International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-1, 2019, pp67-74
http://www.bipublication.com

Research Article

Examining the effect of Endosulfan organ chlorine venom on serum levels of sexual hormones and ovarian follicles in female rats

Banafsheh Manouchehr, Reza Ghazanfari, Niloufar Pirdadeh Khani, Ghasem Simorgh and Saeed Salahi

Ischemic Disorder Research Center,
Golestan University of Medical Sciences,Gorgan, Iran

[Received: 29/11/2018; Accepted: 05/01/2019; Published: 07/01/2019]

ABSTRACT

Background and Objectives: One of the most effective insecticides and organ chlorine proteins used in agriculture is endosulfan that its use has most effect on insect control. This poison is absorbed in humans and animals through oral, inhalation and dermal. Endosulfan organ chlorine venom is widely used in plants. This study was carried out to evaluate the effect of endosulfan-anhydrous organ chlorine on serum levels of sex hormones and ovarian tissue changes in female rats.

Materials and Methods: In this study, 40 female vistar rats were randomly divided into four groups, experimental group 1 and 2, which received Endosulfan organ chlorine with 5 and 10 mg / kg body weight for 4 weeks, respectively, intra peritoneal. The sham group received olive oil and the control group did not receive any injections. The rats were anesthetized in the proestrus stage and blood was collected from the heart and serum sex hormones were measured by radioimmunoassay. In addition, the ovary was removed and tissue sections were prepared. Data were analyzed by analysis of variance and Duncan test.

Results: The results showed that the levels of estrogen and progesterone hormones, number of corpuscles, number of primary follicles, primary, growing, gravy follicle and acolyte diameter and yellow body, granulose thickness, and repeat thickness in experimental groups were significantly reduced compared to the control group ($P < 0.001$). **Conclusion:** The results show that Endosulfan organ chlorine changes ovarian tissue and decreases estrogen and progesterone hormones and can have adverse effects on oogenesis.

Keywords: Endosulfan Organ chlorine, Ovary, Acolyte, Grapevine Follicle, Female Rat

INTRODUCTION

Over the past fifty years, pesticides have become an essential part of the agricultural world. Although demand for the production and distribution of pesticides to improve the quality and efficiency of agriculture is established, the likelihood of their misuse and unreasonable use is very high. Increasing population and consequently increasing food consumption, especially agricultural products, has led farmers to increase their products. Increasing product cropping has led to an increase in pesticide pesticides. Due to farmers' ignorance of pesticide use, atmospheric leaks, and other factors, agricultural pesticides enter the water of rivers and seas. In this regard, the increased awareness of experts and the general public about the dangers of short-term and long-term contact, including carcinogenesis, diseases of the respiratory system and regeneration, and attracted public attention and policy makers. Today, environmental pollution has become a global issue. Contact with insecticides is considered as a health problem in most urban and rural areas (1).

Considering the fact that every year the spring crops and in recent years the cranberries (watermelons, tomatoes and cucumbers ...) are cultivated in various regions of Iran at a large scale, and everyone in any way try to produce in this short period more crops in land, so they use chemical fertilizers and various chemical pesticides widely (1).

The physical and chemical properties of organic chlorinated toxins and their metabolites make these compounds easy to enter the body. High solubility in the fat and low solubility in water of these compounds leads to their accumulation in fatty tissue. The amount of accumulation in organisms varies by type, duration and concentration of contact in environmental conditions (2).

Toxins of cyclodines (such as endosulfan) affect the receptors, which act on the nerve membrane as 1 GABA as the chlorine ion channel. This kind of toxin is attached to the GABA receptor and reduces the flow of chlorine ions. Indicator marker in this type of poisoning is seizure (3).

Soto et al. showed that endosulfan has estrogenic properties. Endosulfan binds estradiol to binding to estrogen receptors and thereby prevents the proper functioning of hormones (4). Endosulfan affects the female reproductive system and delays sexual maturation and interferes with the synthesis of sex hormones (5).

Jamil et al. (2004) stated that endosulfan has the ability to alter the genetic material specifically for chromosomes in cell culture media (5).

Experimental evidence of side effects of endosulfan on the male reproductive system indicates a delay in sexual maturation and interference with synthesis has sex hormones (6).

Endosulfan is thought to be a disturbing balance of the endocrine system (4).

