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LACTATIONAL EXPOSURE OF METHOXYCHLOR IMPAIRS SPERM QUALITY IN BALB/C MICE

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ABSTRACT
Numerous pesticides derived from human activity are now disseminated in the environment that have been identified as an estrogenic activity that mainly affects the male reproductive system. The objective study was to determine the dose of methoxychlor (MXC) pesticides administered to the mother during the lactation period that would affect the sperm quality of the offspring in mice. Twenty-four BALB/c strains were divided into control and treatment groups. MXC was administered once daily by intraperitoneal to female mice for days 1 to 21 of lactation periods, with 0.14 mg/g, 0.28 mg/g, and 0,42 mg/g doses of MXC. Male litters were killed at postnatal day (PND) 60, and the cauda epididymis was used to observe sperm motility and morphology characteristics. Parameters observed were the number of spermatozoa abnormal and the percentage of progressive spermatozoa. ANOVA showed that the effect of MXC was significantly different in the case of the number of abnormal sperm and the rate of progressive sperm parameters. Duncan's Multiple Range Test (DMRT) showed that D3 (0.42 mg/g) gave the highest effect of MXC on increasing abnormal spermatozoa morphology and decreasing the percentage of motile sperm. These results indicate that exposure to MXC, which showed a physiological response in the lactation period, reduces the sperm quality of offspring.

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1. INTRODUCTION

The organochlorine pesticide is used widely in agriculture to control pest insects (Sparling, 2016). One type of organochlorine group is Dichloro Diphenyl Trichloroethane (DDT), which is more in demand because it is inexpensive, easy to use, and effectively eradicates pests. The use of DDT turns out to have a detrimental impact because of its fat-soluble nature and its difficulty in decomposing (bioaccumulative), which endangers the environment and humans. DDT was then banned from being used for agriculture worldwide, and its replacement is methoxychlor because it is quickly excreted by the body and has lower toxicity than DDT (Annex, 2019).

Methoxychlor (MXC) is an organochlorine insecticide acting as an agonist on estrogen receptor (ER) α and ERβ, or nuclear transcription factors involved in regulating various complex physiological body processes. Estrogen-like endocrine disrupting chemicals (EEDC) are human-made chemicals that alter the endocrine system's functions and interfere with the synthesis, metabolism, binding, or cellular responses of natural estrogens (Gupta et al., 2010). MXC's major metabolite is 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), a more effective ERs agonist than MXC itself. Various research that MXC causes reproductive disorders. Cupp et al. (2003) said that when female rats were exposed to MXC at the age of 7 to 15 days of pregnancy intraperitoneally with doses of 50 and 150 mg/kg/day, causing a decrease in the number of spermatogenic cells per testicle. Exposure of MXC to Perinatal and juvenile rats during development reduces the overall functional spermatogenic capability of the testis in adult animals (Staub et al., 2002).

The pesticides entry into the body will be absorbed quickly from the gastrointestinal tract and and transported through the portal vein to the liver, following a detoxification pathway. In breastfeeding mothers, pesticides may pass the mammary gland tissue, specifically the glands which produce milk and comes out with breast milk during lactation. Some of the pollutants which penetrate the body of pregnant and lactating women will affect the development of behavior in offspring, hormonal disorders, and cancer. Administration of Diethylstilbestrol (DES) to female mice on the 12th day of pregnancy until the 20th day after delivery with a dose of 10 g DES/kg body

weight by gavage, causing the reduction in the number of Sertoli cells and the number of offspring spermatozoa cells (F1) on day 105 and day 315 postnatal day (Fielden et al. 2002).

Numerous studies have reported residue quantifications of methoxychlor in soil, water, sediment, and in a variety of biota. Long after discontinuation of use, MXC still has been found in human tissue samples. Thus, the health effects of MXC remain an important public health concern in humans. The researche of the effect of MXC on the reproductive organs of mice males and females during gestation and the neonatal period has been widely conducted, however research on the impact of MXC given in the lactation period is not reported yet scientifically, Therefore, we decided here to investigate the effect of giving MXC on lactation on the quality of the spermatozoa of mice (Mus musculus L.) which is an important indicator of the male reproductive system.

2. RESEARCH METHOD

Research Animal

Mice used in this study were three-month old treated female of BALB/c strain mice, approximately 25–30g in weight, and male mice F1 offspring from the treated mice, were obtained from Faculty of Medicine, University of Jember.

