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Effect of arsenic on the oogenesis of black Bengal goat (*Capra hircus*) reared at the arsenic polluted area of Mymensingh district in Bangladesh

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ABSTRACT

Arsenic is widely distributed in the environment and available to the human population though various sources including drinking water, food and air. Significant human exposure to arsenic occurs through both anthropogenic and natural sources. Arsenic has recently appeared as a major pollutant of drinking water in several districts of Bangladesh. This study was aimed to observe the effects of chronic arsenic toxicity on the ogenesis and histoarchitecture of female gonads of black Bengal goat. Adult she goats were collected from the arsenic contaminated area and from the arsenic free area of the Mymensingh district of Bangladesh. Ovaries were used for observation of the effects of arsenic on the oogenesis. Histopathological observation was performed on the ovaries after Hematoxylin & Eosin staining techniques. One hundred rounded focuses on the histological slide were selected for the counting of the different stages of ovarian follicles. The mean number of the primordial, growing and mature follicles in control group was 20.040 ± 1.917, 18.930 ± 1.460 and 1.390 ± 0.061 and in arsenic affected group was 17.450 ± 1.701, 13.680 ± 1.911 and 0.750 ± 0.575 respectively. The mean diameter of the primordial, growing and mature follicles of control group were 40.390 ±1.290 µm, 59.21 ± 1.850 µm and 1092.99 ± 12.080 µm and in the arsenic affected group were 35.090 ± 1.650 µm, 52.280 ± 1.730 µm and 1285 ± 7.010 µm respectively. The mean thickness of the granolosa layer of the mature follicle of the control group goat was 57.440 ± 1.630 µm and in arsenic affected group was 73.450 ± 1.130 µm. Thickness of the theca layer of the mature follicle of the control group of goat was 75.230 ± 1.040 µm and in arsenic affected group was 75.240 ± 0.670 µm. Variations of all the parameters between arsenic affected and control group of goats were statistically significant (*P*<0.01) except the mean thickness of the theca layer of the mature follicle. These findings indicate that chronic arsenic exposure alters the oogenesis as well as histoarchitecute of female gonads in black Bengal goat and thereby may affect their reproductive performance.