Endosulfan competes with estradiol to bind estrogen receptors and may stop hormonal activity (7). The estrogenic power of endosulfan increases in the presence of other estrogenic organchlorines (8). Endosulfan also affects the reproductive system and affects sperm mobility; sperm count, spermatogonium cells, and also affects male sex hormones (9). One of the organs that can be affected by the endosulfan pesticides is the ovary, the ovary with the synthesis of hormones and the production of oocytes plays an important role in the reproductive performance of humans and animals. There are controversial reports of the effects of these toxins on ovarian tissue. Some Researchers believe that endosulfan does not change the number of follicle graph in the ovary (12), while others believe that endosulfan increases the number of graph follicles (13). Of course, these complications are not related to the sex of the female, but in the male testicle diameter, sperm tubes reduce the number of spermatogenic cells, Leadig and sertoli, and, by decreasing the amount of testosterone and increasing the levels of LH and FSH in the gonadotrophin hormones, impair the reproductive system in rats (14). According to the World Health Organization (WHO) to increase the infertility rate due to the sensitive structure of reproductive tissues, including theovaries to external factors, such as agricultural toxins, especially endosulfan, and the high consumption of this toxin and high static and on the other, little study in this regard in Iran, the aim of the present study was to investigate the effects of endosulfan on ovarian tissue and hormones of gonadotropin, and estrogen and progesterone hormones in male rats.

MATERIALS AND METHODS

Animals

In this experimental study, 40 male vistar rats with an average age of 12-14 weeks and a weight of approximately 250± 15 were obtained from Tehran University. The animals were kept in the animal room of the Tehran University of Medical Sciences for one week prior to testing. In order to feed the animals, they used compressed food prepared from Pars Animal Feed Company. Animals were polycarbonate transparent in 23±2 ° C, relative humidity of 55-50% and light period (12 hours of light and 12 hours of darkness). In line with this research, all the points related to working with animals were observed.

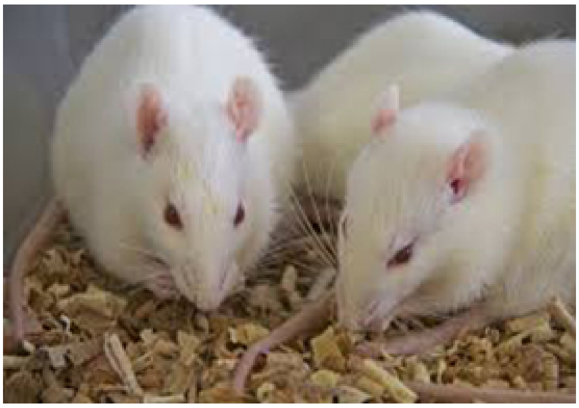

Fig 1. Vistar female rats

Study design

In this study, endosulfanpoison was prepared from Raja Chemistry Company. The endosulfan solution was then injected to the animals at the desired concentrations in olive oil and injected daily for one hour. Animals were randomly divided into four equal groups (n = 10) control, sham and experimental groups 1 and 2. Rats in experimental groups 1 and 2 received doses of 5 and 10 mg / kg (15) of the endosulfan venom for four weeks (five days a week and two days rest) and injected intraperitoneally. Shem group received 1 µl of lubricant (intravenous) for intraperitoneal injection. Control group animals did not receive any medication. After the injection period, the rats were anesthetized in the proestrus cycle with ether and blood samples were taken from their hearts and then the ovary was removed from the body for histological studies. Blood samples were collected at 3000 rpm for 15 minutes and centrifuged and serum removed. Then, using the radioimmunoassay method (RIA), the levels of estrogen, progesterone, LH and FSH were measured.

Textural study

After blood sampling, the rats were dissected and the ovaries were removed from the rat's body and after washing and drying and weighing they were placed in a 10% formalin solution. For consistency in the study, their right ovaries were used. Then routine tissue preparation steps including dehydration, de-desalination, paraffin penetration and molding were done and stained paraffin blocks, 5 micron thick sections of the tissue and serially stained with hematoxylin-eosin. Cutting was designed to evaluate about 80 sections of each part of the ovary tissue for each group. The diameter of the yellow body, the size of the oocyte diameter, the thickness of granulose layers, and the velocity of the granulose layers in the follicle graph were measured by the graticule and counting the number of corpus luteum cells done by using lattice graticule. The number of growing primary follicles was measured using an ocular lens with a 40x magnitude (13).

