Arsenic Toxicity on Duck Spermatozoa and the Ameliorating Effect of L-Ascorbic Acid

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ABSTRACT : The objectives of this study were to understand the possible mechanism of duck sperm toxicity induced by arsenic exposure *in vivo*, and to investigate the roles of the antioxidant L-ascorbic acid in ameliorating the arsenic-induced sperm impairment. To test the acute toxicity, the percentages of mortality of mature drakes treated with different concentrations of trivalent sodium arsenite, As (III), and pentavalent sodium arsenate, As (V) were measured. The LD50 value of As (III) for mature drakes was 4.89±1.49 ppm. Although As (V) didn't cause any deaths even at a concentration of 40 ppm, the chronic toxicity of As (V) on sperm quality was shown by a decreased fertilization rate. When the concentrations of As (V) were above 0.4 ppm, fertilization rates were lower than those of 0.04 ppm and control. Drakes treated with 40 ppm of As (V) had the highest malondialdehyde (MDA) level in the testis tissue, 3.100±0.218 μmole/g testis. This showed that 40 ppm of As (V) significantly induced lipid peroxidation in testis tissue. For the 1.2 ppm As (III) treatment, several significant effects were observed: (1) sperm motility was decreased most dramatically by 52.0±9.1% after three days of incubation; (2) fertilization rate of artificially inseminated semen was the lowest, 26.4±15.4; (3) the MDA concentration in testis tissue, 7.846±0.246 μmole/g testis, was significantly higher than the others ($p<0.05$); (4) the sperm number, 1.17±0.40 ($\times 10^9$), was significantly lower than with the 60 ppb and control treatments ($p<0.05$); (5) a black appearance and soft texture was observed in the testis tissue. The antioxidant L-ascorbic acid administered along with 1.2 ppm As (III) decreased the toxicity of arsenic. The ameliorating effects included: improved sperm motility, increased sperm number and fertilization rate, and decreased MDA concentration in the testis tissue. This study suggests that the toxicity of the trivalent arsenic on sperm quality is partly from free radicals generated by its metabolic pathway, and the antioxidant ascorbic acid ameliorates arsenic-caused sperm impairment. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 1 : 19-25)***

Key Words : Drake, Sperm Quality, Antioxidant, Ascorbic Acid, Fertilization Rate, LD50, Lipid Peroxidation

INTRODUCTION

Arsenic is widely distributed in nature and released into the environment through industrial processes (Farmer and Johnson, 1990) and agricultural usage (Ishinishi et al., 1986). Although almost all of arsenic in the form of organoarsenic compounds are nontoxic and not metabolized to toxic forms in human body, there is arsenic contamination of well water in Taiwan, Minnesota (United States), and Canada. An area on the southwestern coast of Taiwan has been referred to as the blackfoot disease endemic area (Chen, 1980; Tseng et al., 1994). The same area also has a high prevalence of skin cancer (Guo et al., 1994). Both conditions have been shown to be dose-related to the high levels of arsenic in the artesian well water consumed (Chen and Lin, 1994; Tseng et al., 1968; Tseng, 1977). In addition, for bladder, lung, liver, nasal cavity, skin, kidney, and prostate cancer that occurred in the blackfoot disease endemic areas, there were dose-response relations with years of artesian well water use (Brown and Chen, 1994; Chen et al., 1985, 1986, 1988, 1992; Wu et al., 1989). The arsenic content of the well water ranged from 10 ppb to 1.82 ppm (Tseng, 1989).