Experimental Unit

Twenty-four mice used in this study were divided into control and treatments groups. Each treatment group consisted of six mice. Mice were reared in different cages, according to the group.

The Treatments of Animals

Mice were acclimatized for approximately a week to adapt to the new environment. Mice were maintained in the laboratory at room temperature with wood chips. Mice were given water ad libitum throughout the study. Female mice were mated with male mice in one male for three females. As an indicator of pregnancy, a plug is found vagina after 18 hours of mating. Mother mice were kept until they gave birth to offspring for 21 days. The treatment was started to be given to the mother mice the day after the child was born for three weeks of the lactation period. The factor used is the dose of MXC. The control (P1) was given corn oil as a control; three treatment group of MXC with the following doses: 0.14 mg/g (P2), 0.28 mg/g (P3), 0.42 mg/g (P4). The administration volume of injected is 0.1 ml. Test materials are provided intraperitoneally every day for three weeks of the lactation period. After three weeks, the F1 offspring were weaned and separated between males and females. At the 8th week postnatal day, all of the mice offspring were males from the treated broodstock were taken, anesthetized with chloroform inhalation then dissected. The number of mice taken from each group is six male mice. Cauda epididymis is used for observation of motility and morphology of spermatozoa. Observation of spermatozoa motility: cauda epididymis was placed in a petri dish containing 1 ml of 0.9% NaCl. Cauda epididymis was cut until smooth and stirred then it was suspended with 0.9% NaCl to form a spermatozoa suspension. Furthermore, the suspension of spermatozoa dropped on the object's glass using a dropper pipette. Complete the glass object using a closing glass was observed using a light microscope with a magnification of 400 times spermatozoa movements and categorized the results. Observation of spermatozoa morphology: As much as 0.5 ml of spermatozoa suspension was dropped on a slide and incubated fixation with 2% formalin for 10 minutes, then allowed to dry, after stained with 1% eosin for 15 minutes, and rinsed with distilled water Observations were made under a microscope with a 400x to 100 spermatozoa magnification, and the result is in percent. The parameters observed were abnormal spermatozoa. Abnormal spermatozoa include primary and secondary abnormalities.

Experimental Design and Data Analysis

This study used a completely randomised design method that consisted of four treatments and six repetitions. The parameters observed were the number of spermatozoa abnormal and the percentage of progressive spermatozoa. Data were analysed using analyses of variance (ANOVA).

3. RESULT AND DISCUSSION

Based on observation motile spermatozoa of mice. The results showed differences in the number of spermatozoa motility. The number of spermatozoa motility fluctuated in each treatment compared to control. The highest number of spermatozoa motility was 77,16 %, and the lowest number was 43,16 %. Based on the ANOVA test with a significance level of 5% for the average percentage of spermatozoa, the P value (0.000) < 0.05. This indicates that the administration of MXC has a significant effect on the average percentage of motile spermatozoa. The fluctuation of number of spermatozoa motility demonstrated that MXC can decreasing motile spermatozoa. The fluctuation of the percentage number of motile spermatozoa can be seen in Figure 1.

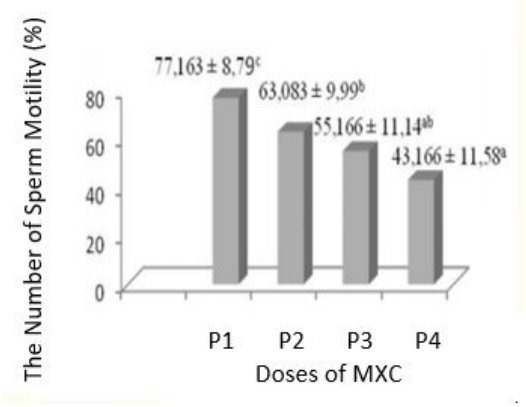

Figure 1
Mean of the number of sperm motility after 3 weeks of MXC injections with the following doses: Corn Oil (P1), 0.14 mg/g (P2), 0.28 mg/g (P3), 0.42 mg/g (P4)

Based on observation morphology of spermatozoa of mice. The results showed differences in the number of morphologies of abnormal spermatozoa. The number of abnormal spermatozoa morphology fluctuated in each treatment compared to control. The highest number of abnormal spermatozoa morphology was 53,60 %, and the lowest number was 15,01 %. The fluctuation of number of abnormal spermatozoa morphology demonstrated that MXC can increasing the number of abnormal spermatozoa morphology. Based on the ANOVA test with a significance level of 5% for the average percentage of abnormal spermatozoa morphology, the P value (0.000) <0.05. These results indicate that MXC has a significant effect on the abnormal morphology of spermatozoa. The fluctuation of the percentage number of abnormal spermatozoa morphology can be seen in Figure 2.