Data analysis

Data were recorded as SE ± Mean and analyzed by SPSS software version 16 and one way ANOVA (Way-OneANOVA) and Duncan's supplementary test were analyzed too. Differences in level ($p < 0.05$).

RESULTS

The examination showed that ovarian weight (Table 1), LH and FSH levels (Table 2) in experimental groups 1 and 2 were not significantly different from the control group ($p < 0.05$). The results of this study showed that the levels of estrogen and progesterone hormones (Table 2), number of corpuscles, number of primary follicles, growth, gravic follicle (Table 1) and oocyte diameter and yellow body (Table 3) in experimental groups 1 and 2 showed a significant decrease compared to control group ($P = 0.001$), but the number of atretic follicles was significantly higher than control group ($p = 0.001$, Table 1). The granulose and rectangular thickness in the experimental groups showed a significant decrease compared to the control group (P = 0.001; Table 2).

Table 1. Average ovarian weight and number of different follicles in experimental groups compared to controls

Parameter/group	Control 1	Witness	Experimental 1 (5mg/kg)	Experimental 2 (10mg/kg)
Ovary weight (g)	0/0287 ± 0/0039[a]	0/0287 ± 0/004[a]	0/028 ± 0/0035[a]	0/0278 ± 0/0041[a]
Secondary follicle number	16/65 ± 1/56[a]	15/8 ± 1/74[a]	10/63 ± 1/39[b]	7/32 ± 1/83[c]
Primary follicle number	7/58 ± 0/95[a]	7/32 ± 1/05[a]	6/33 ± 0/78[b]	5/62 ± 0/91[b]
Growing follicle number	6/23 ± 0/75[a]	6/12 ± 0/63[a]	4/28± 0/68[b]	4/27 ± 0/74[b]
Graph follicle number	2/39 ± 0/34[a]	2/25 ± 0/29[a]	1/65 ± 0/42[b]	0/79 ± 0/21[c]
Atretic follicle number	0/84 ± 0/1[c]	0/89 ± 0/15[c]	1/54 ±0/12[b]	2/23 ± 0/15[a]
Yellow body number	4/24 ± 0/51[a]	4/13 ± 0/48[a]	2/16± 0/33[b]	1.61 ± 0/37[b]

Table 2. the average of sexual hormones in experimental groups compared to control

Parameter/group	Control	Shem	Experimental 1 (5mg/kg)	Experimental 2 (10mg/kg)
Estrogen(IU/L)	45/12 ± 0/92[a]	43/375 ± 0/87[a]	34/3 ± 0/9[b]	32/43 ± 0/92[b]
Progesterone(IU/L)	28/47± 0/9[a]	27/76 ± 0/83[a]	16/2 ± 0/85[b]	12/65 ± 0/9[c]
(Pg/ml)LH	0/33 ± 0/02[a]	0/31 ± 0/022[a]	0/29 ± 0/02[a]	0/27 ± 0/021[a]
(Pg/ml) FSH	0/28± 0/008[a]	0/275 ± 0/006[a]	0/269 ± 0/008[a]	0/27 ± 0/007[a]

Table 3. the average of ooyste diameter and yellow body and the thickness of granulose and teka in experimental groups compared to control

Parameter/group	Control	Shem	Experimental 1 (5mg/kg)	Experimental 2 (10mg/kg)
ooyste diameter (micron)	65/2 ± 2/36[a]	63/85 ± 2/85[a]	51/36 ± 2/32[b]	46/15 ± 3/16[c]
yellow body diameter (micron)	483/64 ± 8/35[a]	478/32 ± 10/43[a]	448/25± 8/63[b]	432/17 ± 8/78[c]
Granova thickness (micron)	48/75 ± 1/32[a]	46/58 ± 1/25[a]	31/43 ± 1/28[b]	25/35 ± 0/95[c]
Teka thickness (micron)	21/34 ± 0/85[a]	20/87 ± 0/93[a]	17/35 ± 0/75[b]	15/69 ± 0/79[b]