However, inorganic arsenic fails to induce tumors in most laboratory animals. And this has led to the suggestion that arsenic may act as a co-carcinogen (IARC, 1980; Leonard and Lauwerys, 1980). Recently, evidence suggesting that oxygen radicals can be produced during arsenic metabolism has been reported. For example, the antioxidant enzymes catalase and/or superoxide dismutase (SOD) were shown effectively to reduce the frequency of arsenite-induced sister chromatid exchanges in human peripheral lymphocytes (Nordenson and Beckman, 1991) and XRS-5 X-ray sensitive cells (Wang and Huang, 1994). Inorganic arsenic also induced oxidative stress in human fibroblasts *in vitro* (Keyse and Tyrrell, 1989; Lee and Ho, 1995) and in mice lung *in vivo* (Yamanaka et al., 1991). Their results suggest that oxygen radicals may participate in the toxicity induced by arsenic. For these reasons, we investigated the protective defense of antioxidants on preventing the toxicity of inorganic arsenic *in vivo*.

There is limited information regarding the effects of inorganic arsenic on reproductive performance in human,

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animals, and poultry. Vodela et al. (1997) reported that reproductive function in hens of broiler breeders is sensitive to adverse effects of contaminated drinking water. A low concentration of a chemical mixture (0.8 ppm arsenic, 1.3 ppm benzene, 5.1 ppm cadmium, 6.7 ppm lead, 0.65 ppm trichlorethylene) significantly decreased egg production and egg weight, and increased percentage of embryonic mortality. It seems to show that the effects of inorganic arsenic on reproductive function of hens indeed exist. However, it does not show that the affect was caused only by arsenic. In addition, male reproductive function wasn't investigated in the paper. We supposed that male reproduction should be affected by the toxicity of inorganic arsenic, because inorganic arsenic may induce oxidative stress to damage sperm functions. Sperm are sensitive to oxidative damage because of high polyunsaturated fatty acid content and the relatively low activity of antioxidant enzymes (Aitken and Clarkson, 1987; Aitken et al., 1993; Alvarez et al., 1987; Iwasaki and Gagnon, 1992).

The objectives of this study are to investigate the effects of inorganic arsenic on the quality of duck sperm and to understand the possible mechanism of its toxicity. An antioxidant of L-ascorbic acid was also administered along with arsenic to understand its roles in the detoxification of arsenic-caused reproductive impairment in sperm quality.

MATERIALS AND METHODS

Treatments of animals

In Taiwan, waterfowl are not able to feed along riversides due to potential excreta contamination. Therefore, groundwater is their main water source. However, there are high levels of inorganic arsenic in the groundwater under some areas in Taiwan. To investigate the effect of only inorganic arsenic, but not the chemical mixture of contaminated groundwater, on the sperm quality of waterfowl, mature brown Tsaiya drakes were chosen. Brown Tsaiya duck (*Anas platyrhynchos*) is laying species, which is the most popular species for production of eggs in Taiwan. Mature brown Tsaiya drakes above 25 weeks of age, body weight average 1.23±0.25 kg, were randomly assigned and housed in individual cages. All the drakes were maintained on a standard diet and drinking public water which contained a low level of arsenic, lesser than 50 μg/liter water. To investigate the toxicity of inorganic arsenic on drakes, injection into leg muscle was chosen to be the route of arsenic administration. This administration could limit the interference of other chemicals and prevent environmental contamination of arsenic in drinking water.

The first trial investigated the acute toxicity of the trivalent sodium arsenite ($NaAsO_2$; As (III)). The different concentrations of As (III) (60 ppb, 0.12 ppm, 1.2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, 12 ppm; ppm=mg chemical/kg body weight) were injected intramuscularly (i.m.) and mortality was recorded 24-48 h after administration. The solvent of both As (III) and As (V) was saline. Each treatment contained 10 drakes. The LD50 value of As (III) was defined. A parallel study was performed to investigate the acute toxicity of pentavalent sodium arsenate ($Na_2HAsO_4 \square 7H_2O$; As (V)), with the levels of 0.04, 0.4, 4, and 40 ppm.

