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Chronic natural arsenic exposure affecting histoarchitecture of gonads in Black Bengal goats (*Capra aegagrushircus)*

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ABSTRACT

Arsenic is a major water pollutant that may cause serious health hazard (*e.g.*, infertility) in human and animal. We evaluated the changes in histoarchitecture of testes and ovaries of adult Black Bengal goats (n=10) reared in arsenic affected areas in Bangladesh. Grossly, we found insignificant variations among the testes and ovaries, but histological evaluation revealed an extensive alteration in morphology of both testes and ovaries in the arsenic affected goats. In testes, the thickening of tunica albugenia and trabeculae, widening of intertubular space between seminiferous tubules, and narrowing in diameter of seminiferous tubules were observed. In ovaries of arsenic affected goats, significant decrease in number of primary follicles and antral follicles were observed. The diameters of secondary and antral follicles were significantly reduced. The granulosa layer of antral follicles showed marked thickening. The findings indicate that chronic arsenic exposure alters the histoarchitecture of both male and female gonads in Black Bengal goat, and thereby may affect their reproductive performance.

Keywords

Arsenic intoxication, Black Bengal goat, Ovary, Reproduction, Testes

ARTICLE HISTORY

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INTRODUCTION

Arsenic is a ubiquitously distributed xenobiotic ranking first in the list of 20 hazardous substances set by the Agency for Toxic Substances and Disease Registry (ATSDR) and United States Environmental Protection Agency (USEPA) (Goering et al., 1999). High level of arsenic in the drinking water were reported in many countries including Bangladesh, Taiwan, China, Chile, Mexico, Argentina, Thailand, Finland, and Hungary (Chatterjee et al., 1995; NRC, 1999; Pott et al., 2001). Thus, drinking water may act as the principal source of arsenic exposure to human and animal, although occupational exposure may occur, particularly in the areas of metal smelting and glass making industries (Bode and Dong, 2002; Yih et al., 2002). The arsenic exposure through feed chain has also been reported (Naidu et al., 2006).

Both human and animal can be equally affected with arsenic intoxication. Black Bengal goat (*Capra aegagrushircus)* is a popular goat breed in Bangladesh comprising >90% of the total goat population in the country. The Black Bengal goats are famous for their very delicious meat and quality skin having high demand in the global market. Among the total contribution of livestock in Bangladesh, the goat ranked second in terms of meat, milk and skin production representing about 28, 23, and 28%, respectively (FAO, 1997).

The flood plain and deltaic areas including the coastal regions of Bangladesh are highly contaminated with

arsenic, although the tableland and hill tract regions of Bangladesh are primarily free from arsenic contamination (Chakraborti et al., 2010). Therefore, the goat rearing in these arsenic contaminated areas are at risk of arsenic intoxication. This situation may hamper their reproduction capacity as like as previous study revealed a decreased sperm motility, low number of sperm in semen, and more critically, fertilization rate may decrease (Lin et al., 2002). Although there are some reports that describe the effects of experimental arsenic intoxication on different organs of experimental animals (Islam et al., 2011; Lin et al., 2002), the effect of natural arsenic intoxication on reproductive organs of Black Bengal goat is yet to be studied. Therefore, we investigated into how extent the gonads of Black Bengal goats rearing in the arsenic contaminated areas are affected at gross and microscopic levels.

MATERIALS AND METHODS

Experimental sample collection: Ten experimental goats (five males and five females) were collected from different arsenic affected areas of Mymensingh district in Bangladesh. For confirmation about arsenic intoxication of the goats, we contacted with the Senior Chemist, Department of Public Health Engineering and collected goats from the Buror Char, Dhobaura, Fulpur and Haluaghat areas (most affected areas) of Mymensigh district. Additional two goats (as control) were collected from Chittagong Hill tract regions (arsenic free areas) of Bangladesh (Khan et al., 2006; Chakraborti et al., 2010). The goats had no developmental anomalies and detectable diseases that would cause any problem in the morphology and physiology of the gonads. The animals were sacrificed by piercing carotid arteries by following the animal welfare guidelines set by Bangladesh Agricultual University, and then it was left for total discharge of blood. The testes from male and ovaries from female goats were collected immediately in the postgraduate laboratory.