DISCUSSION AND CONCLUSION

The results of this study showed that endosulfan intraperitoneal injection decreased the number and diameter of corpuscles, granular thickness, rhosa, number of primitive, primitive, growing and gravicular follicles and oocyte diameter in experimental groups compared to control group. Serum estrogen and progesterone hormones also decreased significantly, but there was no significant change in the level of LH and FSH hormones. In addition, the number of atritic follicles has increased in experimental groups. Reducing these parameters in the experimental group than the control group can be considered as the role of endosulfan in response to cellular macromolecules, such as nucleic acids, and inhibition of DNA replication. Therefore, assuming that this conclusion is achieved, the mitosis is inhibited and the natural process of replacement of the reproductive cells is not done or delayed (8 and 14). Some studies have shown that organ chlorine has an alkylating and electrophilic property that can affect nucleic acids and cell proteins, thereby altering cell function and inducing cell death (16, 17). The results of this study are a reduction in the number of primary, growing, and gravicular follicles. Research shows that Organ chlorine toxins produce free radicals and reactive oxygen species, and increase the peroxidation of lipids, leading to the destruction of cell membranes and cell death (18). Reductions in the number of ovarian follicles appear to be due to either the direct effect of endosulfan and the metabolites resulting from the toxin on the growth and development of these follicles, or the effect on hormones and their production impairment, reducing the number of follicles (to Except the follicle of the atritic). It should be noted that the entry of endosulfan into the tissues of the body can produce reactive oxygen and oxidize the lipids and destroy the cell membrane. In total, these factors can reduce the number and diameter of the yellow body (18 and 19). Johari et al. (12) reported that diazinon at dosages of 50, 100 and 150 mg / kg for 14 days did not significantly alter the number of primary, secondary and gravicular follicles, which differed from the results of this study, and in this study we used endosulfan venom intraperitoneally and may also affect the duration of injection, which lasted four weeks. It should be noted that the complications caused by the toxins depend on the dose, duration, and route of administration (6). The Dutta and Maxwell studies show that endosulfan increases the number of follicular follicles, inter-follicular space, necrosis and vacuolated cytoplasm, and structural and tissue changes in the ovary (16). MohseniKouchesfahani et al. Reported that intravenous injection of 40 mg / kg doses of diazinon decreases primary and growing follicles and increases the number of graph follicles (13); which is consistent with the results of our study on the number of follicles different ovaries (except follicle graph), number and diameter of the corpus luteum, number of atitir and oocyte diameter. As we used the endosulfan instead of diazinon in this study, in addition to decreasing the diameter of the yellow body, its number declined, which may be indicative of degeneration. Since the number of yellow bodies is directly related to the number of oocyte released, its reduction will reduce the number of oocytes. It was also found that the number of granulose and teka cells in the experimental group receiving endosulfan decreased significantly. A decrease in the number of these cells may be related to the production of free radicals and various reactive oxygen species, increased lipid peroxidation, and ultimately induction of endosulfan cell death. Studies have also shown that endosulfan can react with cellular macromolecules, such as proteins and nucleic acids, and inhibit DNA replication and stop the mitosis, which may lead to a reduction in granulose cells and rheumatoid arthritis. Another result of this study is the reduction of serum estrogen levels. Maxwell and Dutta reported that diazinon at a dose of 60 µg / L up to 1 week would reduce estrogen (20), which is consistent with the results of the present study. Granulose and teka cells produce estrogen with each other. Therefore, after reducing the granulose and teka cells that are responsible for secretion of the estrogen hormone in the female, it is expected that the amount of this hormone will also decrease in serum. In fact, with the reduction of estrogen levels, the first issue is the changes that have occurred in granulose cells. It seems that these cells should either be degenerated or that they have lost the ability to secrete estrogen due to atrophy. Although in our study, either endosulfan can be cited, but it seems that decreasing the number of cells further suggests that granulose cells are caused by prolonged endosulfan injections, degenerated and removed from the environment. The chemical compounds present in the environment can degrade the endocrine system and may inhibit their activity (21). Regarding the role of estrogen in the growth and development of follicles (22), the decrease in the amount of this hormone in the experimental group receiving endosulfan decreases the growth and development of follicles and may also have a negative effect on ovulation. After ovulation, the remaining granulose cells in the follicle wall are torn and the single-cell cells turn into luteal cells, and these cells form the yellow body and secrete progesterone. Reducing the number of yellow body and their diameter in this study can be due to the reduction of granulose cells in the follicles. Due to the reduction in the number of yellow body, progesterone secretion is expected to decrease, which also indicates the results of this study. Also, there was no significant change in the level of LH and FSH hormones in this study. Gonadotropins secreted from the pituitary and estrogen and progesterone hormones produced from the ovary, with positive and negative feedback can affect the level of gonadotropins secretion in the estrus cycle. Studies show that endosulfan has no significant effect on gonadotropins (12, 13), which is consistent with the results of this study. Considering that in this study the amount of gonadotropins decreased, but it was not statistically significant, it may be possible to increase the level of these hormones by increasing the dose and duration of exposure to poison, which requires more studies. The results of this study indicate that endosulfan decreases the number of cells by histologic changes. It seems that endosulfan has two pathways on the ovary and its function. It has a direct effect on the ovarian tissue and induces oxidative stress and decreases ovarian cells and follicles and ultimately reduces ovulation and in the other way affects Endocrine system and imbalance in the secretion of sex hormones cause disturbances in the reproductive system. The destructive effects of endosulfan in this study are dose dependent. According to the results of this study, it is suggested that the use of this poison be monitored and managed properly by the Plant Protection Organization and the Ministry of Health in order to reduce its adverse effects on different tissues.

