# BIOCHEMICAL INDEXES OF SEMEN AND SPERMATOZOON FUNCTIONAL ACTIVITY AFTER EXPERIMENTAL INFLUENCE OF PHENOXYHERBICIDE ECOTOXICANTS

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## Introduction

The problem of the environmental aggressive factor influence on male reproductive system is very actual because testes are enough sensitive to different toxic agents [5,10]. Nowadays entering and accumulation of dioxincontaining organochlorines including dichlorphenoxyacetic acid (2,4-D) compounds effective and widely spread herbicides in water and food is of special meaning [8]. One of the organochlorine intoxication signs is the androgen insufficiency syndrome [1]. It has been reported that this appearance is spread not only in the ecologically unfortunate regions but also among the man population as whole [9]. The opinion of WHO experts is that modern comprehension of the male reproductive system function and regulation especially in the extremal conditions are fragmentary in many ways and not enough full that dictates the necessity of wide fundamental researches in this field.

It was shown that 2,4-D intoxication is followed by spermatogenesis disturbances, gamete number decreasing, reducing of their mobility and fertility [4]. As far as one of the main criteria of the reproductive system pathology diagnosis objectivisation is spermoplasma biochemical structure providing sperm normal metabolism and vitality our study aim was experimental evaluation of 2,4-D influence on some ejaculate biochemical indexes and gamete functional qualities.

## Materials and Method

Sexually mature male rats were used in the experiment. Chronic intoxication was provided by means of 2,4-DA (dimethylamine salt) daily peroral administration during 1 and 4 months in total doses of 1/10 $LD_{50}$ and $LD_{50}$. Spermatozoon functional state was estimated by their number, character and moving duration. The biochemical investigation of spermoplasma included determination of the main sperm energetical substrate - fructose - and its hour expense – fructolysis [6]. Fructose oxidation product in gametes – lactate – was also investigated in the ejaculate samples [7]. The activity of energy metabolism enzymes as lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and amino acid metabolism ones as aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT), testosterone and prolactin content - was studied at the same time using standard test kits.

## Results and Discussion

2,4-DA caused spermatozoon amount decreasing (Table) correlated with the exposure duration and dose. Sex cell amount was 74-80 % from the control level in the 1- month experiment, but these shifts were not statistically significant. In the 4-months experiment more essential and reliable changes were observed: sperm number decreased to 54-66% of the normal level.

As spermatozoon leading functional characteristics are motion activity figures, the fertility mainly depends on the mobility but not on the germinative cell number [2]. In our study the 2,4-DA influence resulted in the limiting of gamete motion activity which was the smallest at 4-month intoxication in the LD50 total dose (59% of the control level).

**Table**
**Herbicide 2,4-DA Influence on Spermoplasma Biochemical Indexes and Spermatozoon Mobility**

| Indexes | Group of animals | | | | |
|---|---|---|---|---|---|
| | control group | 1/10 $LD_{50}$, 1 month | $LD_{50}$, 1 month | 1/10 $LD_{50}$, 4 months | $LD_{50}$, 4 months |
| Spermatozoon amount, $*10^6$ | 41,3±6,2 | 33,6±5,9 | 30,8±4,1 | 28,7±3,9* | 22,3±3,5* |
| Spermatozoon mobility, min | 260±14,9 | 266±19,1 | 241±13,8 | 215±14,5* | 153±10,4* |
| Testosterone, nmol/l | 1,63±0,21 | 1,68±0,39 | 1,34±0,32 | 1,28±0,29 | 0,90±0,15* |
| Prolactin, μg/l | 8,13±0,63 | 8,09±0,52 | 8,22±0,67 | 7,51±0,94 | 4,33±0,82* |
| Fructose, mmol/l | 10,6±1,2 | 10,9±2,1 | 10,3±1,6 | 12,4±1,4 | 13,6±1,7 |
| Fructolysis, mmol/l/h | 0,64±0,10 | 0,60±0,12 | 0,52±0,14 | 0,29±0,07* | 0,26±0,08* |
| Lactate increasing, mmol /l for 4 hours | 2,94±0,64 | 2,56±0,48 | 2,61±0,33 | 1,09±0,34* | 0,86±0,39* |
| LDH, μmol/min per 1 mg of protein | 0,65±0,08 | 0,59±0,04 | 0,59±0,07 | 0,50±0,05* | 0,41±0,06* |
| MDH, μmol/min per 1 mg of protein | 0,24±0,05 | 0,25±0,04 | 0,22±0,06 | 0,10±0,02* | 0,11±0,04* |
| AST, nmol/min per 1 mg of protein | 25,3±3,2 | 18,5±4,4 | 16,9±2,9* | 15,8±3,5* | 12,6±4,8* |
| γ-GT, nmol/min per 1 mg of protein | 7,56±0,83 | 8,15±0,59 | 6,99±0,61 | 11,7±0,63* | 15,4±0,96* |

