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# RADIOACTIVITY, HEAVY METAL AND OXIDATIVE STRESS MEASUREMENTS IN THE FOLLICULAR FLUIDS OF CATTLE BRED NEAR A COAL-FIRED POWER PLANT

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## ABSTRACT

Coal-fired power plants release heavy metals via gas and dust discharges and can radioactive pollution. This can harm human and animal health. Livestock are often exposed, and the reproductive system is one of the most commonly affected systems. In this study, we measured heavy metals and radioactivity levels as well as oxidative stress parameters in the ovarian follicular fluids from cattle bred near coal-fired power plants. Samples were from the slaughter houses near the coal-fired power plants in Elbistan (n=25) samples from Elazig (n=14)—300 km from the plant—served as the control. Cadmium (Cd), lead (Pb), and mercury (Hg) measurements were performed using atomic absorption spectrophotometry. Malondialdehyde (MDA), Glutathione (GSH), and Glutathione Peroxidase (GSH-Px) levels were measured colorimetrically with a spectrophotometer. $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K were measured with a gamma counter. It was conclude that the Pb concentration was high in the follicular fluids of the cattle bred near the Elbistan coal-fired power plant (22.27±1.73 µg/dL, P<0.05). However, MDA concentration was high in the cattle from Elazig (85.77±10.40 µmol, P<0.05). The Cd, Hg, GSH levels and GSH-Px activity were not different (P>0.05). No difference was found between the groups with regard to radioactivity parameters. These assays may be of value to animal breeders and authorities working near the Elbistan Power Plant.

**Keywords:** Cattle, follicular fluid, radioactivity, oxidative stress, heavy metals.

## INTRODUCTION

Environmental pollution frequently targets the reproductive system, and coal-fired power plants are one of the important factors contributing to this pollution. Gases and dusts released from coal-fired power plants spread Hg, Cd and Pb. These elements may reach the reproductive system after entering the body (Sener and Yildirim, 2000). Vidovic *et al.*, (2005) showed that the release of heavy metals into the atmosphere through coal-fired power plants increases metal load in soil. In turn, this can lead to plant accumulation and eventually high species (animal, human) contamination. Cd, Pb and Hg are some of the most common heavy metal contaminants.

Heavy metal pollution has important negative effects on the reproductive functions of both animals and humans. The negative effects of these toxic elements are accumulated in the follicular fluid and harm granulosa cells. This leads to impaired production of hormones that are critical for female fertility. In addition, increased heavy metals in the follicular fluid are known to negatively affect oocyte quality (Capcarova and Kolesarova, 2012).

Hg, Pb and Cd are known to have negative effects in various reproductive activities in different species (Monsefi and Fereydouni, 2013; Nampoothiri and Gupta, 2006). Lead is suggested to cause ovarian changes in sheep and delay sexual maturation despite a mechanism that remains unclear (Bires *et al.,* 1995). Massányi *et al.,* (2007) reported that vacuolization, congestion and necrosis are formed in the uterus and ovary of females exposed to Cd. Lafuente *et al.* (2003) suggested that pineal hormone secretions disrupted based on Cd dose. Cadmium was shown to completely hinder secretion by inhibiting calcium channels in the myometrium of rats, cats and pregnant cattle (Kara *et al.*, 2003).

Mercury has been shown to accumulate in human follicular fluid (Al-Saleh *et al.,* 2008). Hg, Pb and Cd exist in different concentrations in the fluids of small and large follicles. The levels in follicular fluid and blood are similar during follicular development. High heavy metal concentrations in the fluid of small follicles indicate long-term exposure to these elements (Silberstein *et al.,* 2009). Heavy metal levels are twice more high in serum than follicular fluid (Yuan and Tang, 2001). Lead is a reproductive toxin to humans and animals. Sterility, habitual abortion and irregular menstruation occur in women who are working in lead-based industries. Blood levels of 40 µg Pb/100 mL cause ovulation disorders (Winder, 1993). Angell and Lavary (1983) reported that premature rupture of membranes; premature delivery and elevated risk of preeclampsia are higher among pregnant women with approximately 25 µg/100 mL of Pb concentration.

