3' RP 5' TGAATCTTTCACCGGCAGG 3'	58	481

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Western blotting was carried out according to procedure of[19]. Proteins (50-100µg) were electrophoresed on 10% SDS–polyacrylamide gels and transferred onto nitrocellulose membranes using a semi-dry transfer method. After completion of transfer, the membranes were blocked in 5% non-fat milk in PBS, pH 7.4 containing 0.025% Tween-20 and incubated overnight at 4°C with appropriate dilution of the primary antibody. Membranes were washed several times with PBS containing 0.05% of Tween–20 (PBST) to remove the non-specifically bound antibodies and incubated with the corresponding horse radish peroxidase-conjugated secondary antibody for one hour. Following extensive washing in PBST, the antigen bound antibodies were detected by Enhanced Chemi-Luminiscence (ECL) detection system using the protocol recommended by the manufacturers (Amersham Biosciences, UK). Antibodies used in the study are compiled in TABLE 2.

TABLE 2 : List of antibodies employed for Western blot analyses in bull

Antibody	Source	Antibody type	Dilution of primary Ab	Dilution of secondary Ab	Protein size (kDa)
ERα	Santa Cruz SC-542	Polyclonal, rabbit	1:100	1:5000 (Anti-rabbit)	66
CK19	Novocastra CK-19	Monoclonal, mouse	1:100	1:5000 (Anti-mouse)	40
AQP1	Santa Cruz SC-9879	Polyclonal, rabbit	1:100	1:5000 (Anti-goat)	28
SLPI	Santa Cruz	Polyclonal, rabbit	1:100	1:5000 (Anti-rabbit)	12

Histological study

The tissue samples collected for histological study from the caput, cauda and corpus regions of epididymis of intact and castrated bulls were immediately fixed and processed by routine paraffin embedding technique. Sections of four to five micron thickness were cut using Bright Rotary Microtome with disposable blades. These sections were then stained with routine hematoxylin and eosin method.

RESULTS

Considering the fact that little is known about the expression of genes in different regions of epididymis of bull, the present study was restricted to genes known to be regulated by androgens and estrogens. Castration results in a drastic decrease in androgen and the estrogen, since androgens are precursor for estrogens. It can be seen that the expression of cyclophilin mRNA, when analyzed in parallel for all the genes studied by RT-PCR, remained unchanged in all three regions of the epididymis in intact bull (Figure 1) and caput and cauda regions of intact and castrated bull (Figure 2). The expression of cyclophilin was drastically reduced in the caput to cauda region of the castrated bull when compared to the intact bull (Figure 2).

The ERα was found to be expressed in all three regions of the bull epididymis. The results revealed a decrease in the intensity of expression of ERα from caput to cauda region of the epididymis in intact bull (Figure 3). Concomitant with the ERα mRNA expression, ERα protein expression was also observed in all the three regions of the epididymis in intact bull by western blot analysis (Figure 5). The ERα gene expression in the epididymis was evaluated in the samples collected from caput and cauda region of castrated bull. It can be seen that the expression of ERα mRNA in the castrated bull was less in the caput region, while the cauda region showed complete absence of ERα mRNA (Figure 4). Further, the western blot analysis for ERα protein revealed no expression for the same in all three regions of castrated bull epididymis (Figure 5).

The expression of CK19 mRNA was found to be more or less equal in all the three regions of the intact bull epididymis (Figure 6). Similarly, CK19 protein expression was also observed in all the three regions of the epididymis by western blot analysis (Figure 8). The expression of CK19 was drastically reduced in the caput and cauda region of the castrated bull when compared to the intact bull. However, slight expression of CK19 mRNA was observed in the caput region but the cauda region showed complete absence of CK19 mRNA in the castrated bull epididymis (Figure 7). The western

blot analysis for CK19 protein did not reveal any difference in the expression of all the three regions of intact and castrated bull epididymis (Figure 8).

The expression of AQP1 mRNA was observed only in caput region of the intact bull epididymis. There was no mRNA expression for AQP1 in the corpus and cauda region (Figure 9). The PCR amplification for AQP1 mRNA in castrated bull epididymis did not reveal any signal in both caput and cauda region (Figure 10).

