

Effect Of Polyvinyl Chloride On Activin Hormone In Mice

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ABSTRACT

The study was conducted to find out the effect of polyvinyl chloride (PVC) on the concentration of the hormone activin, which is important in many reproductive processes in the body. Twenty-seven female mice were dosed after making sure that pregnancy had occurred with PVC throughout the pregnancy period and then the breastfeeding period. The pregnant females were divided into three groups, the first was the control group, the second group was treated with PVC material at a concentration of 50 mg/kg b.w.; and the third group was treated with the same substance at a concentration of 100 mg/kg b.w., blood samples were taken from sixty newborns of both sexes after puberty, and the results showed a significant increase in the concentration of the hormone activin in the groups of females for both concentrations of PVC compared to the control group, while the concentration of activin decreased significantly in males for both concentrations of PVC compared to the control group. The results of this decrease were effective in decreasing the weight of the testes and the number of sperm, which decreased compared to the control group.

Key words : Poly vinyl chloride , activin , testes weight , sperm count

الخلاصة

أجريت الدراسة لمعرفة تأثير البولي فينيل كلوريد على تركيز هرمون الأكتيفين ، وهو مهم في العديد من العمليات التناسلية في الجسم . جرعت سبعة وعشرون فأرة بمادة البولي فينيل كلورايد بعد التأكد من حملها طوال فترة الحمل ثم فترة الرضاعة. قسمت الإناث الحوامل إلى ثلاث مجموعات ، المجموعة الأولى هي مجموعة السيطرة ، والمجموعة الثانية جرعت بمادة البولي فينيل كلورايد بتركيز 50 ملغ / كغم من وزن الجسم ، و المجموعة الثالثة بنفس المادة بتركيز 100 ملغ / كغم . و بعد انتهاء فترة الرضاعة تركت الحيوانات المولودة حديثا لحين البلوغ ثم جمعت عينات الدم ولكلا الجنسين لاجراء الفحوصات المطلوبة ، وأظهرت النتائج ارتفاع معنوي في تركيز هرمون الأكتيفين في الإناث البالغة ولكلا التركيزين من البولي فينيل كلورايد مقارنة بمجموعة السيطرة. في حين انخفض تركيز الأكتيفين بشكل معنوي في الذكور البالغة ولكلا التركيزين مقارنة بمجموعة السيطرة وانخفاض في وزن الخصيتين وعدد الحيوانات المنوية لنفس المجموعة مقارنة بمجموعة السيطرة.

INTRODUCTION

Modern industries are based on the introduction of a lot of plastic in many of the materials used by humans, and these materials have multiple risks to human health. An example of these materials is polyvinyl chloride (PVC), which is used in the manufacture of many materials such as credit cards, floor coverings, food containers, plastic bottles, children's toys, windows, doors, and more. (1)

PVC is a synthetic resin made from vinyl chloride ,it is a member of a large family of polymers broadly referred to as "vinyls.". (2) Exposure to PVC occurs either through the use of plastic materials that are included in its composition, such as drinking water in plastic bottles, or through the presence of factories specializing in these materials, such as petrochemical and plastic factories. PVC is non-toxic in its pure state, but its toxicity occurs during its manufacturing stages. (3) Where some substances such as phthalates are added, which are substances that have harmful effects on health and can cause many diseases of the nervous and vascular systems, may be carcinogenic, and have effects on the respiratory and reproductive systems in addition to genetic disorders, (4) Studies have confirmed the effect of these substances on mucous membranes, the heart, the muscular system, and the brain. (5, 6). It affects body tissues such as connective tissue, inhibits the immune system, leads to mutations, affects the endocrine system, and causes defects in mental development in children. (7) It also leads to poor fertility in males and females. (8)

activin, which is one of the hormones responsible for reproduction. (9) It was discovered in 1980 as a reproductive protein that stimulates follicular stimulating hormone (FSH), which is produced by the pituitary gland (10) Activin is secreted from several

cells during the stages of embryonic development. (11) It has recently discovered its role in the development of stem cells and the development of the embryo. Studies have confirmed its role in the development of the gonads in adults. (13) It also has a role in the immune cells development in the body. (14) It has a wide range of vital effects in the tissues and organs of the body from the stage of embryonic formation until puberty. (15)

THE AIM OF THE STUDY

Current study aims to determine the effect of PVC on fertility in laboratory mice through its effect on the hormone activin, which is necessary for the functioning of the reproductive system and the formation of sex cells in male and female mice.

2: MATERIALS AND METHODS

2.1. Experimental Animals: Adult female mice were housed with males for one night and monitored for the appearance of a vaginal plug the next morning. Noontime on the day of the vaginal plug was considered as day 0.5 of gestation, after birth and lactation period, the newborn stay to puberty then Sixty healthy male and female mice 8 weeks old weighted (27 - 30 gm) were used in this study. The mice housed in stainless steel wire bottom cages and kept under constant environmental conditions and fed on fresh standard pellet and given tap water with 12 hours light/dark cycle.