The Elbistan Coal-Fired Power Plant produces 2800 MW of power by burning 18 million tons of brown coal annually. Coal-fired power plants lead to radioactive pollution in addition to heavy metal pollution depending on the natural radioisotope concentration within the coal that is used (Buke, 2003; Zeevaert *et al.*, 2006; Gencer, 2003; Constantin, 2010). Radioactive elements that naturally are safe underground are deposited in the surface in the form of ash (Sahin *et al.*, 2002).

In this study we investigated studied the concentrations of heavy metals, radioactivity and oxidative stress parameters in the follicular fluids of cattle bred near a coal-fired power plant in Turkey. This work characterizes one of the limitations of fossil fuels.

## MATERIALS AND METHODS

**Collection of follicular fluids:** We collected the follicular fluids from 78 cows of different ages and breeds. The ovaries were obtained from animals slaughtered near the Elbistan Coal-Fired Power Plant. It has been confirmed that these animals were reared and maintained in this area since they were born. Control animals were from Elazig, which is 300 km from any coal-fired plant. The experimental animals near the plant had a physical examination. Ovaries were transferred to the laboratory on ice within one hour after slaughter. Follicles with cysts or the other pathologies were not used. The follicular fluid was aspirated from the follicles 4 mm or above using a 19 gauge needle. The resulting follicular fluids were stored at -20 $^0$C until analysis.

**Experimental design**

**Test 1:** The Cd, Pb and Hg levels were measured in 25 follicular fluid samples from the Elbistan cohort (Group 1). The control group included 14 follicular fluid samples from the Elazig cohort (Group 2). The $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K ratios were also measured in these samples.

**Test 2:** Additionally 25 follicular fluids from different experimental animals near Elbistan (Group 3) and 14 control samples from Elazig (Group 4) were measured for MDA, GSH and GSH-Px activity. Such a design was planned, as sufficient amount of follicular fluid could not be obtained.

**Heavy metal measurement in follicular fluid:** The Cd, Pb and Hg measurements were done with inductively coupled plasma atomic absorption spectrophotometry (Patra *et al.*, 2008).

**Oxidative stress parameter measurements in follicular fluid:** MDA, GSH level measurements and GSH-Px activities were performed with spectrophotometry as described in Tietz Textbook of Clinical Chemistry and defined by Turk *et al.* (2010).

**Radioactivity measurements in follicular fluid:** The measurement of $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K ratios in follicular fluid is summarized below.

**Sample preparing and counting:** Firstly, the planchettes were cleaned and soaked in water for 1day. Any residue was removed with a spatula. The planchettes were soaked in nitric acid for 2 hours and then cleaned by distilled water. The samples were then transferred to the 4.6 cm$^3$ aluminum planchettes. Samples on the planchette were evaporated at 60$^o$C until sediment formed. The samples were then characterized by gamma counting.

**Measuring of gamma radioisotopes:** There are two general types of gamma spectrometers: 1) sodium iodide or thallium-activated crystalline NaI (Tl), and 2) germanium or lithium-doped germanium Ge (Li) detectors Knoll 1989. Here in, we used 2˝×2˝ NaI (Tl) well-type detectors to determine the gamma radioactivity. This is housed in a cylindrical lead shield that was 13.7 cm diameter and 15.5 cm length. The lead shield thickness is about 3.5 cm and is suitable to block the gamma background. The detector entrance window consists of 0.50 mm aluminium. The photo peak efficiency of the NaI (TI) detector is 24% (Kulahci and Sen, 2008; Kulahci and Sen, 2009a; Kulahci and Sen, 2009b). The counting time was 1000 seconds.

The $^{210}$Pb concentrations were measured via 46.52 keV gamma (γ) rays, $^{232}$Th detection was via the 583.1 keV γ rays emitted by $^{208}$Tl, and $^{40}$K activity is based on 1460 keV γ rays. The $^{238}$U activity concentrations are based on detection of 609.32 keV γ rays emitted by $^{214}$Bi, and the determination of $^{129}$I activity is based on 30 keV γ rays (Paolo *et al.*, 2013). To minimize errors, the window interval is regulated as 10 - 20 keV. Thus, the energy interval is adjusted for $^{210}$Pb activity concentrations of 40±10 keV, for $^{129}$I activity of 30±10 keV, for $^{238}$U activity of 609±20 keV, 575±15 keV for $^{232}$Th, and 1460±10 keV for for$^{40}$K.