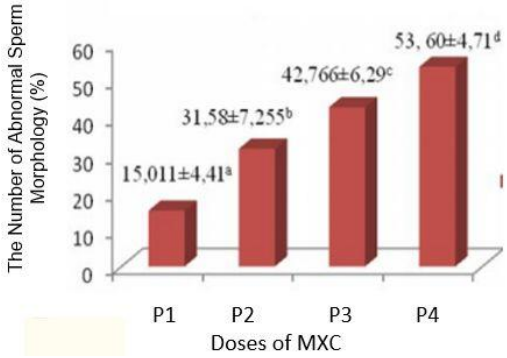

Figure 2
Mean of the number of morphologies of abnormal sperm after 3 weeks of MXC injections with the following doses: Corn Oil (P1), 0.14 mg/g (P2), 0.28 mg/g (P3), 0.42 mg/g (P4).

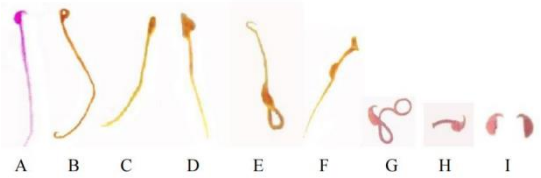

Figure 3
Figure of primary (B and C) and secondary (D-I) abnormalities of mice spermatozoa after administration of MXC in the lactation period. (A) normal spermatozoa; (B) round head spermatozoa; (C) flat head spermatozoa; (D) head spermatozoa ruptured, (E) spermatozoa neck curled, (F) spermatozoa neck fractured, (G) spermatozoa neck and tail curled (H) spermatozoa tail fracture; (I) headless spermatozoa.

The observation of the quality of spermatozoa showed that the administration of MXC during the lactation period decreased the percentage of motility and increased spermatozoa abnormalities in mice. Reduction in sperm quality and count which may be the result be due to the presence of MXC metabolites are transferred from mother to offspring via breast milk. According to Kunin and Beckerman (2022), chemical substances entering the peritoneal cavity will pass through the peritoneal wall by passive diffusion into capillary blood vessels. The blood passes through the hepatic portal vein to the liver from the capillaries for detoxification. The toxic chemicals through detoxification in the liver called biotransformation, which will convert these substances into metabolite

products. Some of the metabolites will be excreted, and some will be excreted enter the bloodstream (Yunita et al. 2007). MXC (HPTE) metabolites in the blood pass through the hepatic vein to the inferior vena cava, which then through the heart and is circulated throughout the body, including the mammary glands. Breast milk is channeled from the alveolus to the ductal system and then flows through the lactiferous ducts into the baby's mouth (Lin et al. 2009). MXC metabolites could be transferred from lactating mothers to newborns via breast milk. Methoxychlor is detected in human breast milk, and its metabolites may cross the placenta.

Along with mechanical dysfunctions, decreased sperm quality due to MXC metabolites promoted both directly and indirectly. Direct influence can occur by MXC metabolites induces oxidative stress in the epididymis and epididymal sperm by decreasing antioxidants. The free radical-induced oxidative stress contributes significantly to producing and increasing abnormal sperm and decreasing the number of sperm and fragmenting and transforming sperm DNA. These changes in sperm DNA result in infertility. Indirect effects can occur hormonally through inhibition of Leydig cell function. According to Phaniendra et al (2015), free radicals are molecules containing unpaired electrons, which cause unstable atoms that can damage cells. Latchoumycandane & Mathur (2002) stated that oral administration of MXC in male rats at a dose of 100 mg/kg day for 45 days could induce oxidative stress caused by increased hydrogen peroxide (H2O2) lipid peroxidase in the testes.

The spermatozoa membrane is the major target of ROS and lipids are potential targets. Spermatozoa plasma membrane lipids have high levels of phospholipids, which makes spermatozoa very susceptible to ROS (Chianese and Pierantoni., 2021). High levels of ROS too can oxidize lipids, proteins, and DNA. Lipid oxidation in spermatozoa membranes produces malondialdehyde (MDA) compounds, which are toxic to cells, causing damage to spermatozoa membranes. The damage to the plasma membrane of spermatozoa disrupts cell metabolism, thereby increasing spermatozoa abnormalities. The spermatozoa membrane damage could decrease sperm motility.

