



ISSN Print: 2664-9926
ISSN Online: 2664-9934
NAAS Rating: 4.82
IJBS 2025; 7(6): 381-384
www.biologyjournal.net
Received: 22-03-2025
Accepted: 27-04-2025

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Minerals profiles in the epididymal luminal milieu with respect to maturation of buck (*Capra hircus*) spermatozoa

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i6f.402>

Abstract

The aim of the present study was to investigate about micro environment of epididymis towards maturation of buck spermatozoa. Normal testes attached with intact epididymis collected from adult and healthy bucks (n=60) from local abattoir immediately after slaughter were used for assessment of minerals profiles in the epididymal luminal fluid. It was revealed that in caput, corpus and caudal regions, the level of Zn and Cu found to be increased gradually, however, the level of Fe, Na and K diminished steadily. Concentration of Zn and Cu was highest in caudal segment, whereas concentration of Fe, Na and K was highest in caput segment. The overall relationship of spermatozoan motility was found to be positive and highly significant ($p < 0.01$) with the total concentration of Cu and at 5% level with Na and Zn, however, negative but not significant ($p < 0.01$) relationship was observed in case of Fe and K. The study concluded that alteration in the levels of Zn, Cu, Fe, Na and K in the epididymal luminal fluid are obvious along with other factors in regulating the intraluminal milieu that are responsible for sperm maturation process in the epididymis of Black Bengal buck.

Keywords: Epididymis, Buck, Mineral Profile, Spermatozoa

Introduction

In mammals, testicular sperm are not able to fertilize an oocyte and require additional post testicular changes during their transit through the epididymis to achieve fertilization competence. Proper luminal microenvironment of the epididymis is essential and plays pivotal role for successful sperm maturation^[15]. Maturation of sperm occurs by the interaction of sperm cells with the unique epididymal luminal environment during epididymal transit^[19]. Numerous reports have demonstrated that during sperm maturation process, many epididymal secretory proteins including hormone, enzymes, vitamins, ions, cations, trace elements are associated with the sperm surface and play important roles in male reproduction^[9]. Several studies have postulated that trace elements are also present in epididymal luminal fluid (ELF) assuming their probable roles in sperm maturation process^[16, 20, 21]. Trace elements which often act as the cofactors of many enzymes, play important roles in the process of normal spermatogenesis and testicular development and are indispensable elements in semen of male animals^[7]. Depending on the concentration, trace/inorganic elements influence the epididymal spermatozoan characters during maturational phases and play key roles on the metabolism of carbohydrates, proteins, lipids and with other biomolecules. However, the influence and / or mode of action by which these elements act are not completely understood due to complexity in mode of action of the metallo-biomolecule's and the neurohormonal relationship. Hence, the aims of present study on buck was to estimate the physiological levels of zinc (Zn), copper (Cu), Iron (Fe), Sodium (Na) and Potassium (K), in the ELF collected from different segments of epididymis viz. caput, corpus and cauda from the testis of the slaughtered bucks.

Materials and Methods

Source of the material and retrieval of epididymal spermatozoa

Testes with intact epididymis from healthy bucks (n=60) immediately after slaughter were collected for present study.

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Tunica albuginea, fat-pad, blood vessels, adipose and connective tissues were removed and washed thoroughly with NSS. Ligatures were placed unilaterally at the proximal end of the vasdeferenses, ampulla and cauda epididymis separately and distal to caput epididymis and vassefferentia. After ligations, epididymis along with vasdeferenses were dissected out from each testis and washed thoroughly by NSS. Each ligated portion of the right epididymis was cut gently into three parts, viz caput, corpus and cauda. The individual portions were minced carefully and luminal content from each segment was collected by giving gentle pressure on the excised tissues into the medium with separate clean glass rods. The resultant suspensions were aspirated and filtered through individual nitex membrane (150 mm pore size) into glass test tubes, centrifuged (Remi, India) at 500 g for 10 min. and the supernatants were discarded. Finally, each sperm pellet was re-suspended with 2 ml of PBS separately, vortexed for 3s and kept at 37°C into an incubator provided with 5% CO₂ in air for 20 min. allowing sperm cells to swim up into the medium.

Assessment of spermatozoan motility

Right epididymis was considered for evaluation of spermatozoa motility by standard subjective ranking method [28].

Collection of epididymal luminal fluid

Left epididymis of each animal was considered for mineral estimation. Each portion of the ligated caput, corpus and cauda epididymis was cut gently and placed individually into the sterile dry watch glasses and weighted. A total 3.5 g of each caput, corpus and cauda from each epididymis was weighted followed by carefully mincing of each portion. 2 ml of chilled PBS was then poured into each watch glass. Individual glass rods were used to give gentle pressure on the excised tissues, and tissues were thoroughly flushed several times with same PBS solution remaining into each watch glass with separate syringes. Whole content of each segment including the PBS solution were transferred to coded sterile test tubes and cold centrifuged at 15,000 g for 30 min. After centrifugation supernatant from each test tube was collected very carefully and aliquoted and kept in 1.5 ml Eppendorf tubes. All the tubes were then properly labelled, and kept at -20°C till experimentation.