Gross morphological study: Immediately after collection of gonads, the gross morphology (color and shape), and biometry (length, width, breadth and weight) were measured and recorded.

Histological study: For histological studies, the tissues obtained from the genital system were fixed in the 'Bouin's fluid' (Gridley, 1960) for 24 h, and were dehydrated in series of ascending grade of alcohol followed by clearing in three changes in xylene. The tissues were infiltrated with different grades of melted paraffin in the oven as well as embedded in paraffin. The sections were cut at 6 µm thickness using a sliding microtome (MIC-509, Euromex, Japan). After cutting, the sections were floated on luke-warm water in a floatation bath at 37^0C for stretching, and then the sections were mounted on clean slides using an adhesive (egg albumins), and dried on a slide warmer at 37^0C. The sections were stained using Mayer's Hematoxylin and Eosin (H&E). The histological structures of the gonads were observed using a light microscope. Photographs from the selected specimens were taken using photomicrographic camera (Olympus, PM-C 35 model, Japan) for better illustration of the results. Then, the measurements of different histological structures of the gonad tissues were performed by the calibrated stage micrometer (in µm).

Statistical analysis: Results were expressed as mean±SEM. Statistical comparisons were made by Student's *t*-test (SPSS software, version 16.0). Statistical significance was accepted when the *p* values were <0.05.

RESULTS AND DISCUSSION

In the present study, the effects of natural arsenic intoxication on reproductive organs have been studied. We observed insignificant variations in length, width and breadth of both testes and ovaries of goats from arsenic affected areas as compared to control goats (**Table 1**). The difference in the wet weight, including color and shape, of testes and ovaries between arsenic affceted goats and control goats was also insignificant. We next wonder whether chronic natural arsenic exposure could affect histoarchitecture of gonads. Microscopic examination revealed extensive alteration in both testes and ovaries in the arsenic affected goats. In the testes of arsenic affected goats, tunica albbugenica was markedly thickened as compared to control (**Figure 1A-a**). The lesion was characterized by densely irregular connective tissue, collagen and elastic fibers. The tunica albugenia was thickened in arsenic affected goats by 18% (**Figure 1B-a**). The septula testis was thicker and more prominant in arsenic affected goat than that of control goat (**Figure 1 A-b**). Trabeculae at the origin was more thicker than that in the intertubular space (**Figure 1A-b**). The trabecualae was thickened in arsenic affected goat by 64% (**Figure 1B-b**). The intertubular space between the seminiferous tubules were wider in arsenic affected goats than that of control (**Figure 1A-c**). The diameter of seminiferous tubules was narrower in arsenic affected goats as

Table 1. Comparison of mean of length, width, breadth and weight of gonad, testes and ovary, between arsenic affceted and control group of goats.

Parameters	Testes		Ovary		Interpretation
	Control	Arsenic affected	Control	Arsenic affected	
Length (cm)	5.95 ± 0.04	5.88 ± 0.07	1.87 ± 0.02	1.77 ± 0.08	NS
Width (cm)	3.82 ± 0.02	3.77 ± 0.03	1.43 ± 0.02	1.48 ± 0.04	NS
Breadth (cm)	9.75 ± 0.15	9.93 ± 0.06	3.16 ± 0.17	3.20 ± 0.05	NS
Weight (g)	36.97 ± 0.65	35.26 ± 0.93	0.92 ± 0.02	0.81 ± 0.12	NS