Introduction

Environmental pollutants include a wide range of chemicals, mostly man-made, which are present in products that are used on a daily basis, such as detergents, pesticides and adhesives or derived from the combustion of hydrocarbon fuels. Arsenic is a xenobiotic metalloid and ubiquitously distributed in nature. Arsenic and its compounds are best known as a deadly poison (ATSDR, 2005). Drinking water is the principle source of arsenic exposure to human and animal, although occupational exposures occur particularly in the metal smelting and glass making industries (Bode and Dong, 2002; Yih *et al*., 2002). Now a day, arsenic exposure through feed chain has been reported (Naidu *et al*., 2006; Kile et al., 2007). It has been established that the significant portions of the Ganga-Meghna-Brahamaputra plain in India and Bangladesh encircling a large area of 56974 9 sq km with a population of over 500 million are at risk with arsenic poisoning (Chakraborti *et al*., 2004). The arsenic disaster of Bangladesh has been called the most terrible environmental catastrophe of the twentinth century. World Health Organization (WHO) described the condition as the largest mass poisoning of a population in history. It was estimated that 59 out of 64 districts and about 29% of the total tubewells in Bangladesh are contaminated with arsenic (Khan *et al*., 2006; Chakraborti *et al*., 2010; Neumann *et al*., 2010) and about 85 million people are at risk of drinking arsenic contaminated water and foodstuffs (Hossain, 2006; Wahidur, 2006). In a recent report, Chakraborti *et al*., (2010) showed that hand tube wells of the tableland and hill tract regions of Bangladesh are primarily free from arsenic contamination, while the flood plain and deltaic region including the coastal region are highly contaminated with arsenic. The extent of this environmental disaster is greater than any other recorded in human history. Although the exact time of onset of arsenic exposure in Bangladesh is mysterious, but suspected that it was started during the 1960s and 1970s when government of Bangladesh in collaboration with UNICEF started to install hand-pumped tube wells to provide pathogen-free drinking water to the people (Smith *et al*., 2000). Access to safe drinking water is the basic human right. Now-a-days, one of the most serious worldwide environmental problems is drinking water polluted by arsenic. Arsenic can enter into food chain causing wide spread distribution throughout the plant and animal kingdoms (Ulman *et al*., 2004). The detection of arsenic in milk and meat is a new finding (Awal, 2007). Organic arsenic exposure can also occur by eating food. Inorganic arsenic trioxide is a component of geologic formations and can be washed out into the ground water. Arsenic poisoning can be related to human activities such as mining and ore smelting but is more often associated with dissolved solids naturally endemic in the aquifer environment. Chronic arsenic toxicity due to drinking of arsenic contaminated ground water is a major environmental health hazard throughout the world (Mazumder, 2008). Chronic arsenic poisoning results from drinking contaminated well water over a long period of time. This is due to arsenic contamination of aquifer water. Acute arsenic exposure may promote immediate gastrointestinal tract infection (Goebl *et al.,* 1990), whereas chronic effects may exert degenerative, inflammatory and neoplastic changes of respiratory, haematopoetic, cardiovascular and nervous systems (Neiger & Osweiler, 1989). The effects may include shortened life expectancy, decrease in reproduction, and behavioural changes. In arsenic toxicosis, excitement, restlessness, ruffled hair coat, ataxia, in coordination, muscle tremor, paralysis and severe skin lesions were observed in rats following administration of arsenic trioxide (Awal, 2004). It is observed that heavy congestion in liver, spleen, kidney and heart with severe hemorrhagic enteritis and rose-red inflammation in the stomach. Arsenic is a recognized reproductive toxicant in humans and induces malformations. Recently arsenic intoxication in experimental animals has been found to be associated with hepatic tumors (Waalkes *et al.*, 2003), inhibition of testicular steroidogenic function (Sarkar *et al.*, 1991) and spermatogenesis (Sukla & Pandey, 1984), as well as with severe metabolic disorders such as diabetes in humans (Longnecker & Daniels, 2001, Tseng *et al.*, 2002). Although health risk assessments of arsenic typically focus on cancer, several recent studies have examined non-cancer health outcomes in association with environmental arsenic exposure, primarily in drinking water (Argos *et al.,* 2010; Chen *et al.,* 2010a, Chen et al 2010b; Mazumder *et al.,* 2005; Parvez et al., 2011). The mode of action of arsenic toxicity may also involve a continuum of non-cancer effects leading to tumor formation with sufficient dose and duration. The relationship between arsenic and cardiovascular disease (CVD) effects has been studied in populations exposed to elevated arsenic levels in drinking water (Chen *et al*., 2011) and in patients receiving high doses of arsenic trioxide as a chemotherapy drug for specific leukemia sub-types. The present study was undertaken to observe the effects of chronic arsenic on the oogenesis in the Black Bengal Goat (*Capra hircus)* reared at the arsenic prone area of the Mymensingh district of Bangladesh.

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Materials and Methods

Experimental animals

Ishwarganj upazilla was consider as most of the arsenic affected area of Mymensingh district and Nirlakha char area of Guripur Upazilla under Mymensingh district was considered as no arsenic contaminated area. A total of 12 adults female black Bengal goats were used to investigate the effect of arsenic on the oogenesis by using the light microscopy. Among of these 6 (six) adult female black Bengal goats were collected from the Ishwarganj Upzilla of the Mymensingh district for using as a target group. Another 6 (six) adult female were collected from the Nirlakha Char area of the Guripur Uzilla under Mymensingh district for using as a control group. The right ovaries were collected after the animals euthanized at the Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh. Gross morphology of the ovaries was studied. Small pieces of the ovaries were preserved in the Bouin’s fluid for the histopathological examination. The specimens were kept at 4^0c for overnight. After several washes with phosphate buffer solution the specimens dehydrated with graded series of ethanol and embedded in paraffin. The paraffin blocks were cut at 5 µm thickness and dried at 40˚C overnight. They were stained with Hematoxylin and Eosin (H & E) staining teqniques for general histological studies. Total count of one hundred (N=100) focuses on slide was selected for counting different stages of ovarian follicles in this study. The diameter of the follicle was measured using calibrated microscope and data were tabulated and analyzed for drawing the effects of arsenic on the oogenesis in the black Bengal goat. The experiment was conducted at the Post-graduate Laboratory, Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. In the histological study, number of primordial, growing and mature follicles, diameter of the primordial follicle, growing follicle and mature follicles and finally thickness of the theca layer and granulosa layer of the mature follicles were determined with calibrated microscope to observe the effects of arsenic on oogenesis.