Mineral estimation

All the samples were thawed first. Each sample was digested in a microwave (MLS-1200 MEGA, Milestone, USA) using a 5 ml of nitric oxide and 1ml of HCl/g, then assessed by spectrophotometer (Unicam Solar 939 USA). The flame conditions were those recommended by the instrument manufacturer for Zn Cu, and Fe (wavelength 324.8 nm, 213.0 nm, 248.3 nm, respectively). Analyzing reference material (MBH Anal.Ltd.UK) were employed to test the reproducibility of the method. The graphite furnaces were optimized for maximum absorbency and liner response while aspirating as per known strands. The concentration of Na and K from the ELF were estimated by Flame photometry [2].

Statistical Analysis

All the data were subjected to analysed by SPSS 21.0 (Statistical Package for Social Science) and Pearson's correlation coefficient. The mean were compared using Duncan Multiple Range tests [14].

Results

Zinc: The levels of Zn (µg/dl) recorded were 124.25 ± 0.76, 130.45 ± 0.75 and 138.40 ± 1.28 in caput, corpus and cauda epididymis respectively (Table 1). Concentration of Zn was highest caudal segment. Highly significant differences ($p < 0.01$) were found among the three different segments. The correlation of coefficients (Table 2) showed significant differences ($p < 0.05$) between caput and corpus ($r = 0.468$), caput and cauda ($r = 0.489$) and between corpus and cauda ($r = 0.725$) the differences varied highly significantly ($p < 0.01$).

Copper: The mean Cu (µg/dl) concentration were 36.54 ± 0.73, 46.52 ± 1.31 and 51.25 ± 1.21 in caput, corpus and cauda epididymis respectively with highly significant difference ($p < 0.01$) among three different segments (Table 1). Concentration of Cu was found highest in caudal segment. The correlation of coefficients (Table 2) between caput and corpus ($r = 0.526$), caput and cauda ($r = 0.578$), and between corpus and cauda ($r = 0.639$) varied highly significantly ($p < 0.01$).

Iron: The levels of Fe (µg/dl) recorded were 181.62 ± 1.62, 168.38 ± 1.21 and 136.27 ± 1.12 in caput, corpus and cauda epididymis respectively with highly significant difference ($P < 0.01$) among the three segments (Table 1). Concentration of Fe was highest in the caput segment. The correlation of coefficients (Table 2) between caput and corpus ($r = 0.427$), caput and cauda ($r = 0.498$) were found significant ($p < 0.05$) and between corpus and cauda ($r = 0.567$) was highly significantly different ($p < 0.01$).

Sodium: The mean Na (mEq/l) concentration were 152.34 ± 1.29, 147.68 ± 1.25 and 132.69 ± 1.18 in caput, corpus and cauda epididymis respectively with highly significant difference ($p < 0.01$) among the three segments (Table 1). Concentration of Na was found highest in the caput. The correlation of coefficients (Table 2) between caput and corpus ($r = 0.463$), caput and cauda ($r = 0.537$) and corpus and cauda ($r = 0.613$) varied highly significantly ($p < 0.01$).

Potassium: The levels of K (mEq/l) were 15.82 ± 0.64, 12.42 ± 0.72, 8.96 ± 0.81 in caput, corpus and cauda epididymis respectively with highly significant difference ($P < 0.01$) among the three segments (Table 1). Concentration of K was found highest in the caput segment. The correlation of coefficients (Table 2) between caput and cauda ($r = 0.524$) was found highly significant ($p < 0.01$), whereas between caput and corpus ($r = 0.314$) and between corpus and cauda ($r = 0.305$) were non significant.

In the epididymis overall relationship of spermatozoan motility (Table 3) was found to be positive and highly significant ($p < 0.01$) with the total concentration of Cu ($r = 0.626$) and at 5% level with Na ($r = 0.384$) and Zn (0.743), however, negative but not significant ($p < 0.01$) relationship was observed in case of Fe ($r = 0.301$) and K ($r = 0.296$).