Legend: NS=Non-significant, Number of sample; Arsenic affected=10, Control=2

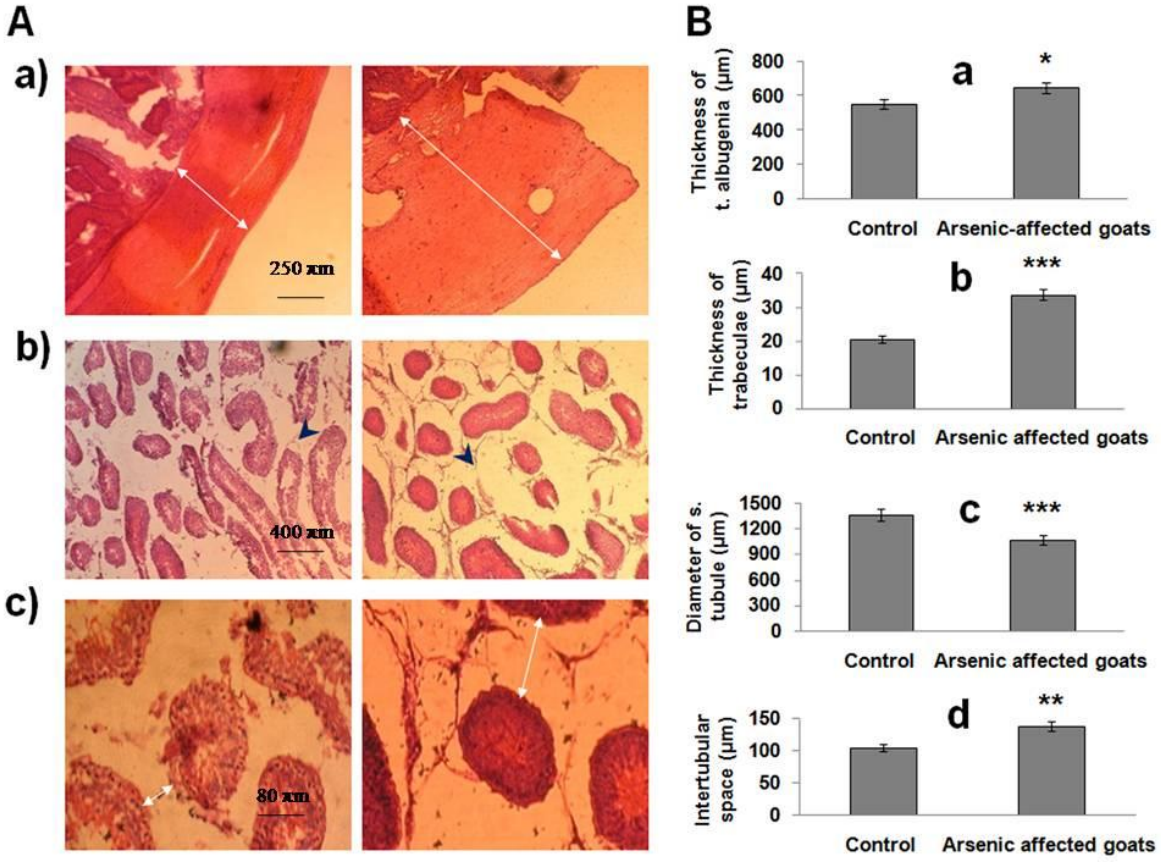

Figure 1. Effects of chronic arsenic exposure on histoarchitecture of testes of goats. A. Microscopic images showing thickening of tunica albugenia (a) and trabeculae (b), and redundant seminiferous tubules (c) in arsenic-affected goats (right panel) compared to that of control group (left panel). The septula testis was thicker and more prominant in arsenic affected goat (arrow head). H&E Stain. B. Measurement of the thickness of tunica albugenia (a) and trabeculae (b), the diameter of seminiferous tubules (c), and the length of intertubular space (d). Statistical significance compared with vehicle: *p<0.05, **p<0.01 and ***p<0.001 (Student's t-test). Data points represent the mean±SEM.

compared to control goat (**Figure 1A-c**). The interstitial cell was fewer in arsenic affected goats than that of the control group. The diameter of seminiferous tubules was reduced in arsenic affected goat by 22% (**Figure 1B-c**). Moreover, the length of intertubular spae was increased in arsenic affced goat by 36% (**Figure 1B-d**).