Data collection

The data was calculated as mean ± SD. Significance between groups (P<0.01) was determined by single factor analysis of variance (ANOVA) with fishers least significant difference test comparison using Stat View software (Abacus Concepts Inc. Berkeley, USA).

Results and Discussion

Ovaries are the principal reproductive organs in the female species within which oogenesis takes place. The histological organizations of the ovaries and hormone titer of the body and the environment are the main factors for the process of the oogenesis. The effects of arsenic on the oogenesis were studied. The effects can be resulted in two ways as gross effects and histological effects on which the process of oogenesis depends. Arsenic changes normal histoarchitechture of the gonad along with the hormonal homeostasis which directly impacts on the oogenesis resulting reproduction failure in the Black Bengal Goat. It was observed by light microscopic examination that arsenic effects directly on the general histoarchitecture of the ovaries and normal process of the oogenesis in the Black Bengal Goat. Histological feature of the different types of follicle were found to be different between arsenic affected and control goats. The minimum alteration of ovarian histology due to arsenic poisoning may negatively influence their production of the black Bengal goat. The mean number of primordial follicle in the cortical region of the ovaries in each focus of the control group of goat was 20.040 ± 1.917 and the mean number of primordial follicle of the cortical region of ovaries in each focus of the arsenic affected group of goat was 17.450 ± 1.701. It is found that the differences between the number of the primordial follicles in each focus of the cortical region of the ovaries of the control group of goat and arsenic affected group of goat was statistically significant ($P<0.01$). The mean number of primordial follicles in the cortical region of the ovaries in each focus of the control group of goat was 18.930 ± 1.465 and the mean number of growing follicle in the cortical region of the ovaries in each focus of arsenic affected of goat was 13.680 ± 1.911. The observation was that the differences between the mean number of the growing follicles in the cortical region of the ovaries of control group of goat and arsenic affected group of goat was statistically significant ($P< 0.01$). The mean number of mature follicles in the cortical region of the ovaries of the control group of goat was 1.390 ± 0.601 and the mean number of the mature follicle in the cortical region of the ovaries of arsenic affected goat was 0.750 ± 0.575. The observation was that the differences between the number of the mature follicles of the cortical region of the ovaries of the control group of goat and arsenic affected group of goat were statistically significant ($P< 0.01$). The mean diameter of the primordial follicle of the ovaries of the control group of the goat was 40.390 ± 1.290 micrometer and the mean diameter of the primordial follicle in the ovaries of the arsenic affected group of goat was 35.090 ± 1.650 micrometer. It is also noticed that the differences between the mean diameter of the primordial follicle of the control group of goat and arsenic affected group of goat were statistically significant ($P<0.01$).The mean diameter of the growing follicle in the ovaries of the control group of the goat was 59.21 ± 1.850 micrometer and the mean diameter of the growing follicle in the ovaries of the arsenic affected goat was 52.280 ± 1.730 micrometer. The observation was that the differences between the mean diameter of the growing follicle of the arsenic affected group of goat and control group of goat were statistically significant ($P<0.01$). The mean diameter of the mature follicle in the ovaries of the control group of the goat was 1092.99 ± 12.080 micrometer and the mean diameter of the mature follicle of the ovaries of the arsenic affected group of goat was 1285.44 ± 7.010 micrometers. The observation was that the differences between the mean diameter of the mature follicle of the control group of goat and arsenic affected group of goat was statistically significant ($P<0.01$).The mean thickness of the granulosa layer of the mature follicle of the ovaries of the control group of goat was 57.440 ± 1.630 micrometers and the mean thickness of the granulosa layer of the mature follicle of the ovaries of the arsenic affected group of goat was 73.450 ± 1.130 micrometer. It is observed that the differences between the mean thickness of the granulosa layer of the mature follicle of the arsenic affected group of goat and control group of goat were statistically significant ($p<0.01$). The mean thickness of the theca layer of the mature follicle of the ovaries of the control group of the goat was 75.230 ± 1.040 micrometer and the mean thickness of the theca layer of the mature follicle of the arsenic affected goat was 75.240 ± 0.670 micrometer. The differences between the mean thickness of the mature follicle of the control group of goat and arsenic affected goat was statistically insignificant.