It is well established that SLPI is one of the androgen regulated gene in epididymis and conversely the level of expression of SLPI was monitored in the intact and castrated bull epididymis. It can be seen that the present study revealed the expression of SLPI mRNA in all three regions of the intact bull epididymis (Figure 11) and complete absence of SLPI mRNA expression in the caput and cauda region of the castrated bull epididymis (Figure 12).

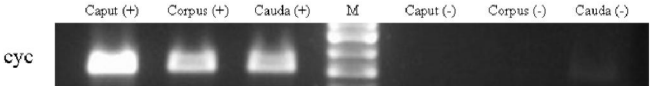

Figure 1: Expression of cyclophilin (cyc) in caput, corpus and cauda regions of intact bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

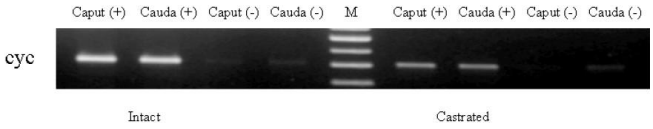

Figure 2: Expression of cyclophilin (cyc) in caput and cauda regions of intact and castrated bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

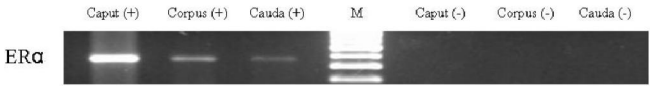

Figure 3: Expression of ERα in caput, corpus and cauda regions of intact bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

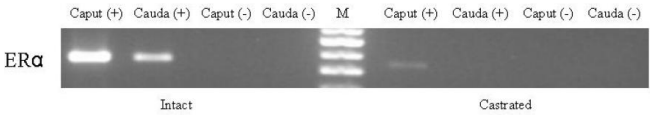

Figure 4: Expression of ERα in caput and cauda regions of intact and castrated bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

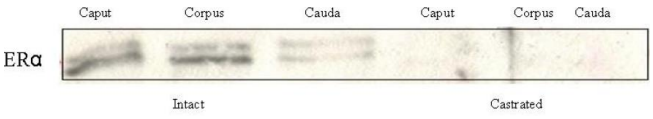

Figure 5 : Western blot analysis of ERα in the caput, corpus and cauda regions of intact and castrated bull epididymis.

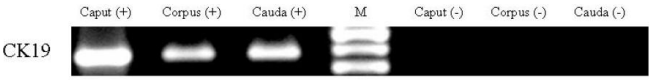

Figure 6 : Expression of CK19 in caput, corpus and cauda regions of intact bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

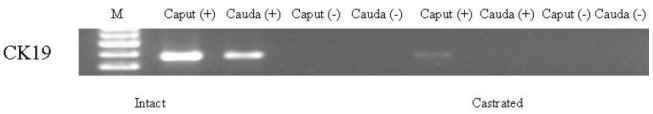

Figure 7 : Expression of CK19 in caput and cauda regions of intact and castrated bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

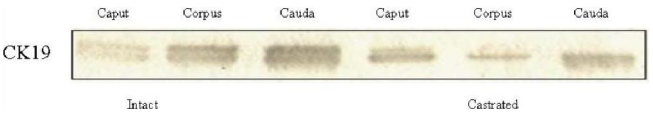

Figure 8 : Western blot analysis of CK19 in the caput, corpus and cauda regions of intact and castrated bull epididymis.