The activities are calculated according to the following equation for all radionuclides (Kulahci and Sen, 2008; Kulahci and Sen, 2009a; Kulahci and Sen, 2009b).

$$A(Bq/l, Bq/kg) = \frac{C}{\xi \times P_\gamma \times M_s}$$

Here, $C$ is the counting rate of gamma rays (counts per second), ξ is the detector efficiency for the specific γ ray, $P_\gamma$ is the absolute transition probability of γ decay and $M_S$ is the mass of the residue in kg or liter.

**Statistical analyses:** The Mann-Whitney U and independent samples t tests were used to compare group parameters. Statistical analysis was done with SPSS 11.5.

## RESULTS

The Pb concentration was 22.27±1.73 µg/dL in follicular fluids from animals near the plant and 20.42±8.90 µg/dL in the Elazig controls. The Hg (<0.075 µg/dL) and Cd (<1 µg/dL) concentrations were below detection limit in both groups (Table 1).

The follicular fluid MDA, GSH and GSH-Px concentrations were 10.60 ± 2.19 µmol, 3.40 ± 1.48 µmol and 77.20 ± 8.836 nmol/min/mL, respectively, in the coal-plant group. These values were 85.77 ± 10.40 µmol, 2.47±0.20 µmol and 107.42±22.50 nmol/min/mL in the control animals bred in Elazig (Table 2).

The Pb concentrations were statistically elevated in the follicular fluids of the coal plant groups versus controls. The MDA concentration was higher in the Elazig region versus Elbistan (p<0.05). The Hg, Cd, GSH and GSH-PX concentrations were not different (Table 1,2) (P>0.05). In this study, $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K concentrations were measured by gamma counter. Obtained results are given in the Table 3. In inter-group comparisons, no difference was found between the follicular fluid levels of $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K (Table 4) (P>0.05).

**Table1. Comparison of heavy metal levels in follicular fluids.**

| | Lead (µg/dL) | Cadmium (µg/dL) | Mercury (µg/dL) |
|---|---|---|---|
| **Group 1 (n=25)** | 22.27±1.73[a] | BDL | BDL |
| **Group 2 (n=14)** | 20.42±8.90[b] | BDL | BDL |
| **P** | * | - | - |

- No significant differences between groups (P>0.05).
* (P<0.05).
[ab]The difference between different letter-carrying averages is significant (P<0.05).
Group 1: Elbistan Coal-Fired Power Plant region, Group 2: Control region (Elazig), BDL: Below detectable level.

**Table 2. Comparison of oxidative stress parameters in follicular fluids.**

| | MDA (µmol) | GSH (µmol) | GSH-PX (nmol/min/mL) |
|---|---|---|---|
| **Group 3 (n=25)** | 10.60 ± 2.19[a] | 3.40 ± 1.48 | 77.20 ± 8.83 |
| **Group 4 (n=14)** | 85.77 ± 10.40[b] | 2.47 ± 0.20 | 107.42 ± 22.50 |
| **P** | * | - | - |

- No significant differences between groups (P>0.05).
* (P<0.05).
[ab]The difference between different letter-carrying averages is significant (P<0.05).
Group 3: Elbistan Coal-Fired Power Plant region, Group 4: Control region (Elazig). Such a design was planned, as sufficient amount of follicular fluid could not be obtained.

**Table 3. The activity concentrations of $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K (Bq kg$^{-1}$) in follicular fluids.**