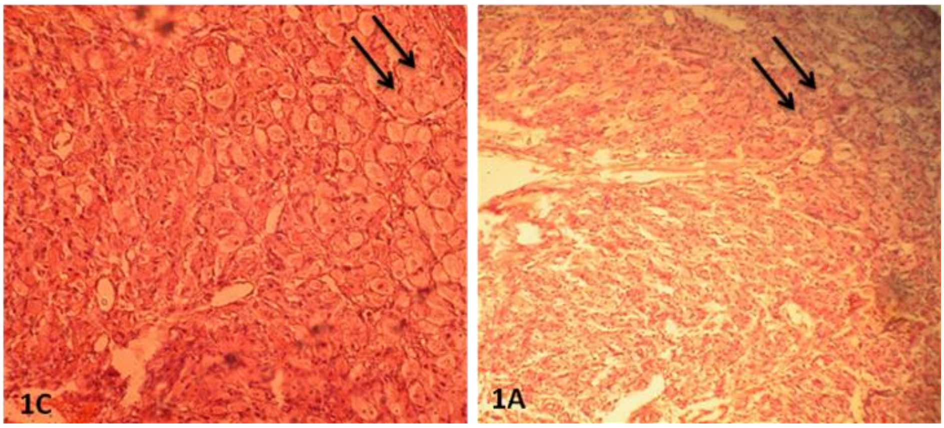

Figure 1. Histology of the ovaries showing the decreased number of the primordial follicles in the arsenic affected group of goats (1A) compared to the number of the primordial follicles in the control (1C) group of goats (H&E stain).

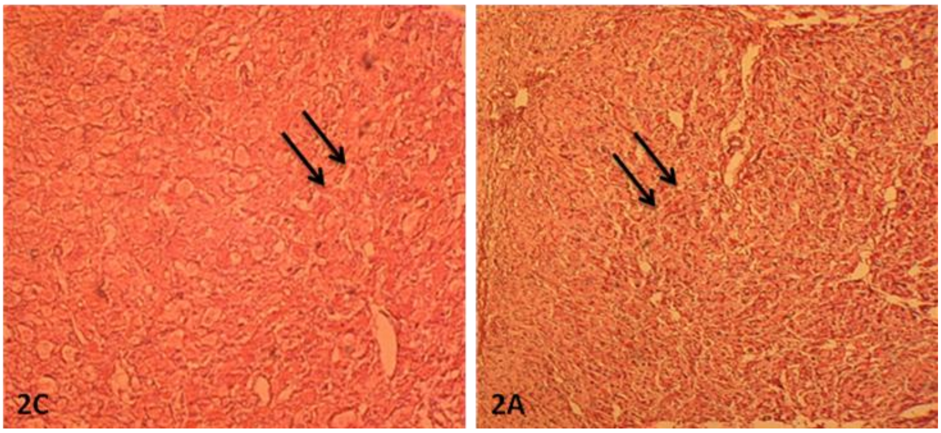

Figure 2. Histology of the ovaries showing increased degree of fibrosis in the cortical region of the ovaries resulting decreased number of different stages of follicles in the arsenic affected group of goats (2A) compared to the less degree of fibrosis and increased number of the different stages of the follicles in the control (2C) group of goats.