REFERENCES

1- Oliveira-Silva JJ, Alves SR, Meyer A, Perez F,Sarcinelli PN, da Costa Mattos RC, et al. [Influenceof socioeconomic factors on the pesticides poisoning,Brazil]. Rev SaudePublica. 2001;35(2):130-5.Portuguese.

2- Hela DG, Lambropoulou DA, Konstantinou IK, AlbanisTA. Environmental monitoring and ecologicalrisk assessment for pesticide contamination andeffects in Lake Pamvotis, northwestern Greece.Environ Toxicol Chem. 2005;24(6):1548-56.

3- Betancourt M, Reséndiz A, Fierro EC. Effect of twoinsecticides and two herbicides on the porcine spermmotility patterns using computer-assisted semenanalysis (CASA) in vitro. ReprodToxicol. 2006;22(3):508-12.

4- Soto AM, Sonnenschein C, Chung KL, FernandezMF, Olea N, Serrano FO. The E-SCREEN assay asa tool to identify estrogens: an update on estrogenicenvironmental pollutants. Environ Health Perspect.1995;103 Suppl 7:113-22.

5- Jamil K, Shaik AP, Mahboob M, Krishna D. Effectof organophosphorus anorganochlorine pesticides(monochrotophos, chlorpyriphos, dimethoate, andendosulfan) on human lymphocytes in-vitro. DrugChemToxicol. 2004;27(2):133-44.

6- Sinha N, Adhikari N, K Saxena D. Effect of endosulfanduring fetal gonadal differentiation on spermatogenesisin rats. Environ ToxicolPharmacol. 2001;10(1-2):29-32.

7- Grünfeld HT, Bonefeld-Jorgensen EC. Effect of invitro estrogenic pesticides on human oestrogen receptoralpha and beta mRNA levels. Toxicol Lett.2004;151(3):467-80.

8- Walsh LP, Webster DR, Stocco DM. Dimethoate inhibitssteroidogenesis by disrupting transcription ofthe steroidogenic acute regulatory (StAR) gene. JEndocrinol. 2000;167(2):253-63.

9- Vittozzi L, Fabrizi L, Di Consiglio E, Testai E.Mechanistic aspects oforganophosphorothionatetoxicity in fish and humans. Environ Int. 2001;26(3):125-9.

10- Shah MD, Iqbal M. Diazinon-induced oxidative stress and renal dysfunction in rats. Food ChemToxicol. 2010;48(12):3345-53.

11- Anbarkeh FR, Nikravesh MR, Jalali M, Sadeghnia HR, Sargazi Z, Mohammdzadeh L. Single dose effect of diazinon on biochemical parameters in testis tissue of adult rats and the protective effectofvitE. IJRM. 2014;12(11):731-6.