The motility of spermatozoa decreased with increasing doses In this study. Spermatozoa motility is expected if the percentage of motile spermatozoa in the category (2+3) is 50% (Ogli et al., 2009). The average percentage of spermatozoa motility in the control group, MXC dose 0.14; and 0.28 included in the normal category because the value was above 50%, while the MXC dose was 0.42 mg/g has an average percentage of 43.16%, which means below the standard percentage. The decrease in spermatozoa motility was thought to be due to free radicals inhibiting the process of oxidative phosphorylation. Oxidative stress is caused by increased ROS production (reactive oxygen species), which causes disturbances in the oxidative phosphorylation process in spermatozoa (Wardani et al. 2012). Oxidative phosphorylation is an energy formation process that involves an enzyme complex found in the inner mitochondrial membrane. Spermatozoa mitochondria are located in the middle of the spermatozoa, while the neck and tail function in the movement of spermatozoa. After being synthesized in the mitochondria, ATP is transported to the axoneme at the tail of the spermatozoa, then converted by dynein in the axoneme, which will decompose ATP into energy for the movement of spermatozoa. The inhibition of the release of ATP to the axoneme results in unfulfilled or reduced energy requirements to move the tail, further resulting in spermatozoa being unable to move quickly or not moving at all (Astuti et al., 2009).

The indirect effect of giving MXC on quality spermatozoa can occur hormonally through inhibition of Leydig cell function. MXC as an endocrine disruptor has an estrogenic activity to provide negative feedback to the hypothalamic pituitary axis. MXC metabolites will bind to the estrogen receptor causing inhibition of GnRH synthesis. Decreased GnRH synthesis causes a decrease in FSH and LH secretion (Rochira et al., 2006). Reduced levels of LH cause interference with Leydig cells to produce testosterone.

Testosterone and FSH are synergistically required normally for the process of spermatogenesis. If the secretion of testosterone and FSH is inhibited, spermatogenesis is also disrupted, resulting in an increase in primary abnormalities in spermatozoa. Inhibition of testosterone secretion also causes impaired maturation of spermatozoa in the epididymis. Spermatozoa maturation is one of the endogenous factors that affect spermatozoa motility (Astuti et al. 2009), so disturbances in this process can reduce spermatozoa motility and increase secondary abnormalities in spermatozoa.

The rate forwarding movement and motility of spermatozoa are closely related to the morphological conditions. If the morphology of the spermatozoa is abnormal, the movement of the spermatozoa will be disturbed. The motility of spermatozoa would take place well if the sperm support by its morphology. Setyadi (2006) states that spermatozoa are considered normal if less than 30% of abnormal forms are found. The observations showed that the average percentage of spermatozoa dose was 0.14; 0.28; and 0.42, including abnormal because the value is above 30%. The administration of MXC significantly affected the spermatozoa abnormalities of mice. The percentage of spermatozoa abnormalities increased with the higher dose of MXC given (Figure 2), and between doses of MXC treatment showed a significant difference.

Spermatozoa with normal morphology are have 'hook-shaped head with standard size, long tail not circular or double. The results of the observation of the morphology of spermatozoa showed that there were primary abnormalities in the morphology of the spermatozoa of mice, such as round heads and flat heads. The secondary abnormalities found were broken head, neck curled, broken neck, curled tail, broken tail, head without a tail (Figure 3).

Primary spermatozoa abnormalities due to disruption of the process of Spermatogenesis occurs in the seminiferous tubules of the testes, including the big head, small head, round head, short head, elongated flat head, head double, and double tail. Secondary abnormalities are morphological abnormalities in spermatozoa that occur during the maturation process of spermatozoa in the epididymis, characterized by the severed tail, split head, and head without a tail.

The results of the observation of spermatozoa abnormalities showed that the percentage of primary abnormal morphology is higher than the percentage of abnormalities secondary, which means that the influence of MXC on spermatozoa morphology is more significant when the spermatozoa are in the testes than in the epididymis. In the neonatal period, the testes have not yet formed spermatozoa.