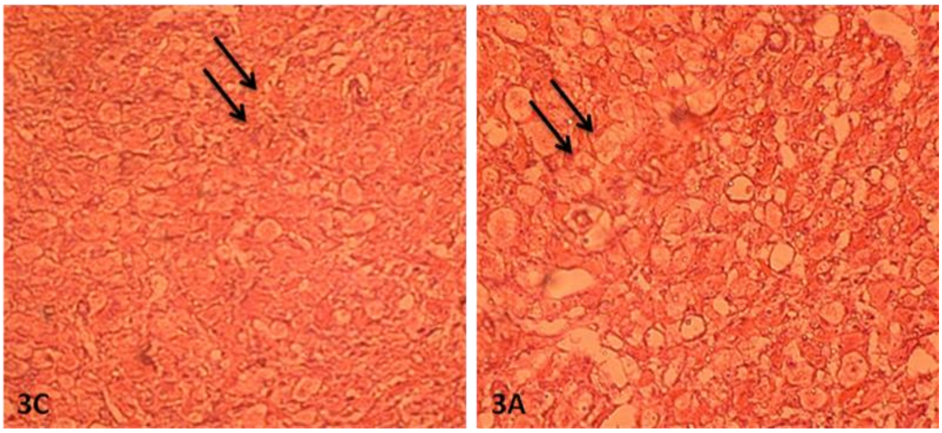

Figure 3. Histology of the ovaries showing the decreased number of the growing follicles in the arsenic affected group of goats (3A) compared to the number of growing follicles in the control (3C) group of goats.

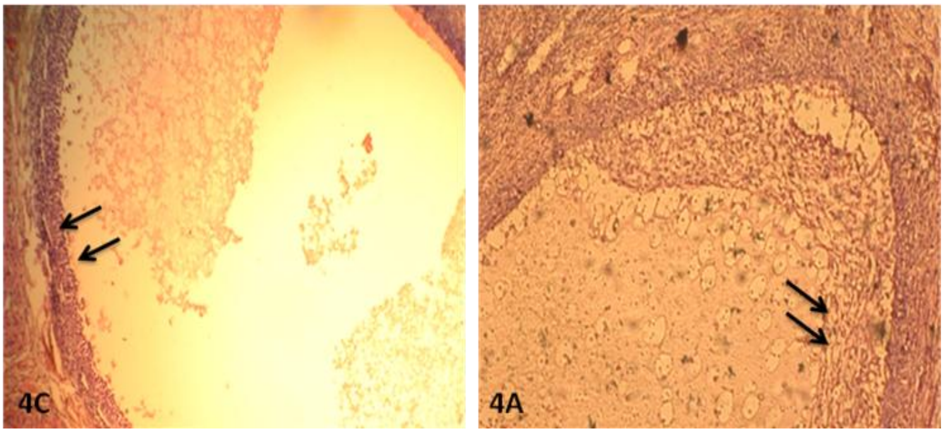

Figure 4. Histology of ovaries showing the increased thickness of the granulosa layer of mature follicles of the arsenic affected group of goats (4A) compared to the thickness of the granulosa layer of the control (4C) group of goats (H&E stain).