In the second trial, As (V) at concentrations of 0.04, 0.4, 4 and 40 ppm and As (III) at concentrations of 60 ppb, 0.12 ppm and 1.2 ppm were chosen to test the chronic effects of arsenic on sperm generation. In addition, 1.2 ppm of As (III), injected in right leg, was administered along with 20 ppm or 40 ppm of L-ascorbic acid (dissolved in phosphate buffer solution, pH 7.0) injected in left leg at the same time. Each treatment of As (V) and A (III) contained 6 and 5 drakes, respectively. The intra-muscle injections of As (V) and As (III) to brown Tsaiya drakes were twice a week for five months to test the chronic toxicity for sperm. Semen was collected for acclimation once a week throughout this study. At the end of treatment, sperm parameters of ejaculated semen were examined, including sperm concentration and decreased percentage of motility after three days of storage in Modena extender at 4°C. Modena extender, a commercial product (provided by Swine Genetics International, LTD., Cambridge, Iowa), contains 15 mM citric acid, 28 mM sodium citrate, 12 mM sodium bicarbonate, 7 mM EDTA, 139 mM glucose and 47 mM Tris. Since ejaculated semen of brown Tsaiya drake is dense, 1 ml Modena extender was used to dilute it. Sperm concentration for each drake and total sperm number per drake was calculated.

Artificial insemination (AI)

After 3 days of storage at 4°C, the collected semen was artificially inseminated into female ducks' vagina to obtain fertility, hatchability, and embryo deformity. Semen of each drake was artificially inseminated (AI) with 2 females of brown Tsaiya ducks. Eggs were collected for 3 days beginning from day 2 after AI (the day of AI = day 0). The fertilization rate was derived from the number of fertilized eggs after 7 days of incubation at 37.2°C divided by eggs incubated. The hatchability % was derived from the number of hatched eggs after 28 days of incubation at 37.2°C divided by fertilized eggs.

Assay for lipid peroxidation

This assay was modified from the procedure described by Esterbauer et al. (1991). In order to explore if arsenic induces lipid peroxidation, malondialdehyde (MDA) in the testis was measured after sacrifice. Two grams of testis tissue was homogenized in 10 ml buffer containing 20 mM Tris buffer, pH 7.4 and 5 mM butylated hydroxytoluene

(BHT). The homogenate was then centrifuged with 3,000 g at 4°C for 10 min. Two hundred µl of samples, standards (2.5-20 µM 1, 1, 3, 3-tetramethoxypropane in 20 mM Tris-HCl, pH 7.4), or water (blank) in a polypropylene microcentrifuge tube was taken. In each tube, 10 µl of 0.5 M BHT (in acetonitrile) and 650 µl of R1 reagent (10.3 mM N-methyl-2-phenylindole in acetonitrile:methanol=3:1) were added and then mixed gently by vortex. After the addition of 150 µl of 12 N HCl, tubes were mixed well, and then incubated at 45°C for 60 min. Turbid samples were centrifuged (15,000 g for 10 min) to obtain a clear supernatant. The absorbency at 586 nm in a spectrophotometer was used to measure the level of MDA.

Statistical analysis

Angular transformation was performed for the discrete quantitative parameters including the fertilization rate %, hatchability % and decreased sperm motility %. The data of total sperm numbers per drake and MDA weren't transformed. All of those data passing homogeneity of variance and normality tests were analyzed with the Duncan's new multiple range test by one way analysis of variance (ANOVA) using the SAS program (1997).

RESULTS

The percentage of mortality of mature drakes treated with different concentrations of As (III) is shown in figure 1. After PROBIT transformation, it showed the LD50 value of As (III) for mature drakes was 4.89±1.49 ppm. A black appearance and soft texture was also observed in testis tissue of 1.2 ppm As (III) treatment (figure 2). However, As (V) didn't cause any deaths and toxic syndromes in organs, even at a concentration of 40 ppm (data not shown). After 5 months of administration, the chronic toxicity of As (V) on sperm quality was shown as the decreased fertilization rate (table 1). When the concentrations of As (V) were above

