Bull. Environ. Contam. Toxicol. (1990) 45:824–828

Bulletin of Environmental Contamination and Toxicology

Detection of Lead in Blood, Seminal Plasma, and Spermatozoa of Bulls. Effect *in vitro* of Lead Acetate on Sperm Motility

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Lead compounds are common pollutants in many areas and can impair the reproductive function of the male by various mechanisms (Uzych 1985). Reduced spermatogenesis in rat (Chowdhury et al. 1984; Sokol et al. 1985; Wiebe et al. 1985) and mouse (Al-Hakkak et al. 1988) after exposure to lead compounds has been reported. Prenatal and neonatal exposure to lead reduces the binding of FSH and LH to their respective receptors in male rats (Wiebe et al. 1982) and significantly suppresses testicular synthesis of testosterone in rats (Wiebe et al. 1982; Sokol et al. 1985) and mice (Rodamilans et al. 1988a). Reduced concentrations of testosterone in the blood were found in human populations occupationally exposed to lead (Braunstein et al. 1978; Rodamilans et al. 1988b), whereas no change in blood testosterone concentration in such populations was seen in other studies (Lacranjan et al. 1975; Cullen et al. 1984), although an impairment of the hypothalamic-pituitary axis was evidenced (Braunstein et al. 1978; Cullen et al. 1984). Evidence for reduced spermatogenesis in men exposed to toxic levels of organic lead has also been presented (for ref. see Barlow and Sullivan 1982). Reduced fertility with increased frequency of asthenospermia, hypospermia and teratospermia was found in two groups of lead-exposed workers (Lacranjan et al. 1975). It seems, from all these experimental and epidemiologic studies, that the effect of lead compounds on the function of the male reproductive system might be direct and/or indirect.

The present study was undertaken a) to investigate the presence of lead in the blood and semen of bulls in a center for artificial insemination located close to Thessaloniki, Greece and b) to evaluate *in vitro* the possible effect of various concentrations of lead, as lead acetate, on sperm motility.

MATERIALS AND METHODS

Blood and semen samples were taken from 14 Holstein bulls (3-5 years old). For each animal the blood and semen sampling were performed the same day.

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The determination of lead in blood was performed by the diammonium hydrogenphosphate-Triton X-100-flameless atomic absorption spectrophotometric procedure (Subramanian 1987). A mixture of 100 µl heparinized blood, 100 µl of a 5% solution of Triton X-100 and 100 µl of a 5% solution of $(NH_4)_2HPO_4$ was brought to 1 ml with double distilled deionized water.

Lead concentrations in seminal plasma and spermatozoa were determined by acid digestion and flameless atomic absorption spectrophotometry (Umeyama et al. 1986). After the estimation of sperm density, the semen was centrifuged to 3000 rpm for 15 min, the supernatant was tranferred and the sediment was washed with normal saline solution. Two hundred (200) µl of a solution containing 12.5% HNO_3 and 12.5% $HClO_4$ were added to 200 µl of seminal plasma and the mixture was brought to 1 ml with double distilled deionized water. The pellet of spermatozoa was digested overnight by adding 2 ml of a mixture of equal volumes of nitric and perchloric acids, centrifuged and the supernatant diluted to 1:20 with double distilled deionized water.

A Perkin-Elmer Model 2380 atomic absorption spectrophotometer equipped with a Perkin-Elmer HGA-400 graphite furnace and a deuterium lamp for background correction was used for the measurements.

Sperm motility or better sperm velocity (the speed of forward sperm motion) (Fakin et al. 1986) was determined by a modification of a sperm penetration test (Gaddum-Rosse et al. 1980; Smokovitis et al. 1987). Lead acetate was added in concentrations of 2.5 µg Pb/ml or 0.25 µg Pb/ml to bovine semen and the samples were remained at room temperature for 60 min. Thereafter, seminal plasma was drawn up into capillary tubes (i.d. 1.7 mm, length 133 mm). The tubes were then positioned vertically with their lower end immersed in a sample of semen (bovine) contained in a small beaker and placed in a water bath (37°C). The upper end of the tube was sealed with vaseline. Control samples of semen contained instead of lead acetate an equal volume of the diluent (Tris-buffer pH 7.4, molarity 0.1). Fifteen and 30 min later the tubes were examined under a binocular dissecting microscope to measure the distance travelled by the leading spermatozoa. A total number of 400 tubes from 20 samples of semen was studied (800 determinations of migration rate).

Statistical analysis was performed by the F-Distribution and Duncan's test; $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Lead was found in blood, seminal plasma, and spermatozoa of bulls in concentrations of 21.7 ± 4.3 ng/ml, 16.9 ± 10.0 ng/ml and 92 ± 56 ng/$1x10^9$ spermatozoa, respectively. The *in vitro* effect of lead, as lead acetate, on sperm motility (velocity) is shown in Table 1. Concentrations of 2.5 µg Pb/ml reduced significantly ($P<0.005$) sperm motility, while smaller concentrations (0.25 µg Pb/ml) had no effect on sperm motility compared to controls ($P>0.05$).

Table 1. Distance (mm) travelled by the leading spermatozoa in 30 min (after the addition of lead acetate or the corresponding diluent to semen and incubation for 60 min). The values are mean ± S.D.

Control	Lead concentration	
	2.50 µg Pb/ml	0.25 µg Pb/ml
22.47 ± 4.01	16.86 ± 4.36*	19.80 ± 5.61

*$P<0.005$

Lead was detected in blood, seminal plasma and spermatozoa of all bulls studied. The concentrations of lead determined reflect the relatively low concentration of lead at the area (e.g. 413 $\mu g \cdot g^{-1}$ in dust, Misaelides et al. 1989); however, in other areas around Thessaloniki higher concentrations of lead have been occasionally measured or cases of acute lead toxicosis in farm animals have been documented (*unpublished data*).

The addition of lead acetate to the semen in the concentration of 0.25 µg Pb/ml did not affect sperm motility (or better sperm velocity), but a higher concentration (2.5 µg Pb/ml) reduced sperm motility significantly. In two groups of lead-exposed workers an increased frequency of asthenospermia was found (Lacranjan et al. 1975). However, no difference in seminal lead comcentrations was noted between fertile and infertile men (Butrimovitz et al. 1983; Umeyama et al. 1986), but infertility is a multifactorial condition.

Lead in seminal plasma could affect sperm function, whereas lead during spermatogenesis might have fundamental effects on sperm morphology and function (Lacranjan et al. 1975). In our study, the addition of lead acetate to the semen might have influenced directly and/or indirectly mechanisms located mainly in mitochondria or in other structures of the sperm, resulting in decreased motility (Prierley 1977; Mann and Lutwak-Mann 1981; Tash and Means 1983; Thomas and Brogan 1983; Fowler 1978). The role of sperm mitochondria in trapping, concerving and supplying energy for sperm motility is well known (Mann and Lutwak-Mann 1981). In semen used for artificial insemination, the seminal plasma is removed shortly after ejaculation or extended; therefore, the presence of lead in seminal plasma could not have any significant biological effect on sperm function. It is of interest, however, that most semen extenders have as main constituent egg yolk, which might contain lead (Kirkpatrick and Coffin 1975).

The results of this study show that although the presence of lead in the semen of farm animals could affect semen quality, this would happen only in areas with heavy pollution of the environment with lead or in case of acute lead toxicosis, since only in higher con-

centrations than those found in the semen of non-exposed animals (about 25 times higher) lead reduced in vitro sperm motility.

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Received February 8, 1990; accepted July 16, 1990.