In ovaries of arsenic affected goats, there was a significant reduction in the primary and antral follicles (p<0.01) as compared to control goat. On the other hand, the numbers of primordial and secondary follicles were almost unaffected (**Figure 2A-a**, **b** and **2B-a**). The diameters of secondary and antral follicles were significantly (p<0.001) reduced in arsenic affected goats (**Figure 2A-b** and **2B-b**). The granulosa layer of antral follicles showed marked thickening, but the theca layer remained unaffected (**Figure 2A-c**). The granulosa layer of antral follicles was thickened in arsenic affected goat by 28% (**Figure 2B-c**). In addition,

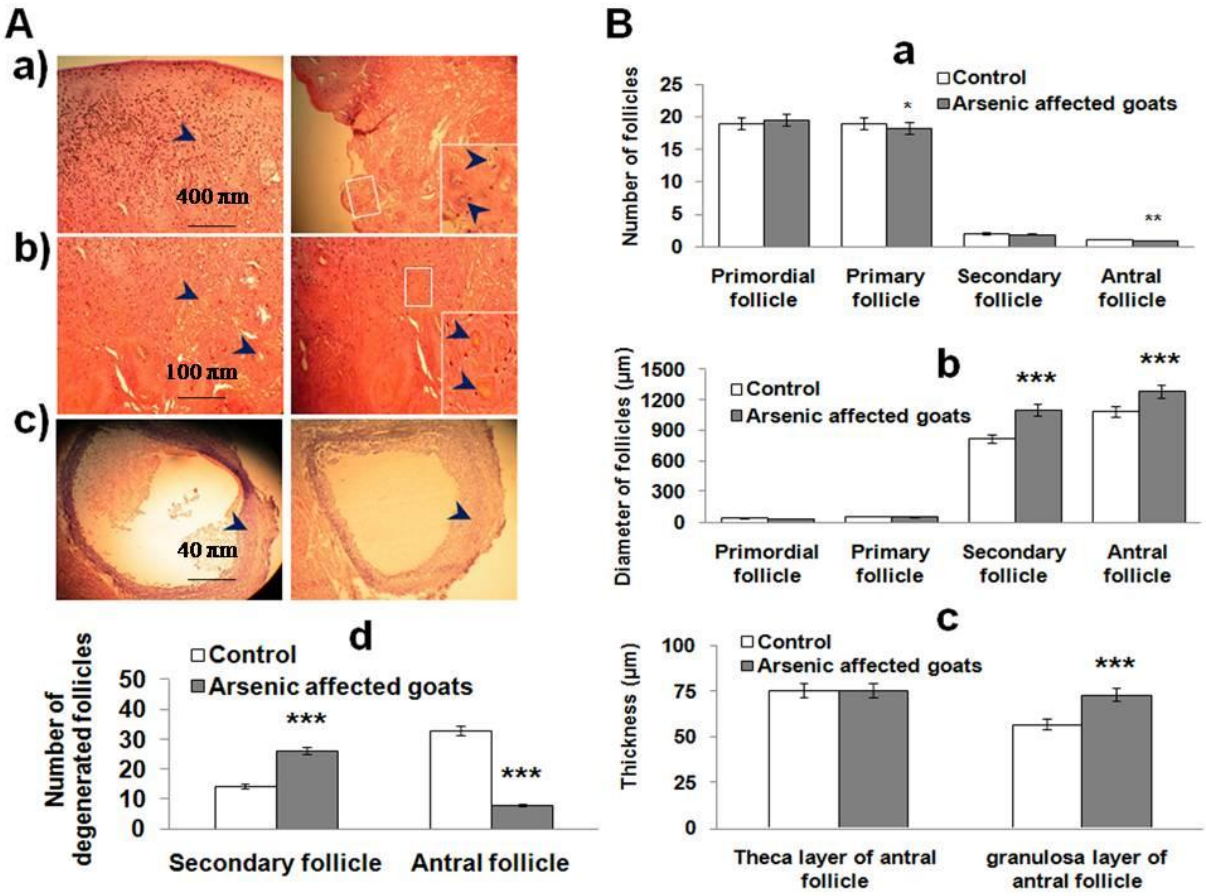

Figure 2. Effects of chronic arsenic exposure on histoarchitecture of ovaries of goats. A. Microscopic images showing reduced number of primary follicles (arrow head) (a), antral follicles (arrow head) (b), and thickening of granulosa layer of antral follicles (arrow head) (c) in arsenic-affected goats (right panel) compared to that of control group (left panel). H&E Stain. B. Measurement of the number (a) and diameter (b) of follicles, the thickness of granulosa layer of antral follicles (c), and the number of degenerated follicles (d). Statistical significance compared with vehicle: *p<0.05, **p<0.01 and ***p<0.001 (Student's t-test). Data points represent the mean±SEM.

there was a significant increase in the degenerated secondary follicles (p<0.001) and antral follicles (p<0.001) in arsenic affected goats (**Figure 2B-d**).

Arsenic intoxication is one of the silent killers in human history. Both human and animals are affected with arsenic intoxication. The detrimental effect of arsenic has been well established. However, most of the experimental researches were conducted on laboratory animals like rat, mice, etc. Here, we investigated into the toxic effect of chronic natural arsenic exposure on the testes and ovaries of Black Bengal goat for the first time. Our investigation showed that both male and female gonads are affected with arsenic intoxication. Our studies were in agreement with the previous reports describing that arsenic exposure caused testicular dysgenesis comprising testicular carcinoma *in situ* and acrosomal dysgenesis characterized by sharing of a dysplastic acrosome by two or more spermatids resulting in characteristic sperm acrosomal-nuclear malformations in rabbit (Veeramachaneni, 2008). In particular, increased intertubular space and decreased diameter of seminiferous tubule indicate testicular dysgenesis. In addition, Chinoy et al. (2004) found that administration of arsenic trioxide caused structural alterations in mice testis, affecting spermatogenesis.