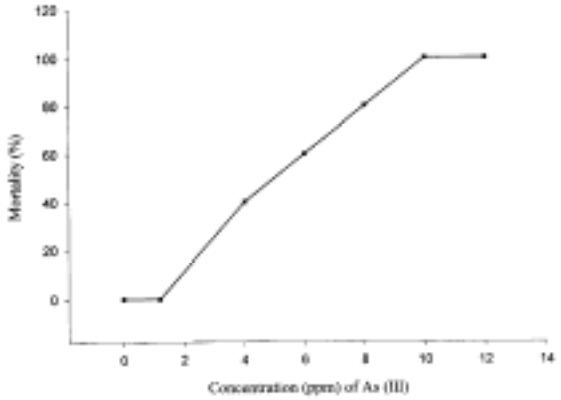

Figure 1. The acute toxicity of sodium arsenic; As (III) resulted in the death of mature drakes. Each treatment contained 10 drakes. Arsenite doses (ppm=mg/body weight kg) were intra-muscle injected and mortality was recorded after 24-48 h

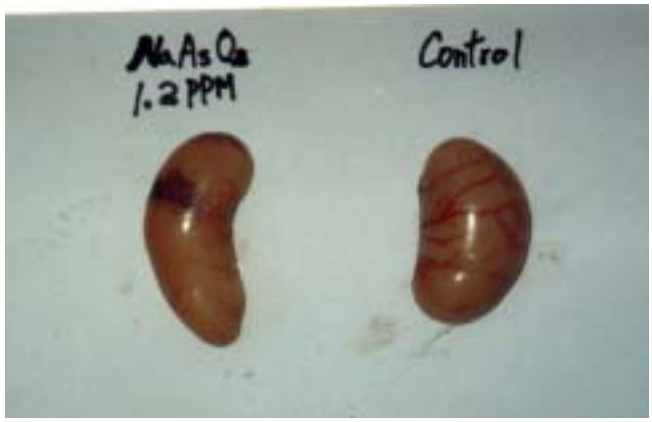

Figure 2. The picture shows the appearance of testis. (Left): As (III) treatment. (Right): Control group

Table 1. Effects of the pentavalent sodium arsenate, As (V), on quality of duck semen

As (V) treatment	Sperm numbers per drake[1] ($\times 10^9$)	Fertilization rate %[2] (Eggs fertilized/eggs incubated)	Hatchability %[3] (Eggs hatched/eggs fertilized)
Control	2.20±1.04	95.1±1.7^{ab} (96 / 101)	91.5±1.9 (87/96)
0.04 ppm	1.51±0.84	96.3±1.7^{a} (106 / 110)	91.1±1.7 (96/106)
0.4 ppm	2.81±1.97	84.2±10.2^{bc} (103 / 123)	92.0±2.1 (93/103)
4 ppm	1.80±0.50	83.6±2.7^{c} (104 / 125)	91.7±1.8 (93/104)
40 ppm	2.93±0.70	86.8±9.4^{abc} (80 / 94)	89.8±2.3 (71/80)

[1]Arsenate (ppm=mg/kg body weight), dissolved in saline, was intra-muscle injected once a week for 5 months. In control, saline was injected. There were 6 drakes in each treatment.

[2] Each drake's semen, diluted by Modena, was AI with the same species of two female. Eggs were collected for 3 days beginning from day 2 after AI (the day of AI = day 0). The fertilization rate was derived from the number of fertilized eggs after 7 days of incubation at 37.2°C divided by eggs incubated. Data refer to means ±SD of six drakes in each treatment.

[3] The hatchability %was derived from the number of hatched eggs after 28 days of incubation at 37.2°C divided by fertilized eggs. Data refer to means±SD of 6 drakes in each treatment.

[a,b,c] Data within the same column without the same superscripts differ significantly ($p<0.05$).

0.4 ppm, fertilization rates were lower than those of 0.04 ppm and control. However, there were no significant differences among the fertilization rates of 0.4, 4, and 40 ppm of As (V) (table 1). For hatchability, there was no difference within all the treatments. Drakes treated with 40 ppm of As (V) had the highest MDA level (table 2). It showed that 40 ppm of As (V) significantly induced lipid peroxidation in testis tissue.