There is a spermatogonium as future spermatozoa, while Spermatogenesis only begins at puberty. In this study, metabolite MXC is thought to affect germ cells in new-born male mice (neonatal) so that it affects the quality of spermatozoa at puberty or the possibility of MXC metabolites transferred from mother to offspring through breast milk to settle in the child's body and affect the quality of spermatozoa as adults. Rats given MXC at a dose of 50 mg/kg/day had normal fertility, but their offspring had an abnormal reproductive function.

4. CONCLUSION

Based on the study, MXC caused decreasing motily and increasing abnormality of morphology sperm in mice. The dose of MXC that caused the highest number of abnormalities of morphology sperm and decreasing number of motilities was P4 (0,42 mg/g). Reduction in sperm quality and count which may be the result be due to the presence of MXC metabolites transferred from mother to offspring via breast milk. Decreased sperm quality due to MXC metabolites promoted both directly and indirectly. Direct influence can occur by MXC metabolites inducing oxidative stress in the epididymis. The free radical-induced oxidative stress contributes significantly to producing and increasing abnormal sperm, while indirect effects can occur hormonally through inhibition of Leydig cell function.

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6. REFERENCES

Annex E. 2019. Information (Risk Profile) on methoxychlor. Submission of information from Parties and observers as specified in Annex E to the Stockholm Convention pursuant to Article 8 of the Convention.

Astuti, S., Muchtadi, D., Astawan, M., Purwantara, B. dan Wresdiyati, T. 2009. Kualitas Spermatozoa Tikus yang diberi Tepung Kedelai Kaya Isoflavon, Seng (Zn) dan Vitamin E. Media Peternakan, 32(1): 12-31.

Chianese R and Pierantoni R. 2021. Mitochondrial Reactive Oxygen Species (ROS) Production Alters Sperm Quality. Antioxidants (Basel). 10 (1): 92.

Latchoumycandane, C., dan Mathur, P. P. 2002. Effect of Methoxychlor on the Antioxidant System in Mitochondrial and Microsome-Rich Fractions of Rat Testis. Toxicolog, 176 (1-2): 67-75.

Lin, H., Lian, Q.Q., Xin Hu., Jin, Y., Zhang, Y., Hardy, D.O., Chen, G.R., Qiu Lu, Z., Sottas, C.M., Hardy, M.P., dan Shan Ge, R. 2009. In Utero and Lactational Exposures to Diethylhexyl-Phthalate Affect Two Populations of Leydig Cells in Male Long-Evans Rats. Biology of Reproduction, 80 (5): 882-888.

Ogli, O., Enyikwola dan Odeh S. O. 2009. Evaluation of the Efficacy of Separate Oral Supplements Compared with the Combined Oral Supplements of Vitamins C and E On Sperm Motility in Wistar Rats. Nigerian Journal of physiological sciences, 24 (2): 129-135.

Phaniendra, A., Jestadi, DB.,and Periyasamy, L. 2015. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem, 30 (1): 11–26.

Rochira, V., Zirilli, L., Genazzani, A. D., Balestrieri, A., Aranda, C., Fabre, B., Antunez, P., Diazzi, C., Carani, C., dan Maffe, L. 2006. Hypothalamic– Pituitary- Gonadal Axis in Two Men with Aromatase Deficiency: Evidence that Circulating Estrogens are at the Hypothalamic Level for the Integrity of Gonadotropin Negative Feedback. European Journal of Endocrinology,155: 513–522.

Setyadi, A. 2006. Organ Reproduksi dan Kualitas Sperma Mencit (Mus musculus L.) yang Mendapat Pakan Tambahan Kemangi (Ocimum basilicum) Segar. Skripsi.

Staub, C., Johnson, L., Chou, K., and Zacharewski, T.R. 2002. Gestational an Lactation Exposur of Male Mice to Diethylstilbestrol Causes Long-Term Effects on the testis, Sperm Fertilizing Ability in Vitro, and Testicular Gene Expression. Endocrinology, 143(8): 3044-3059.

Wardani, E.T., Hayati, A., dan Pidada, R. 2012. Pengaruh Ekstrak Jahe (Zingiber officinale Rosc.) Var. Gajah terhadap Jumlah dan Viabilitas Spermatozoa Mencit (Mus musculus L.) yang Terpapar 2-Methoxyethanol. Surabaya: Universitas Airlangga.

Yunita, A., Fatmawati, U. dan Budi, Y.T. 2007. Absorpsi, Distribusi, Metabolisme dan Ekskresi Toksikan. Surakarta: Universitas Sebelas Maret.