* - $p<0,05$

Fructose spermoplasma content playing the main role in motility and gamete fertility supporting was not significantly differ from the same one of the control animals in the experiment. Fructolysis process investigation was found more informative for judgement on spermatozoon functional full value. It was determined that after 2,4-DA 1 month dose-independent exposure fructose utilization in sex cells was not significantly differ from the normal level. Longer herbicide introduction caused fructose expense decreasing to 41-45% from the control level.

Fructolysis study results are confirmed by the data of lactate accumulation dynamics research. One of the main gamete life products significantly evaluated in the intact animal sperm samples after 4-hour exposition from 1,75±0,37 to 4,69±0,82 mmol/l. It testifies high glycolysis process aimed at

their motion activity energetical providing. Sex cells kept their ability for high carbohydrates anaerobic oxidation ratio: lactate increase after 1 month intoxication was close to the control data. Another lactic acid level dynamics was found after 4-month 2,4-DA introduction. Lactate cumulation in these conditions was only 29 and 37% from the control figures after herbicide influence in LD50 and 1/10LD50 total doses accordingly. The lactate synthesis decreasing in sperm straight correlated with gametes mobility reducing ($r1=+0,73$; $r2=+0,66$, $p<0,05$). These data testify that energetic supplement processes deterioration connected with the ability limitation to utilize main oxidation substrate – fructose- is one of the probably reasons of spermatozoon fertility disturbance (their motion activity in particular).

Another group of markers characterizing germinative function is presented by the different sperm enzymes. We arranged one turned studied oxidoreductase activity changes both LDH and MDH. There were not detected reliable deviations due to 1 month intoxication, in the same time enzyme activity significantly decreased after 2,4-DA chronic introduction. LDH is the terminal enzyme of the glycolytical oxidoreduction and changes we revealed are corresponded to the opinion about glycolysis role reduction in the sex cell energetical metabolism. In its turn low activity of MDH which is one of the Krebs cycle enzymes may testify mitochondrial link disruption of the oxidative reactions in the germinative cells. This hypothesis may be confirmed by the activity investigation of AST accelerating aspartate conversion into oxaloacetate – citric acid predecessor and the citric cycle key metabolite. According to our data AST was inhibited in whole period of our observation.

The activity of γ-GT which is the glutathione katabolism and interorganic circulation main enzyme had not undergone essential changes after the 1 month herbicide daily entrance. Longer intoxication during 4 months caused γ-GT spermoplasma activity increasing. It is known that reducible glutathione play central role in the conjugation of toxic and reactive products generated in the xenobiotic biotransformation reactions. Likely, that γ-GT reaction ratio increasing reflects the interorganic glutathione exchange and has an adaptive meaning. Besides, γ-GT realizes the aminoacid transport into spermatozoons. Therefore, γ-GT activity rising may testifies aminoacid energetic role increasing in the germinative cells in the glycolysis blockade conditions.

The sperm fertility also depends on the testosterone and prolactin spermoplasma existence. Our study showed that spermoplasma content of these hormones correlates with the spermatozoon concentration: influence time as well as toxicant dose increasing resulted in testosterone and prolactin level reducing with the oligozoospermia developing. Testosterone prevents the premature sperm acrosome reaction until the fertilization site will be reached [3]. Prolactin is necessary for normal development and functional activity survival of testicles and additional gonads. Consequently, determined testosterone and prolactin spermoplasma content decreasing may also limit gamete fertilization ability. It is necessary to emphasize that any way significant correlative interconnections between testosterone and prolactin levels in the blood serum and sperm were not discovered, that is evidently connected with their unequal role in the fertility determination mechanisms, influence to spermatozoon different metabolism links and unequal drawing degree into the pathological process.

Thus, sperm fertilization property reduction after chlororganic substance intoxication is conditioned by not only spermatozoon concentration decreasing and their motion activity limitation but also with the considerable changes in the sperm biochemical spectrum. Obviously, discovered disruptions are reflections of more common and deep changes that requires to analyse in details the masculine reproductive system on the all levels of its hierarchic organization and regulation in the strong technogenic influence on the environment conditions.

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