In table 3, the effects of 1.2 ppm As (III) treatment were significantly different from those of the other treatments, except for the data of hatchability. Its sperm motility was decreased dramatically by 52.0±9.1% after three days of incubation and fertilization rate when artificially inseminated was the lowest, 26.4±15.4%; the average sperm number per drake, 1.17±0.40 ($\times 10^9$), was significantly lower than those of the 60 ppb and control treatments ($p<0.05$). However, when the antioxidant L-ascorbic acid was administered in left leg muscle along with 1.2 ppm As (III) in right leg muscle, there was a significantly decrease in the toxicity of arsenic in the treatment of 1.2 ppm As (III): (1) the decreased percentage of sperm motility was lower, (2) the average of sperm numbers was higher, and (3) the fertilization rate was higher than those of the treatment of 1.2 ppm As (III) only.

The MDA concentration in testis tissue of 1.2 ppm As (III) treatment, 7.846±0.246 μmole/g testis, was significantly higher than the others ($p<0.05$) (table 4). However, the ratios of testis weight/ body weight between different treatments of As (V) or As (III) were not significantly different ($p>0.05$) (data not shown). When at least 20 ppm L-ascorbic acid was added into the treatment of 1.2 ppm As (III), the MDA level in testis was significantly decreased ($p>0.05$).

Table 2. Effects of the pentavalent sodium arsenate, As (V), on lipid peroxidation of duck testis tissue[1]

As (V) treatments	MDA[2] (μmole/g testis)
Control	0.383±0.283[a]
0.04 ppm	0.367±0.351[a]
0.4 ppm	1.100±0.833[a]
4 ppm	1.450±0.517[a]
40 ppm	3.100±0.218[b]

[1] The procedure was the same as the previous description in footnote [1] of table 1.

[2] The concentration of malondialdehyde (MDA) in testis tissue was tested after 5 months of As (V) administration. Data refer to means±SD of 6 drakes in each treatment.

[a,b] Data within the same column without the same superscripts differ significantly ($p<0.05$).

DISCUSSION

Recently, many studies have shown that the cellular toxicity induced by inorganic arsenic is related with oxidative stress (Blair et al., 1990; Chang et al., 1991; Lee et al., 1985, 1989; Lee and Ho, 1995; Wang and Huang, 1994; Yamanaka et al., 1989a, b, 1990, 1991). Spermatozoa would be sensitive to oxidative stress because of their high polyunsaturated fatty acid content and the relatively low activity of antioxidant enzymes (Aitken and Clarkson, 1987; Aitken et al., 1993; Alvarez et al., 1987; Iwasaki and Gagnon, 1992). Lipid peroxidation has also been associated with decreased motility of human spermatozoa treated with the exogenous system of xanthine and xanthine oxidase (Aitken et al., 1993). Excess amounts of exogenous H_2O_2 have also been shown to inhibit the acrosome reaction and reduce hyperactivation in human spermatozoa (Griveau et al., 1994; Oehninger et al., 1995). Very few papers studied the effects of inorganic arsenic on sperm fertilizing ability. It was for this reason that this study focused on the arsenic

Table 3. Effects of the trivalent sodium arsenite, As (III), and its combined administration with L-ascorbic acid on quality of duck sperm[1]

