Structural Modification of the Black Bengal Buck
(Capra hircus) Acrosome During Post-testicular
Maturation of Spermatozoa

Modificación Estructural del Acrosoma de la Cabra Black Bengal
(Capra hircus) Durante la Maduración de los Espermatozoides

Uttam Datta; Soumendra Kumar Bandopadhyay** & Manik Lal Hembram***


SUMMARY: The present study was conducted to measure various biometric parameters of intact/normal acrosomes (AC) collected respectively from caput, corpus and cauda epididymis and vas deferens of Black Bengal buck. Giemsa stained acrosomes were measured after camera lucida drawings. Observations revealed dimensional characters of the acrosomal cap diminished gradually and significantly (p<0.01, p<0.05) during spermatozoa maturation phases in the different regions of the excurrent duct. Shape and size of the AC were also found to be influenced significantly (p<0.01, p<0.05) by the age and body weight of the animals. The structural modification along with decrease in the morphology of the AC reflected one of the maturational indexes of the male gametes in Black Bengal buck.

KEY WORDS: Black Bengal buck; Epididymis; Acrosome; Biometry.

INTRODUCTION

During propagation of the sperm cells through different regions of the male reproductive tract, various physical and chemical alterations in the sperm plasma membrane (SPM) including its lipid and protein contents (Yanagimachi, 1994) as well as sperm structural components undergo changes (Bearer & Friend, 1990). These changes enable spermatozoa to fertilize egg (Glover, 1974; Katz et al., 1989). One of the important changes in the spermatozoon morphology during maturation phases is structural modifications of the AC that alters the shape and size of the sperm head (Fawcett & Hollenberg, 1963; Guraya, 1987; Hafez, 1987). Ultrastructural investigations revealed that the appearance and topographical configuration of the SPM are altered during epididymal transit (Bedford, 1965; Bedford & Nicander, 1971). These alterations typically involve in reduction in size and a change in the apical segments, change in the appearance of acrosomal contents (Olson et al., 2003), and change in the density and distribution of the membrane particles (Lopez et al., 2007). However, Sperm morphology in combination with other objective traits is useful to determine the fertility index of an individual. Abnormal bull sperm morphology has been found correlated with reduced fertility (Sekoni & Gustafsson, 1987; Correa et al., 1997). In particular, the occurrence of abnormal sperm head morphology is associated with lower fertility in bull (Saacke & White, 1972; Sekoni & Gustafsson). Therefore, evaluation of spermatozoan morphology provides information on testicular function and fertility potential of a male (Melrose, 1962; Hann et al., 1969). Moreover, it has been reported that age and body weight of an individual also influence the dimensional characters of the ejaculated spermatozoa (Beatty, 1969; Pangawkar & Mittal, 1978; Bandopadhyay, 1981).

Hence, present study on the morphological changes of AC and their probable relationship with the age and body weight of the animal during spermatozoan maturation phases...
was undertaken to investigate the nature of changeable morphometric characteristics of the AC in the different regions of male reproductive tract in Black Bengal buck.

**MATERIAL AND METHOD**

Forty-five adult and healthy Black Bengal bucks were selected from goat breeding farm of the University. The animals were divided into two age groups (Group I – 3 years; Group II – above 3 to 31⁄2 years), and three weight groups (Group 1= 12.5 kg; Group 2= 12.5 to 13.5 kg and Group 3= 13.5 to 14.5 kg) respectively. All the animals were maintained on the standard balanced feed and water was supplied ad libitum.

For the experiment, each buck was castrated by open method (O’Conner, 1980). Immediately after castration, tunica albuginea were removed from both the testis. Ligatures were placed unilaterally as per anatomical positions at the proximal end of the vas deferens and cauda epididymis separately, and distal to the caput epididymis, vas efferentia and vas deferens respectively. After ligations, epididymides including vas deferenses were dissected out from the testes. Each ligated region (caput, corpus, cauda and vas deferens) was cut again and minced separately into 2 ml of 0.15 M phosphate buffer saline (PBS), pH 7.4 at 37ºC into different polystyrene Petri dishes. Luminal content from each portion was collected separately by giving gentle pressure on the excised tissues into the medium with individual clean glass rods. The resultant suspensions were filtered through separate nitex membrane (150 μm pore size) to free the cellular debris. Each filtrate was collected into individual glass test tubes, centrifuged at 500 g for 10 min and the supernatants were discarded. Finally, 200 μl of 0.15 M PBS was added to each sperm pellet separately, vortexed for 3 s and kept at 37ºC into an incubator provided with 5% CO2 in air for 15 min allowing sperm cells to swim up into the medium. After incubation, sperm cells from upper layer of the mediums were aspirated carefully by micropipettes and transferred into individual coded test tubes.

Subjective evaluation (WHO, 1987) of the spermatozoan motility (0–5 scale) from each region was performed under the Leitz phase contrast microscope (x100; x200). Spermatozoan motility >40% were considered for the experiment. However, as the spermatozoa from the caput region are immotile we considered the whole sperm suspension for the experiment.