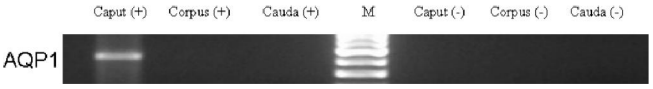

Figure 9 : Expression of AQP1 in caput, corpus and cauda regions of intact bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

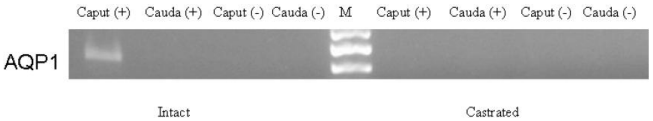

Figure 10 : Expression of AQP1 in caput and cauda regions of intact and castrated bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

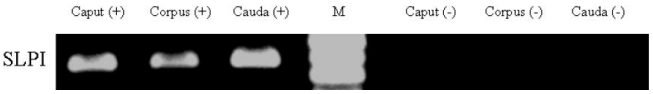

Figure 11 : Expression of SLPI in caput, corpus and cauda regions of intact bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

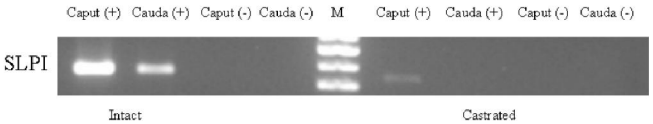

Figure 12 : Expression of SLPI in caput and cauda regions of intact and castrated bull epididymis. M: DNA ladder, (+) indicates RT-positive and (-) indicates RT-negative.

Histological study in the intact bull epididymis

Histologically, the caput region of the intact bull epididymis revealed numerous ductule which were lined by tall columnar epithelial cells (principal cells) showing pseudostratification with nuclei placed at different levels in the eosinophilic cytoplasm (Plate 1a). The apical

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portion of the principal cell showed long stereocilia (microvilli). In addition to principal cells, a few basal cells which were cuboidal with round nuclei placed at the basal region of ductule were also observed (Plate 1c). The lumen of the ductules was regularly oval or circular in appearance and consisted of moderate amount of sperms. These ductules were found to be surrounded by a thin smooth muscle layer and were separated by connective tissue.

The corpus region of the intact bull epididymis revealed almost similar to the microscopic appearance of caput region (Plate 2a) except for a slight decrease in the height of the lining principal cells (Plate 2c). The cauda region of the intact bull epididymis revealed very large ductules lined by flattened principal cells and abundant amount of sperms in the lumen. The lumen varied in the shape and size depending upon the amount of sperms (Plate 3a). The smooth muscle layer around the ductules was comparatively more. In addition, many apoptotic cells with condensed or crescent shaped nucleus surrounded by a halo were also observed in the sperm mass (Plate 3c).

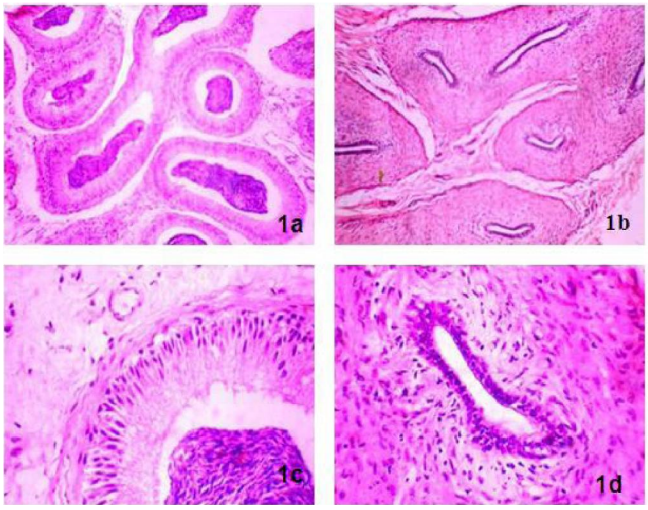

Plate 1: The caput region of intact bull epididymis showing numerous ductules with oval or circular lumen and moderate amount of sperms (1a) H&E X 125 and tall columnar epithelial cells with long stereocilia (1c) H&E X 500. The caput region of castrated bull epididymis showing reduced ductular size lined with flattened cuboidal epithelial cells (1b) H&E X 125 and irregular ductule diameter surrounded with thick layer of proliferative smooth layer (1d) H &E X 500.