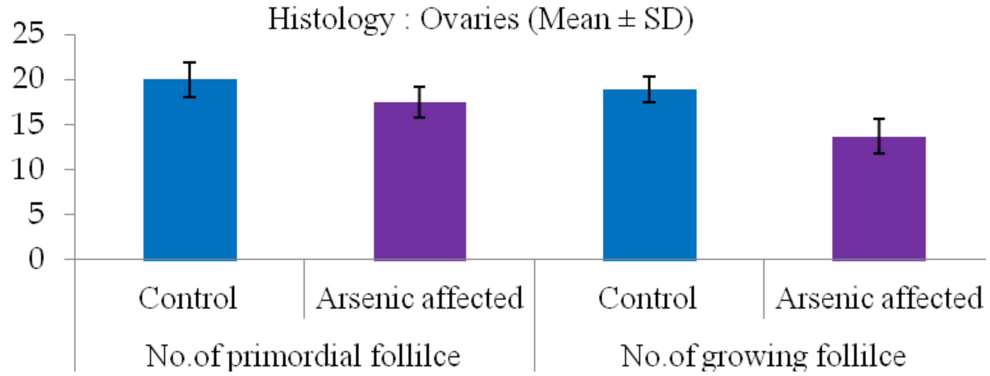

Figure 5. Comparative mean number of the primordial follicles and growing follicles in the ovary of the control and arsenic affected group of the Black Bengal Goats.

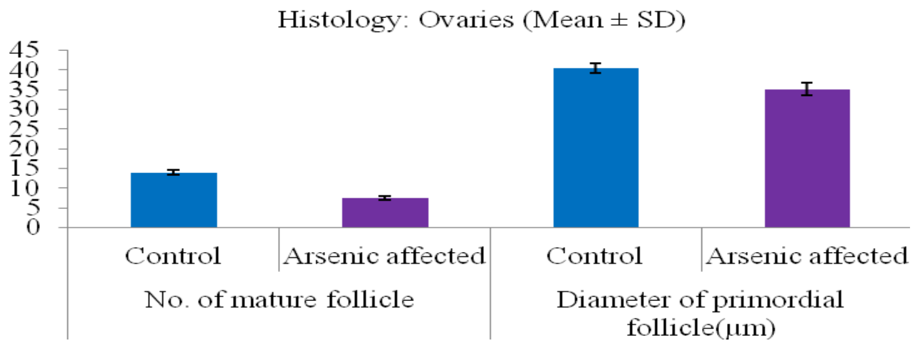

Figure 6. Comparative mean number of the mature follicles and diameter of the primordial follicles (µm) in the ovary of the control and arsenic affected group of the black Bengal goats.

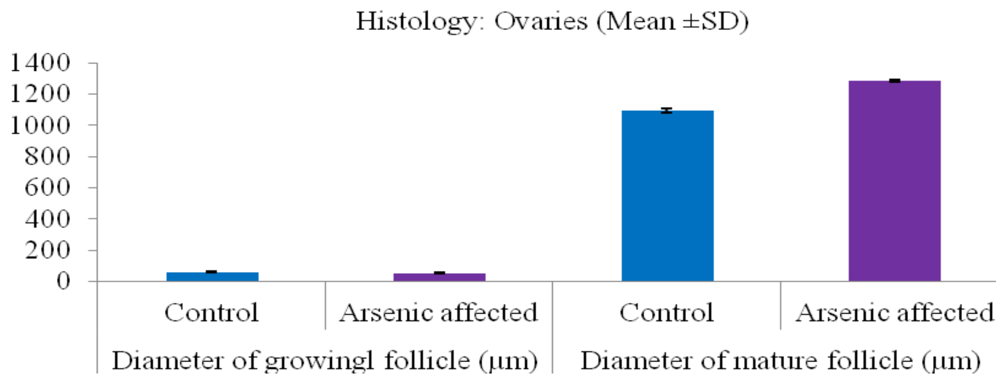

Figure 7. Comparative mean of the diameter of the growing follicles and the mature follicles in the ovaries of the control and arsenic affected group of the black Bengal goats.