Table 1: Concentration of Zn ($\mu\text{g/l}$), Cu ($\mu\text{g/dl}$), Fe ($\mu\text{g/dl}$), Na (mEq/l) and K (mEq/l) in the fluids collected from different regions of epididymis

Parameter	Epididymis region		
	Caput	Corpus	Cauda
Zinc ($\mu\text{g/l}$)	124.25 $\pm$ 0.76 ^a	130.45 $\pm$ 0.75 ^b	138.40 $\pm$ 1.28 ^c
Copper ($\mu\text{g/l}$)	36.54 $\pm$ 0.73 ^a	46.52 $\pm$ 1.31 ^b	51.25 $\pm$ 1.21 ^c
Iron ($\mu\text{g/l}$)	181.62 $\pm$ 1.26 ^a	168.38 $\pm$ 1.21 ^b	136.27 $\pm$ 1.12 ^c
Sodium (mEq/l)	152.34 $\pm$ 1.29 ^a	147.68 $\pm$ 1.25 ^b	132.69 $\pm$ 1.18 ^c
Potassium (mEq/l)	15.82 $\pm$ 0.64 ^a	12.42 $\pm$ 0.72 ^b	8.96 $\pm$ 0.81 ^c

Value expressed as Mean $\pm$ SE (n=60). Different superscripts (a, b and c) differ highly significantly ($P<0.01$)

Table 2: Pearson's Correlation coefficient between different epididymal regions

Parameter Variable	Zinc	Copper	Iron	Sodium	Potassium
Caput and Corpus	.468*	.526**	.427*	.463**	.314
Caput and Cauda	.489*	.578**	.498*	.537**	.524**
Corpus and Cauda	.725**	.639**	.567**	.613**	.305

*Significant at 5% level ($P<0.05$), ** Highly Significant at 1% level ($P<0.01$).

Table 3: Pearson's Correlation Coefficient between Sperm motility (%) with different parameters

N=60	Zinc	Copper	Iron	Sodium	Potassium
Sperm Motility	.743*	.626**	.301	.384*	.296

*Significant at 5% level ($P<0.05$), ** Highly Significant at 1% level ($P<0.01$).

Discussion

The present investigation confirms that Zn levels and sperm motility, which exhibited a good relationship as the concentration of Zn increases gradually from caput to cauda and hence might be used as a simple analytical tool to monitor as one of the indicators for sperm maturation process. An auto metallographic analysis of different sections of the epididymis revealed that Zn^{2+} levels within the lumen increased from caput to cauda [25] which simulate with the present findings. The role of Zn in spermatogenesis and sperm maturation is of great importance. Zn deficiency is related to alterations in sperm parameters that lead to male infertility [22]. Gonadal dysfunction, decreases testicular weight, and shrinkage of seminiferous tubules may occur due to deficiency of Zn [6]. A Zn deficiency could seriously affect spermatogenic process, as well, as primary and secondary sex organs development [24]. Zn is a vital component of enzymes involved in steroidogenesis. Additionally, it has been shown that Zn may act indirectly through the pituitary to regulate the gonadotropins hormones [18].

Present data indicates exhibited a positive relationship between spermatozoan motility and the level of Cu. The level of Cu found to be increased gradually. This may be due to that during journey through the epididymal regions, spermatozoan motility that gradually enhanced requires more ATP for energy metabolism for which more cupro enzymes is required in mitochondria. Moreover, it is also essential for suppression of excessive ROS production to maintain the integrity of sperm cell membrane. Cu is an important trace and essential element for all organisms [11]. Moreover, it is a component of a member of metallo enzymes and metallo proteins [1] which are involved in energy and antioxidant metabolism [17, 4]. The present observation revealed that concentration Fe, Na and K were highest in the caput region and diminished gradually, which may indicate that immature sperm cells probably require more Fe, Na and K for their metabolism than the sperm of other regions. Semen contains certain amount of Fe, as its physiological level is required for normal spermatozoa production. Studies

on the Fe transport into the testis via the transferrin receptors have focused primarily on the Sertoli cell function [26], but the Leydig cell through its storage of Fe via ferritin may also play a role in the Fe homeostasis and may be a primary source of Fe for the Sertoli transport to developing sperm. Storage of Fe in Leydig cells also provides an extra layer of protection to germinal cells and still maintains easy availability of Fe to the Sertoli and germ cells. The demands for Fe are the greatest during sperm production [27]. Levels of Na and K delicately control the sperm motility, and quiescence and viability [8]. The Na concentration of the spermatozoa decrease concomitantly with the level of sodium in the epididymal plasma but an extracellular / intracellular gradient is maintained in the ejaculate [10, 23]. Intracellular potassium has been shown to be related to sperm motility in ram [13]. K plays vital roles in the regulations of sperm motility, chemotaxis and the acrosome reaction [12, 5], an indication of sperm plasma membrane integrity [3] and in situations when spermatozoa encounter a hypo-osmotic challenge upon ejaculation into the female tract [29].

Conclusion

Present study concluded that alteration in the physiological concentration of Zn, Cu, Fe, Na, K, in the ELF are obvious along with other factors in regulating the intraluminal milieu that are responsible for sperm maturation process in the epididymis of bucks. Present investigation also confirms that all the parameters studied might be used as a simple analytical tool to monitor as one of the indicator for sperm maturation process in comparison with other conventional evaluations. Molecular as well as biochemical and physiological mechanism of minerals involved in the male fertility are however require more investigations.

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