Treatment	Sperm numbers per drake ($\times 10^9$)	Decreased percentage of sperm motility%[2]	Fertilization rate%[3] (Eggs fertilized/eggs incubated)	Hatchability %[4] (Eggs hatched/ eggs fertilized)
Control	2.80±1.84[a]	20.0±12.9[b]	85.7±6.0[a] (49/58)	93.2±2.9 (45/49)
60 ppb As	2.54±1.29[ab]	32.5±2.9[b]	77.2±4.6[ab] (52/68)	92.1±2.3 (47/52)
0.12 ppm As	1.39±0.88[bc]	30.0±10.6[b]	70.4±3.5[bc] (49/70)	92.0±2.1 (45/49)
1.2 ppm As	1.17±0.40[c]	52.0±9.1[a]	26.4±15.4[d] (19/82)	91.8±3.2 (17/19)
1.2 ppm As+20 ppm vit C	2.37±0.39[abc]	21.0±8.2[b]	57.8±4.9[c] (38/66)	91.4±2.3 (34/38)
1.2 ppm As+40 ppm vit C	3.30±0.59[a]	7.0±8.4[c]	59.7±5.1[c] (35/59)	90.9±2.7 (32/35)

[1] Arsenite (ppm=mg/kg body weight), dissolved in saline, were intra-muscle injected in right leg once a week for 5 months. In control, saline was injected. At the same time, ascorbic acid was also dissolved in saline and intra-muscle injected in left leg. There were 5 drakes in each treatment.

[2] Values refer to the decreased percentage of motility after sperm were incubated in the Modena medium at 4°C for three days.

[3, 4] The procedure was the same as the previous description in footnote [2, 3] of table 1.

[a,b,c] Data within the same column without the same superscripts differ significantly ($p<0.05$).

Table 4. Effects of the trivalent sodium arsenite, As (III), and its combined administration with L-ascorbic acid on lipid peroxidation of duck testis tissue[1]

Treatment	MDA[2] (μmole/g testis)
Control	0.341±0.058[a]
60 ppb As (III)	0.422±0.074[a]
0.12 ppm As (III)	1.700±0.147[b]
1.2 ppm As (III)	7.846±0.246[c]
1.2 ppm As (III) + 20 ppm Vit C	4.820±0.217[d]
1.2 ppm As (III) + 40 ppm Vit C	4.501±0.227[d]

[1] The procedure was the same as the previous description in footnote[1] of table 3.

[2] The concentration of malondialdehyde (MDA) in testis tissue was tested after 5 months of As (III) administration. Data refer to means±SD of five drakes in each treatment.

[a,b,c,d] Data within the same column without the same superscripts differ significantly ($p<0.05$).

toxic effects on sperm generation of poultry.

These results showed that treatments of inorganic arsenic *in vivo* increased lipid peroxidation in testis tissue and decreased sperm fertilizing ability. They strongly suggested that oxidative stress was induced by inorganic arsenic, which induced testis lipid-peroxidation and damaged sperm fertilizing ability. Although the decreased sperm fertilizing ability resulted from the administration *in vivo* of arsenic, the hatchability of fertilized eggs wasn't affected. It showed that the toxicity of arsenic *in vivo* damaged some percentage of sperm population, but sperm able to fertilize eggs were still healthy. The toxicity of arsenic could affect sperm generation.

In mammals, both As (III) and As (V) are transferred into the embryo in mice and monkeys, As (III) at a slower rate than As (V) (Lindgren et al., 1984). Teratogenicity of arsenic has been described or hamsters, rats, and mice (Baxley et al., 1981; Beaudoin, 1974; Ferm and Kilham, 1977; Hood and Harrison, 1982). In hens, the amount of arsenic incorporated into the egg was extremely low but increased with time (Anke et al., 1982). And so it is possible for arsenic to be incorporated into testis and ovaries of poultry. Although we didn't test the retained arsenic level in testis tissue and semen of drakes, it indeed showed lipid-peroxidation of testis tissue and a low fertilization rate of sperm in drakes injected with As (III) and As (V). In mammals, it has been known that As (V) must be reduced to As (III) before methylation (Vahter and Envall, 1983). Methylation of inorganic arsenic to mono- and dimethylated arsenic was demonstrated in rats, mice, hamsters, rabbits, cats, and dogs. The biological methylation of arsenic is a general phenomenon in mammals (Marafante et al., 1980; Odanaka et al., 1980). Cows and dogs excrete a considerable percentage of inorganic As (III) or As (V) given orally as methylated arsenic in the urine (Lakso and Peoples, 1975). Dogs excrete >95% of the arsenic via the urine. The half-life of the first component (85% of the dose) was 5.9 h; that of the second (14% of the dose) was 2.4 days (Hollins et al., 1979). After 6 h, >90% of the arsenic in the plasma, red blood cells, and urine was present as dimethylarsinic acid (Charbonneau et al., 1979). However, in poultry, the metabolic pathway of arsenic is still uncertain.