| Station | $^{210}$Pb (Bq/kg) | $^{129}$I (Bq/kg) | $^{238}$U (Bq/kg) | $^{232}$Th (Bq/kg) | $^{40}$K (Bq/kg) | Station | $^{210}$Pb (Bq/kg) | $^{129}$I (Bq/kg) | $^{238}$U (Bq/kg) | $^{232}$Th (Bq/kg) | $^{40}$K (Bq/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 EL | 3,083.80 | 28,480.0 | 1,632.10 | 1,376.00 | 22,682.00 | 1E | 1,698.00 | 4,700.00 | 115.92 | 404.71 | 3,544.10 |
| 9 EL | 6,534.80 | 4,880.00 | 2,448.10 | 930.820 | 15,542.00 | 2E | 3,823.20 | 1,269.00 | 306.98 | 237.24 | 5,359.20 |
| 11EL | 6,363.50 | 5,933.40 | 1,051.00 | 370.980 | 36,054.00 | 3E | 1,125.80 | 4,213.30 | 197.83 | 350.74 | 10,221.0 |
| 12 EL | 1,976.80 | 3,876.90 | 855.990 | 1,463.20 | 14,217.00 | 4E | 1,1748.0 | 16,160.0 | 1,187.0 | 728.47 | 33,604.0 |
| 16 EL | 2,377.60 | 2,666.70 | 5,65.230 | 578.150 | 6,400.700 | 5E | 1,229.40 | 3,237.70 | 690.10 | 470.59 | 9,963.90 |
| 17 EL | 7,917.10 | 3,269.60 | 1,548.20 | 985.370 | 27,760.00 | 6E | 4,885.00 | 1,469.40 | 211.96 | 462.52 | 19,031.0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 EL | 2,886.40 | 3,531.00 | 613.950 | 669.860 | 18,250.00 | 7E | 2,097.90 | 3,428.60 | 238.46 | 86.720 | 4,800.50 |
| 22 EL | 2,284.30 | 6,755.60 | 1,978.30 | 1,304.10 | 30,337.00 | 8E | 1,558.90 | 1,550.80 | 730.45 | 87.170 | 4,265.10 |
| 23 EL | 2,977.80 | 3,955.60 | 453.360 | 449.670 | 21,236.00 | 9E | 1,720.20 | 1,920.00 | 529.90 | 381.58 | 6,960.80 |
| 25 EL | 3,216.70 | 9,447.70 | 565.230 | 539.610 | 16,402.00 | 10E | 9,230.60 | 38,629.0 | 847.84 | 3,584.50 | 4,800.50 |
| 26 EL | 5,096.60 | 1,976.50 | 610.940 | 904.640 | 39,534.00 | 13E | 11,454.0 | 35,840.0 | 3,857.7 | 3,399.50 | 55,446.0 |
| 29 EL | 1,3021.0 | 6,506.70 | 1,780.50 | 755.450 | 50,406.00 | 19E | 53,845.0 | 53,867.0 | 3,956.6 | 5,665.90 | 574,060.0 |
| 32 EL | 8,811.00 | 36,946.0 | 1,753.50 | 2,649.00 | 77,899.00 | 24E | 60,943.0 | 73,600.0 | 6,676.7 | 5,665.90 | 109,210.0 |
| 33 EL | 7,067.20 | 6,300.00 | 1,391.00 | 607.060 | 32,029.00 | 25E | 32,894.0 | 17,600.0 | 5,044.7 | 2,266.40 | 89,050.00 |
| 34 EL | 3,970.40 | 4,325.90 | 439.620 | 1,049.20 | 24,892.00 | 26E | 17,296.0 | 14,044.0 | 2,308.0 | 629.54 | 57,873.00 |
| 39 EL | 7,158.90 | 9,400.00 | 1,112.80 | 1,062.40 | 19,952.00 | - | - | - | - | - | - |
| 40 EL | 12,237.0 | 21,876.0 | 989.150 | 674.510 | 8,400.900 | - | - | - | - | - | - |
| 44 EL | 3,793.60 | 10,533.0 | 1,360.10 | 2,293.30 | 73,508.00 | - | - | - | - | - | - |
| 45 EL | 6,950.90 | 5,653.40 | 1,780.50 | 1,025.30 | 14,002.00 | - | - | - | - | - | - |
| 46 EL | 13,706.0 | 27,378.0 | 1,483.70 | 449.670 | 50,406.00 | - | - | - | - | - | - |
| 50 EL | 4,772.60 | 4,640.00 | 1,335.30 | 1,295.10 | 49,145.00 | - | - | - | - | - | - |
| 51 EL | 4,992.90 | 6,080.00 | 2,374.00 | 1,539.40 | 68,047.00 | - | - | - | - | - | - |
| 56 EL | 3,784.20 | 1,538.50 | 970.130 | 996.200 | 58,16.000 | - | - | - | - | - | - |
| 60 EL | 8,076.60 | 10,667.0 | 3,709.30 | 1,753.70 | 22,4020.0 | - | - | - | - | - | - |
| 64 EL | 1,249.80 | 6,161.70 | 694.510 | 430.540 | 14,478.00 | - | - | - | - | - | - |