Histological study in the castrated bull epididymis

Microscopically, the caput region of epididymis in castrated bull revealed ductules with reduced luminal size, lined by flattened cuboidal epithelial cells with basally placed round nuclei (Plate 1b). These ductules were found to be surrounded by thick layer of proliferative smooth muscle fibers and also fibrous connective tissue in interductular region. The lumen

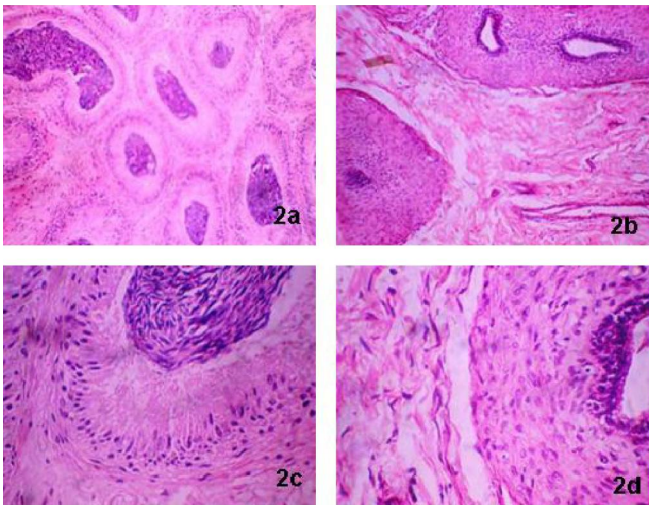

Plate 2 : The corpus region of intact bull epididymis showing numerous ductules lined by columnar epithelial cells with smooth muscle layer (2a) H&E X 125 and reduced height lining the ductule consisting of moderate amount of sperm mass (2c) H&E X 500. The corpus region of castrated bull epididymis showing irregular shaped ductules and thick smooth muscle layer (2b) H&E X 125 and reduced ductules lined by flattened epithelial cells (2d) H&E X 500.

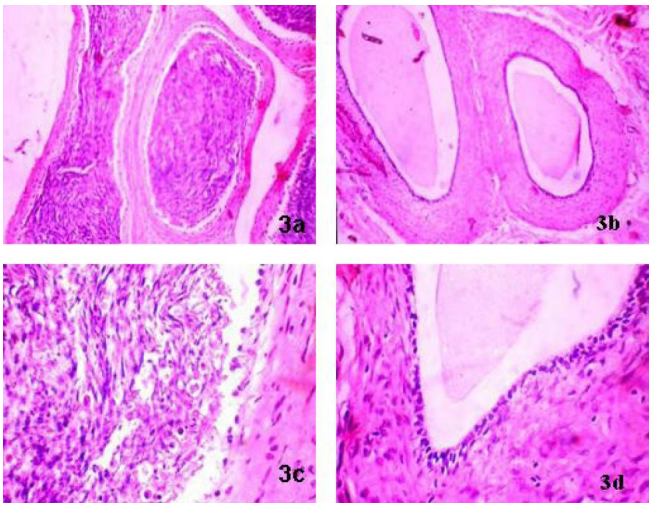

Plate 3 : The cauda region of intact bull epididymis showing ductules of larger size with cuboidal epithelium and abundant amount of sperm mass in the lumen (3a) H&E X 125 and presence of apoptotic cells (3c) H&E X 500. The cauda region of castrated bull epididymis showing thickened smooth muscle layer and eosinophilic fluid in the lumen (3b) H&E X 125 and flattened epithelium (3d) H&E X 500.

revealed complete absence of sperms (Plate 1d).

The corpus region of the castrated bull epididymis revealed similar microscopic appearance of the caput region (Plate 2b) but smooth muscle proliferation around the ductule was comparatively more when compared to caput region (Plate 2d). The cauda region of castrated bull epididymis revealed large ductules with darkly stained flattened epithelium, surrounded by marginally increased smooth muscle fiber layer and abundant amount of interductular connective tissue (Plate 3b). The lumen of the ductule showed absolute absence of sperms that was found to be replaced with eosinophilic fluid (Plate 3d).