Ten normal stained sperm cells with normal and intact AC from each coded slides were drawn by Camera Lucida (x7,700) and measured (Pant & Mukherjee, 1973). Following dimensional characters (Fig. 1) were determined (Pant & Mukherjee, 1973; Ostermeier et al., 2001) for the acrosomal length (al) [the distance between the midpoint of the base line of the AC and its apex, i.e. AB]; acrosomal breadth (ab) [maximum scale reading of the line parallel to the base line, i.e. CD]; acrosomal breadth at base (abb) [maximum scale reading of a line of the acrosomal base, i.e. EF]; acrosomal shape (as) [the ratio of the length and breadth of the cap, i.e. AB/CD] and acrosomal area (aa) which was measured by a planimeter from the Camera Lucida drawings. All the mensuration characters were expressed as μ2 and μm. The mean percentage data were transformed to arcsin value and analyzed statistically (Snedecor & Cochran, 1980).

Acrosome morphology was assessed using Giemsa staining method (Harayama, 2003; Harayama et al., 2004) with some modifications. Uniform smears were drawn on the coded microscopic glass slides (pretreated with 70% alcoh) with 10 μl of sperm suspension prepared from both sides of the different regions of the excurrent duct. The smears were air dried at 37ºC on a hot plate and fixed for 15 min in methanol. Fixed smears were stained for 15 min with 6% potassium dichromate dissolved in 0.01% of aniline blue solution prepared in PBS, pH 6.8. Finally, smears were re-stained in Giemsa solution (0.15 g of Giemsa dye dissolved in 100 ml of PBS, pH 6.8) for 1 h at room temperature in humid condition. All the stained smears were washed with a gentle flow of 0.15 M PBS for 2 min following by a single dip into distilled water. Washed smears were treated with clove oil for 15 sec, dehydrated in a xylene bath for 1 min and mounted with DPX.

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Fig. 1 Diagrammatic presentation (Camera Lucida drawing) of different dimensional characters of acrosomal cap. (“al”-acrosomal length [AB]; “ab”-acrosomal breadth [CD]; “abb”-acrosomal breadth at base [EF]; “as”-acrosomal shape [AB/CD])
RESULTS

The modified Giemsa staining method produced a clear and prominent view for the caprine acrosomal cap (Fig. 2) under the microscope.

Table I depicts the different dimensional characters of the AC from the experimental regions. Highly significant variations (p<0.01) in all the measured dimensional characters were found between the regions. But in “al” between cauda vs. corpus; and in “aa” between cauda vs. vas deferens only significant variation (p<0.05) were found. However, in “al”, “ab”, “abb” and in “as” the variations between cauda and vas deferens were non significant (Table II). Statistical analysis also indicated that all the dimensional characters of the AC diminished significantly during spermatozoan journey from caput to vas deferens. The relationship (Table III) of each dimensional characters of AC among different regions of the excurrent duct exhibited higher significance (p<0.01), than “al” in between cauda vs. vas deferens (p<0.05). For “abb” no such relationship was existed in between corpus vs. cauda.

Least square analysis of variance and Duncan’s Multiple Range tests (DMR test) were performed to find out whether individual’s age and body weight had any influence on the mensuration characters of AC among the different regions. Analysis revealed that due to variation / effect of age, dimensional characters like “ab” in caput, corpus, cauda and vas deferens; “abb” in cauda and vas deferents and “as” in caput in age group I (3 yrs) varied significantly (p<0.01) than age group II (above 3 yrs). Moreover, DMR test indicated that two age groups exhibited significant variations (p<0.01) due to effect of age only in “ab” among the four experimental regions, “abb” in the caudal region and in the vas deferents, and “as” in the caput epididymis (Table IV) respectively.

Morphometric characters of the AC were also found to be influenced by the body weight of the animals significantly (p<0.01) in all the four regions on the “ab”; “abb” (p<0.01) only in the cauda and vas deferens, and “as” (p<0.05) in the caput region only. DMR test also revealed that weight group 1 varied significantly (p<0.01) from weight group 2 and 3, whereas latter two groups did not exhibit any variation in “ab” from caput, corpus and in vas deferens, and “abb” in cauda and vas deferens respectively. However, “as” in caput and

<table>
<thead>
<tr>
<th>Region</th>
<th>Acrosomal Length (μm)</th>
<th>Acrosomal Breadth (μm)</th>
<th>Acrosomal Area (μm²)</th>
<th>Acrosomal Breadth at base (μm)</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caput</td>
<td>4.24±0.008</td>
<td>4.33±0.01</td>
<td>4.16±0.01</td>
<td>16.33±0.1</td>
<td>1.27±0.004</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.17±0.008</td>
<td>4.23±0.01</td>
<td>4.08±0.01</td>
<td>15.36±0.09</td>
<td>1.27±0.005</td>
</tr>
<tr>
<td>Cauda</td>
<td>4.11±0.02</td>
<td>4.09±0.01</td>
<td>3.99±0.01</td>
<td>14.18±0.11</td>
<td>1.29±0.005</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>4.08±0.007</td>
<td>4.08±0.01</td>
<td>3.99±0.01</td>
<td>14.17±0.11</td>
<td>1.29±0.006</td>
</tr>
</tbody>
</table>

n = 45; Values are expressed as mean ± SE.