**Table 4. Comparison of the radioactivity in the follicular fluid.**

| | $^{210}$Pb (Bq/kg) | $^{129}$I (Bq/kg) | $^{238}$U (Bq/kg) | $^{232}$Th (Bq/kg) | $^{40}$K (Bq/kg) |
|---|---|---|---|---|---|
| **Group 1 (n=25)** | 5,772.30±687.95 | 9,311.17±1,843.99 | 1,398.86±152.38 | 1,046.13±114.74 | 38,456.62±8,753.15 |
| **Group 2 (n=14)** | 14,369.93±5,026.33 | 18,101.92±5,781.83 | 1,793.34±540.36 | 1,628.10±515.72 | 65,879.27±37,325.67 |
| **P** | - | - | - | - | - |

- No significant differences between groups (P>0.05).

## DISCUSSION

**Heavy metals in follicular fluid:** Chronic exposure to heavy metals negatively affects reproductive health in animals and humans. Thus, heavy metal measurements are important to determine the concentrations in human and animal organisms. This identifies the risks they carry for public and environmental health. This also minimizes environmental pollution that could lead to greater problems in the future. Capcarova and Kolesarova (2012) suggested that Pb, Cd and Hg are the most common pollutants—these can be detected in many tissues and organs of animals near the pollution source. These heavy metals may also exist on the ovary in developed and developing follicles. In the same study, increasing heavy metals in follicular fluid were suggested to negatively affect the quality of oocytes. In previous studies, Cd, Hg and Pb were shown to be present in the follicular fluid of women (Younglai *et al.*, 2002). In another study (Bires *et al.*, 1995), Pb accumulation was reported in sheep ovarian tissues. However, to the best of our knowledge, there is no report on heavy metal pollution in the follicular fluids of the cattle bred near a coal-fired power plant. In the present study, the average Pb values were found to be higher in animals bred in the Elbistan Coal Plant region (22.27±1.73 µg/dL) versus the Elazig control region (20.42±8.90 µg/dL).

**Radioactivity in follicular fluid:** Various radioactive substances are released into the environment when coal is burned. The concentrations of radionuclides like $^{238}$U and radium ($^{226}$Ra) have already been reported to be very high in the coal used at Elbistan Power Plant (Ozturk and Ozdogan 2004). However, there is no radioactivity data on bovine follicular fluid from this same region. Here we report the levels of $^{210}$Pb, $^{129}$I, $^{238}$U, $^{232}$Th and $^{40}$K found in this fluid—we found no difference between the groups.

**Oxidative stress parameters in follicular fluid:** The disrupted relationship between oxidant and antioxidant mechanisms plays an important role in the continuity of physiological balance, especially the reproductive system. Oxidative stress arises as the result of impaired balance between oxidant and antioxidant mechanisms. Following up on the oxidative stress parameters provides information into the condition of the target tissue and organ system (Angell and Lavary 1983; Lavranos *et al.*, 2012). Here, MDA and GSH measurements as well as GSH-Px activity were calculated to assess oxidative stress. We found that the MDA ratio was higher in Elazig (85.77 ± 10.40 µmol) versus Elbistan (10.60 ± 2.19 µmol). This suggests that the high Pb levels may be inducing oxidative stress in the bovine ovaries. However, this correlation is far from causal and must be studied further. Indeed, there are multiple physiological and pathological conditions that may lead to an increase in MDA both in blood and other tissues.

**Conclusion:** The present study indicates negative effects of heavy metals to reproductive system. In addition, higher Pb concentration in follicular fluids of the cows bred around Elbistan Coal-Fired Power Plant provides information about the level of heavy metal pollution in the region. However, no difference was found with regard to radioactivity parameters in follicular fluids. More studies with a novel approach are suggested to assess fertility problems in the cows bred around Elbistan Coal-Fired Power Plant, animal breeders and the concerned authorities should be warned.

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