DISCUSSION

Epididymis, being a component of excurrent tract of the male reproductive system, is involved in various physiological processes including sperm maturation. Epididymal function is also regulated by various factors at both the gene and protein level. Among the various factors that are regulating epididymal function, androgens have been clearly established as playing a key regulatory role in the synthesis and secretion of specific molecules associated with sperm maturation and storage[8]. In view of this, the present study was taken up to envisage the presence and role of sperm maturation proteins viz., ERα, CK19, AQP1 and SLPI in both intact and castrated bulls.

The macroscopic appearance of drastic reduction in size of the epididymis (Plate 4) in castrated bull when compared to the same in intact bull indicates atrophic changes. This could be probably due to the absence of blood supply to the testicle as well as to the epididymis and the absence of androgens in the castrated bull[2]. The role of ERα has been exemplified by the work conducted in Estrogen Receptor alpha knock out (ERαKO) animals. The most remarkable effect of loss of the ERα function was attributed to fluid accumulation leading to abintact dilatation of the excurrent ducts in the ERαKO mice[10]. Likewise, *in-vivo* studies conducted by employing antiestrogens such as tamoxifen (non-steroidal agent) and ICI 182780 (steroidal agent) were also shown to down regulate the expression of ERα in rat and bonnet monkeys[17]. Hence, the expression of ERα mRNA (Figure 3) and ERα protein (Figure 5) in the caput, corpus and cauda region of the epididymis as evidenced in the present study, suggests the possible role of ERα in regulating fluid absorption, ion and protein concentration in the epididymal fluid, sperm motility and its maturation in the epididymis of intact bull. Further, in the present study, a decrease in the intensity of expression of ERα from caput to cauda region (Figure 4) may be due to the fact that first two segments of the epididymis that are concerned with sperm maturation in the presence of sperm maturation proteins. This was attributed to secretory and absorptive functions, whereas the terminal segment is engaged in sperm storage[9]. Further, in the present study, the expression of ERα protein in the caput, corpus and cauda region of the bull epididymis by western blot analysis was done using antibodies raised against rat ERα protein. Hence, it is of the opinion that the western blot results need further confirmatory studies using antibodies raised specifically against bull ERα protein. Likewise[2], observed a decrease in total protein, RNA and DNA content in the epididymis following orchidectomy. He also opined that the RNA expression in the caput and cauda epididymis was differentially affected[8] reported a decline in the transcripts of several genes in the epididymis, confirming that androgens play an essential role in epididymal function. In the present study, it was also observed that the intensity of expression of ERα mRNA in the caput region of intact bull was higher than that of the same in the castrated bull which indicates that this protein is regulated by androgens. Further, the absence of any expression of ERα mRNA in the cauda region as well as ERα protein in caput, corpus and cauda region of the epididymis of the castrated bull indicates that this protein is androgen dependant.

The presence of CK19 mRNA (Figure 6) and CK19 protein (Figure 8) in all the three regions of the epididymis in the intact bull is probably because CK19 is ductal epithelial cell specific protein. It has also been postulated that CK19 has a predominant role in secretory tissues including epididymis in anchoring several apically placed ion exchangers and proteins[16]. Further, CK19 being expressed more or less in equal intensities in all the three regions of the epididymis is suggestive of its role as an essential cytoskeletal protein irrespective of the region. However in the present study, the identification of CK19 protein by western blot was

done using antibodies raised for rat CK19 and the same needs further confirmation with antibodies raised specifically for bull CK19. In the present study, it was observed that the intensity of expression of CK19 mRNA in the caput region of intact bull was higher than that of the same in the castrated bull (Figure 7), while the same was found to be completely absent in the cauda region of the castrated bull. This result is in conformation with the reports of[2,8,17] suggesting that these proteins are down regulated in the absence of androgens. Surprisingly, CK19 protein expression was observed to be present in all the three regions of the epididymis of the castrated bull (Figure 8). This may be due to the homology of the rat CK19 antibody with some other protein of the bull epididymis. Hence, it is suggested that the same needs further confirmation with antibodies raised specifically for bull CK19.