Table II. “t” (paired) values showing differences in the morphometric characters of the acrosomal cap from different epididymal regions and vas deferens.

<table>
<thead>
<tr>
<th>Region</th>
<th>Acrosomal Length</th>
<th>Acrosomal Breadth</th>
<th>Acrosomal Area</th>
<th>Acrosomal Breadth at base</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauda</td>
<td>3.89±0.06</td>
<td>1.86±0.03</td>
<td>28.61±0.13</td>
<td>16.18±0.11</td>
<td>1.11±0.05</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>25.53±1.18</td>
<td>22.06±1.07</td>
<td>54.48±1.6</td>
<td>34.90±1.6</td>
<td>1.68±0.05</td>
</tr>
</tbody>
</table>

DISCUSSION

Sperm maturation in the epididymis is a gradual process that results in a number of successive changes in the spermatozoa. These successive changes alter the morphology of the spermatozoon, so that their properties and functions are also modified while they move along the excurrent ducts (Briz et al., 1995). Some of the distinctive structural changes during spermatozoal maturation are condensation of acrosome (Fawcett & Hollenberg; Bedford, 1964; Bedford & Nicander; Paüfler & Foote, 1968; Hamilton, 1972; Jones et al., 1974; Jones, 1975; Hanks, 1977), reduction in the acrosomal size (Bedford, 1965) as well as loss of the acrosomal components (Holt, 1980). Significant decreases in the dimensional characters of the AC from the epididymal segments in the Black Bengal buck are also consistent with the above findings.

Present observation also revealed that AC in the caput sperm had the greatest surface area than the spermatozoa from cauda and vas deferens. Compaction of the nucleus and acrosomal material are related for the decrease of the surface area. These observations also simulate with the findings of Fornés & de Rosas (1989) in rat. Moreover, higher osmotic pressure of the epididymal secretion than that of blood (Lindhal & Kihlström, 1952; Glover; Guraya) is also responsible for dehydration of the sperm cells resulting decrease in the permeability of water content of the acrosome (Hamilton;
Salisbury et al., 1978). Thus through progressive loss of water and a corresponding increase in the specific gravity (Lavon et al., 1966; Salisbury et al.), swollen acrosomal membrane in caput gradually decreases in their shape and size during their transit (Hamilton; Jones et al.; Hanks) resulting adherence of AC to the sperm nucleus (Jones, 1971, 1975).

Present experiment also revealed that acrosomal area (aa) reduces further in the vas deferens, the reason might be that acrosome undergoes further dehydration in this region as reported in human (Hinrichsen & Blaquier, 1980) and in rat (Brotherton, 1976).

Statistical analysis also revealed age and body weight of the animal influenced the dimensional characters of the maturing AC in the excurrent duct. The causal factor(s) of this type of influence in adult animals of close age groups is / are not known clearly. So far reviewed, reports on the influence of age and body weight of the individual on the morphology of AC were not found, but, Beatty and Bandopadhyay reported that age and body weight of the animal had more pronounced effect on the dimensional characters of the ejaculated spermatozoa in mice and buffalo bull respectively. Pangawkar & Mittal also stated that quantitative morphological characters which depend on the strain, breed and species could be affected by age, seasons, temperature shock and by the extender. However, alterations in the biophysical and biochemical properties in the epididymal environment could be one of the causal factors in this observed variation.

The gradual diminution and/or alteration in the shape and size of the AC during their sojourn in the male reproductive tract, and the normal dimensions of the AC in the different regions of the reproductive tract in Black Bengal buck could be helpful in judging the epididymal environment as well as abnormal morphology of the AC.


RESUMEN: El presente estudio se realizó para medir diversos parámetros biométricos del acrosoma (AC) intacto/normal recogido desde la cabeza, cuerpo y cola del epidídimo y vas deferens de la Cabra Black Bengal. Los AC teñidos con Giemsa fueron medidos después de la captura con cámara lúcida. Las observaciones revelaron caracteres dimensionales del capuchón acrosomal que disminuyeron gradualmente y de manera significativa (p <0,01, p <0,05) durante fases de maduración espermática en las diferentes regiones del conducto. La forma y tamaño del AC también fueron influenciados de manera significativa (p <0,01, p <0,05) por la edad y el peso corporal de los animales. La modificación estructural junto con los cambios morfológicos del AC refleja uno de los índices de maduración de los gametos masculinos de la Cabra Black Bengal.

PALABRAS CLAVE: Cabra Black Bengal; Epidídimo; Acrosoma; Biometría.

REFERENCES


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