The difference in acute toxicity between inorganic As (V) and As (III) in mature drakes was very significant. When As (V) was muscle-injected in drakes, no mortality was observed, even at the high concentration of 40 ppm. However, 10 ppm of As (III) caused acute death after 5-10 min of injection. It is possible that A (V) is easy to metabolically detoxify in mature drakes, but As (III) is not. In addition, it is possible that As (V) will accumulate in muscle of drakes and cause chronic toxicity. In hens, skeleton muscles stored most of the retained ^{76}As at the measuring points from 45 min to 96 h after oral intake, followed by skeleton, liver, blood, lungs, kidneys, feathers, and ovaries (Anke et al., 1982). The absorbed arsenic is incorporated into different tissues at different rates. Although As (V) didn't result in acute toxicity, it induced a chronic affect on the fertilization rate of artificially inseminated semen and lipid peroxidation in testis tissue. As (V) chronically impairs testis tissue in part by lipid peroxidation and then decreases the fertilizing ability of semen.

The acute toxicity of As (III) gave rise to mortality after 5 min to 24 h of administration. The LD50 of As (III) was 4.89±1.49 ppm. Therefore, low concentrations of As (III) were chosen to test the chronic toxicity on sperm generation. It showed that the 1.2 ppm concentration of As (III) significantly affected quality of sperm and testis tissue. Especially, it decreased the fertilization rate by 60% and increased the MDA level 23-fold over the control. Some of the testis samples in 1.2 ppm As (III) treatment showed a black appearance and soft texture. This phenomenon is similar to the blackfoot disease characterized by a chronic peripheral vascular disease, caused by arsenic toxicity, which progresses to gangrene, blackening and spontaneous amputation of the extremities.

Based on many *in vitro* studies, several antioxidants such as catalase, superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPX), and glutathoine s-transferase (GST) could support the protective function to reduce arsenic toxicity, especially glutathione (Chang et al., 1991; Huang et al., 1993; Lee and Ho, 1994). Lee and Ho (1995) suggested that the additional catalase could partly reduce the toxicity of As (III) in the cell culture of human fibroblasts *in vitro*. However, in our preliminary experiments, when As (III) along with glutathione was injected into fertilized eggs of ducks, glutathione couldn't

reduce the arsenic impairment on hatchability (data not shown). Therefore, L-ascorbic acid, instead of glutathione, was chosen to test the detoxification for the *in vivo* toxicity of As (III) and the result indeed showed it to be beneficial.

Although the low concentration of 1.2 ppm As (III) induced significant effects on the fertilizing ability of sperm and lipid peroxidation in testis tissue, L-ascorbic acid could reduce both of the chronic impairments ($p<0.05$). It suggested that the damage of lipid-peroxidation, induced by arsenic, could be reduced by non-enzymatic antioxidants, such as L-ascorbic acid. In ducks, ascorbic acid could be produced by the birds but its level alone without provision in feed couldn't detoxify the lipid-peroxidation of arsenic (as the control). However, the additional high dose of ascorbic acid injected into drakes could work. L-ascorbic acid played a role as antioxidants scavenging reactive oxygen species (ROS) generated by inorganic arsenic.

In summary, it is suggested that the toxicity of the trivalent arsenic on sperm quality is partly from free radicals generated by its metabolic pathway, and that the antioxidant L-ascorbic acid ameliorates arsenic-caused sperm impairment.

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