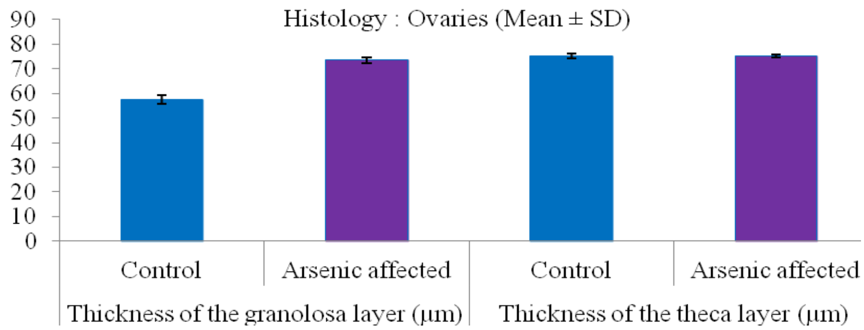

Figure 8. Comparative mean of the thickness of the granulosa layer and theca layer of the mature follicles in the ovary of the control and arsenic affected group of the black Bengal goats.

In the present study, the effects of natural arsenic intoxication on reproductive organs have been studied. Ovaries of goats from arsenic affected areas as compared to control goats. The difference in the wet weight, including color and shape of ovaries of arsenic affected goats and control goats were also insignificant. Microscopic examination revealed extensive alteration in ovaries in the arsenic affected goats. In the ovaries of arsenic affected goats, germinal epithelium was markedly altered as compared to control group of goat. The lesion was characterized by densely irregular connective tissue, collagen and elastic fibers. The distinct demarcation of cortex and medulla is damaged in the ovaries of arsenic affected goat compared to control group of goat as connective tissue of the ovaries has been altered due to arsenic toxicity. The blood vessels, nerves along with connective tissue have become altered in normal organization due to arsenic toxicity in arsenic affected goat compared to the control group of goat. It is shown in the ovary that vacuolization of stroma, atretric follicle and pycnotic follicular cell which is supported by Jhala *et al.,* 2004. The Arsenic directly effects on the cortical region in folliculogenesis resulting the decrease in the number of primordial, growing and graffian follicles seen in the current study could be due to a lack of available protein necessary for cell division, growth, and differentiation of germ cell during oogenesis (Fig.1A).These findings are similar to the findings of Islam *et al* 2011. Large number of occyte of ovarian follicles has become degenerated and necrosed with the toxic effects of arsenic in the ovaries collected from the arsenic affected goat. The follicular cells of the different stages of follicles also become degenerated and necrosed due to arsenic toxicity which is supported by the Jhala *et al,* 2004.The diameter of the primordial cell decrease in the ovaries of arsenic affected goat due to necrosis of the stromal and follicular cells. The theca interna, externa and granulosa cells of the growing follicles have become disorganized resulting the decrease of the diameter of the growing follicles due to arsenic toxicity on the ovaries of arsenic affected group compared to the control group of goat (Fig. 3A). A large number of oocyte is in the small antram for the disruption of granulosa cell of the growing follicles in the ovaries of arsenic affected goat which is present in the normal position in the ovaries of control group of goat. The degenerative changes are distinct in the different stages of the ovarian follicles in ovaries the arsenic affected goat compared to the ovaries of the control group of goat (Fig. 3A). The cells of the corona radiata, cumulus oophorus, membrane granulosa layer has become degenerated and necrosed resulting cell are accumulated in the follicular fluid of the antram. Our findings are supported by experiments in other animal models such as recently arsenic intoxication in experimental animals has been found to be associated with hepatic tumors (Waalkes *et al.*, 2003), inhibition of testicular steroidogenic function (Sarkar *et al.*, 1991) and spermatogenesis (Sukla & Pandey, 1984), as well as with severe metabolic disorders such as diabetes in humans (Longnecker & Daniels, 2001, Tseng *et al.*, 2002). Over all effects of arsenic on the ovarian follicles may have great impact on the reproductive performances for the disorganization of ovarian structure as well decrease number of different stages of ovarian follicles.

Conclusion

Toxic effects of environmentally persistent arsenic on the oogenesis of black Bengal goats were observed. The mechanism of action of this compound on the ovarian tissues was not clearly understood. Further study is underway to elucidate the possible mechanism of action of this compound on the ovarian tissue in goats. Finally, it can be suggested here that black Bengal goats (*Capra hircus*) can be used as laboratory animal model to elucidate the toxic effects of arsenic on the female reproduction.

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