The present study revealed the expression of AQP1 mRNA only in the caput region of the epididymis of intact bull, while no expression was observed in the corpus and cauda region of the epididymis (Figure 9) suggesting that AQP1 exists in caput region of the intact bull, probably to help in fluid reabsorption process as suggested by[17]. The absence of AQP1 expression in corpus and cauda region probably could be due to the fluid reabsorption process in this region being taken up by other isoforms of this protein and its regulators and / or due to the presence of various other water channels[13]. The AQPs are said to be regulated by hormones and other factors[3]. In this context[17], has reported that tamoxifen, an antiestrogenic agent, significantly reduces the expression of AQP1 in caput region of rat and bonnet monkey epididymis. The present study revealed complete absence of an expression of AQP1 mRNA in the caput and cauda region of castrated bull epididymis (Figure 10). This absence of AQP1 expression suggests that AQP1 is androgen dependent and require androgens to maintain their intact levels of expression.

The presence of SLPI in the mammalian epidydimis to date has been reported only in the rats[4]. Caldamone et al.[17] reported copious amount of elastase in the semen sample of genital tract infected male. Further, it is also reported that elastase could inhibit sperm motility and SLPI has inhibitory action on elastase and restore the sperm motility. This indicates that the protease-inhibitory function of SLPI was necessary for sperm motility and in turn on its maturation capacity. Apart from this, it was also opined that SLPI has antimicrobial activity[11]. In view of this, the SLPI transcript was studied in the bull epididymis and it was found to express in all the three regions of the intact bull epididymis (Figure 11) indicating that it does have a role in sperm motility in the bull epididymis. Further, the present study also revealed complete absence of SLPI mRNA expression in the caput and cauda region (Figure 12) of castrated bull epididymis. This absence of SLPI expression suggests that it is also androgen dependent and/or requires androgens to maintain the intact levels of expression in the epididymis of the bull.

The microscopic similarities observed between the caput and the corpus region of the bull epididymis could be probably because both these regions are mainly involved in the secretory and absorptive function[9]. The cauda region of intact bull revealed abundant amount of sperms in the lumen suggesting its storage function[9]. The presence of apoptotic cells within the lumen of the epididymal duct suggests the spermiophagy function of the cauda epididymis. The spermiophagy function of the cauda regions helps in quality control of the sperms, preventing mis-shapen, genetically abintact sperms from entering the ejaculate[18]. There is abundant increase in the interductular connective tissue of castrated bull epididymis compared to intact bull epididymis. Hence, from this study it is of the opinion that further studies could be focused on identifying specific markers for these proteins that may pave way for diagnosis and treatment of infertility in domestic species as well as in the design of specific contraceptive agents.

The expression of ERα mRNA and ERα protein in the caput, corpus and cauda region of the epididymis in the present study, suggests the possible role of ERα in regulating fluid absorption, sperm motility and its maturation in the epididymis of intact bull. It was also observed that the intensity of expression of ERα mRNA in the caput region of intact bull was higher than the castrated bull which indicates that this protein is regulated by androgens. Further, the absence of any expression of ERα mRNA in the cauda region as well as ERα protein in caput, corpus and cauda region of the epididymis of the castrated bull indicates that this protein is androgen dependant.

The presence of CK19 mRNA and CK19 protein in all three regions of the epididymis in intact bull is suggestive of its role as an essential cytoskeletal protein. The present study revealed the expression of AQP1 mRNA only in the caput region of the epididymis of intact bull, while no expression was observed in the corpus and cauda region of the epididymis suggesting that AQP1 probably may help in fluid reabsorption process. The present study revealed complete absence of an expression of AQP1 mRNA in the caput and cauda region of castrated bull epididymis indicating that AQP1 is androgen dependent.

The presence of SLPI in all three regions of the intact bull epididymis indicates that it does have a role in sperm motility in the epididymis. Further, the present study also revealed complete absence of SLPI mRNA expression in the caput and cauda region of castrated bull epididymis. This absence of SLPI expression suggests that it is also androgen dependent. Further studies are required on identifying specific markers for these proteins that may pave way for diagnosis and treatment of infertility in domestic species as well as in the design of specific contraceptive agents.

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