Given that follicular count provides better prognostic information of the female fertility, we analyzed ovarian follicles. Both the number and morphology of ovarian follicles particularly the primary follicles and antral follicles were altered in goats in arsenic affected areas. It was reported that arsenite could affect ovarian follicles by reducing its size and numbers (Chattopadhyay et al., 2003). Reduced size of secondary and antral follicles along with the increased number of atretic follicle due to arsenic exposure in our

study substantiates the previous findings. The genotoxic effect of arsenic on ovarian cells has been reported earlier (Akram et al., 2009), and reduced size of ovarian follicles might be due to apoptosis of ovarian cells by arsenic (Navarro et al., 2004). Increase numbers of degenerating follicles in the antral class of control goats may be physiological. Numbers of different types of follicles in this study could not validate due to lack of information. However, in our finding, only the visible follicles with clear nucleus in the oocyte were counted. In spite of the detrimental effect of arsenic on ovarian tissue, the insignificant differences of ovarian weight between control and arsenic affected goats might be due to the exposure of arsenic at relatively smaller dose with shorter duration, and also self detoxification capacity by this species.

In many ways, the alterations by arsenic may affect the reproductive capacity. Definitely, fewer number of interstitial cell produce smaller amount of testosterone which directly hampers male reproduction. Similarly, augmented intertubular space as well as narrower seminiferous tubule are also important as they produce less quantity of sperm which drop fertility rate. In case of female, higher number of degenerated follicle, lower number of normal follicle, reduced diameter of follicle, and thickened layer of antral follicle caused fewer ovulation; this could be a grave problem for reproduction.

CONCLUSION

Chronic arsenic exposure has detrimental effects on histoarchitecture of both testes and ovaries in Black Bengal goats, suggesting that arsenic may have negative impact on fertility which ultimately may exert threat on the livelihood of marginal farmers. Analyses of large numbers of samples and wide ranges of contamination along with role of green grasses in reducing effects of arsenicosis are suggested.

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