12- Fattahi E, Parivar K, Jorsaraei SGA, Moghadamnia AA. The effect of diazinon in leydig cells and sexual hormones in mice. BabolUni Med Sci J. 2007;9 (4): 15- 22. [In Persian]

13- Fattahi E, Jorsaraei SGA, Parivar K, Moghaddamnia AA. The Effects of a Single Dosage of Diazinon and Hinosan on the Structure of Testis Tissue and Sexual Hormones in Mice. Yakhteh Med J. 2010; 12(3): 405- 410. [In Persian]

14- Yilmaz N, Yilmaz M, Altuntas I. Diazinon induced brain toxicity and protection by vitamins E plus C. ToxicolInd Health. 2011;28(1):51-7.

15- MalijiGh, JorsaraeiSGh, Zabihi E, Fattahi E, Rezaie E, SohanFaraji A. Diazinon alters sex hormones, Interferon-gamma, Interleukin-4 and 10 in male Wistar rats. GorganUni Med Sci J. 2014;16:22-28[In Persian].

16- Fattahi E, Jorsaraei SGA, Parivar K, Moghadamnia AA. Influence of diazinon on spermatogenesis in mice. Semnan Uni Med Sci J. 2007; 9(1): 75-82. [In Persian]

17- Sargazi Z, Nikravesh MR, Jalali M, Sadeghnia HR, RahimiAnbarkeh F. Apoptotic Effect of Organophosphorus Insecticide Diazinon on Rat Ovary and Protective Effect of Vitamin E. IJT. 2016;10(2): 37- 44.

18- Slotkin TA, Tate CA, Ryde IT, Levin ED, Seidler FJ. Organophosphate insecticides target the serotonergic system in developing rat brain regions: disparate effects of diazinon and parathion at doses spanning the threshold for cholinesterase inhibition. Environ Health Perspect. 2006; 114(10): 1542-6.

19- Pesando D, Huitorel P, Dolcini V, Angelini C, Guidetti P, Falugi C. Biological targets of neurotoxic pesticides analysed by alteration of developmental events in the Mediterranean sea urchin, Paracentrotuslividus. Mar Environ Res. 2003; 55(1): 39-57

20- Newcomb RD, Gleeson DM, Yong CG, Russell RJ, Oakeshott JG. Multiple mutations and gene duplications conferring organophosphorus insecticide resistance have been selected at the Rop-1 locus of the sheep blowfly, Luciliacuprina. J MolEvol. 2005;60(2):207-20.

21- Johari H, Shariati M, Abbasi S, Sharifi E, AskariHM.The effects of diazinon on pituitary–gonad axis and ovarian histological changes in rats. IJRM. 2010;8(3): 125-30.

22- Mohsenikouchesfahani H, Parivar K, Tahamtani Y. Effect of diazinon as a pesticide on oogenesis and ovary structure of Balb/C mice st

23- - Fattahi E, Parivar K, Jorsaraei SGA, Moghadamnia AA. The effects of diazinon on testosterone, FSH and LH levels and testicular tissue in mice. IJRM. 2009; 7(2): 59-64.

24- Al-Zubaidy MHI, Mohammad FK. Metoclopramide protection of diazinon-induced toxicosis in chickens.J Vet Sci. 2007; 8(3): 249–54.

25- Maxwell LB, Dutta HM. Diazinon-induced endocrine disruption in bluegill sunfish, Lepomismacrochirus. Ecotoxicology and environmental safety. 2003; 60(1):21-7.

26- Sokkar SM, Mohammed MA, SolimanFA.Toxic effects of diazinon on the gonads of fowls. ZentralblVeterinarmed A. 1975;22(7):557-63.

27- Shah MD, Iqbal M. Diazinon-induced oxidative stress and renal dysfunction in rats. Food ChemToxicol. 2010;48(12):3345-53.

28- Richards Js. Hormonal control of gene expression in the ovary. Endocr Rev. 1994; 15(6):725-51

29- Dutta HM, Maxwell LB. Histological examination of sublethal effects of diazinon on ovary of bluegill, Lepomismacrochirus. Environ Pollut. 2003;121(1):95- 102.

30- Kelce WR, Gray LE, Wilson EM. Antiandrogens as environmental endocrine disruptors. ReprodFertil. 1998; 10(1):105-11.

31- Fattahi E, Jorsaraei SGA, Gardaneh M, TahmasbpourMarzony E. The Effect of 8-Methoxypsoralen on Pituitary-Gonad Axis and Ovarian Function in Mice. Cell J. 2013;15(3): 206-11.