2.2. tested substances: polyvinyl chloride was purchased from Sigma-Aldrich Company, Egypt. It is a fine white powder, odorless, stable under normal conditions, insoluble in water, and soluble in fat, oil, and most organic solvents. Corn oil was used to dissolve PVC and administered to animals under experiment. Median lethal dose (LD_{50}) of PVC is 260 mg/kg for mice.

2.3. Exposure to PVC before and after childbirth: pregnant Mice were divided into 3 groups, each of which contained 9 pregnant female mice:

- First group (control): pregnant female mice were given corn oil orally every day.
- second group: 9 pregnant female mice were given PVC orally (every day after dissolving it in corn oil) at a dose of 50 mg/kg/b.w.
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- The third group: 9 pregnant female mice were given PVC orally at a dose of 100 mg / kg /b.w. daily after dissolving it in corn oil.

Pregnant females (separately) were given a daily dose of PVC according to their control group from the sixth day of pregnancy until the end of the 21-day lactation period. After reaching newborn to puberty (60 days), each group (male and female) was sacrificed, and blood and organ samples were taken for the purpose of conducting hormonal tests.

2.4. Sample collection:

The mice were weighed before sacrifice and anesthetized by anesthetic chloroform. abdominal cavity was opened by making a midline in the abdomen to obtain the organs (testes and epididymis from males) used in the study.

2.5. Blood samples

Samples of blood were collected using the heart puncture method, It was placed in a tube without an anticoagulant, then placed in a centrifuge (3000 cycles per minute) for 15 minutes. To obtain blood serum for the purpose of conducting hormonal examinations, all samples were collected in the morning to reduce diurnal changes in the level of hormones.

2.6. Hormonal tests

Measuring the concentration of activin hormone using the ELISA technique

The basis of this method is based on the use of an enzyme to investigate Ag-Ab complexes, as the enzyme changes from a non-colored substance to a colored product, referring to a link between antibodies and antigens (Ma et al., 2006). (16)

Determination of activin in serum (ng/ml) The ready-made test kit from CUSABIO BIOTECH CO., LTD.

2.7. Calculating sperm concentration

Sperm concentration was calculated according to the method of Robb et al. (17). using a Neuber counting chamber used for counting blood cells.

*The method of work:

- The epididymis, after being cut into small pieces of 6–10 pieces, is placed in a Petri dish containing 0.9% physiological solution.
- The suspension is filtered through a clean cloth into a clean test tube.
- One drop is taken from the filtered solution in the test tube, placed on the counting chamber, and covered with a cover slip.
- The number of sperms calculated $\times 10,000$ is calculated to know the concentration of sperm in one mm^3 .

The number of sperm in one mm^3 is the number of sperm calculated $\times 10,000$.

2.8. Calculation of testes weight:

The weight of the testes of male mice treated with PVC during pregnancy and lactation was calculated after reaching the puberty stage, after they were removed with a scale, and compared to the normal weights of the control group.

2.9.statistical analysis :The results were analyzed using SPSS software

3-RESULTS

(Table 1) concentration of the hormone activin in both sexes of mice whose mothers were treated with PVC during pregnancy and lactation.

Groups	Female treated with PVC	Male treated with PVC	Mean differences
Concentration of activin (mean $\pm$ SE)	826.139 $\pm$.813	738.844 $\pm$.813	87.295*

*. The mean difference is significant (p value) at the 0.05 level.

The table showed that there was a significant increase in a hormone activin concentration of treated females compared to males and for both concentrations (T1, T2).

(Table 2) concentrations of activin in males and females whose mothers were treated with PVC during pregnancy and lactation compared to the control group.

type	treat	Mean	Std. Error
male	Cont.	1294.083	1.408
	T1	646.217*	1.408
	T2	276.233*	1.408
female	Cont.	118.667	1.408
	T1	1164.750*	1.408
	T2	1195.000*	1.408

*. The mean difference is significant (p value) at the 0.05 level.

The table shows a significant decrease in the concentration of activin in males treated with PVC at both concentrations compared to the control group. And shows a significant decrease in activin concentration for group T1 (50 mg/PVC) compared to group T2 (100 mg/PVC). While we notice a significant increase in the concentration of activin for females treated with both concentrations of PVC compared to the control group,

(Table 3) the effect of PVC on testes weight and sperm count in male mice whose mothers were treated with PVC during pregnancy and lactation compared to the control group.

Groups	Testes weight mg/b.w.	Sperm count (x 10 ⁴ sperm /ml)
Control	103.68±1.4	67.61±5.46
T1 PVC 50 mg/kg b.w.	83.35 ±1.2*	54.40 ± 6 .55
T2 PVC 100 mg/kg b.w.	74.29 ±1.5*	42.13± 12.0*

*. The mean difference is significant (p value) at the 0.05 level.

The table shows a significant decrease in testes weights for males treated with both concentrations compared to the control group, a significant decrease in sperm count for the T2 group, and a non-significant decrease for the T1 group compared to the control group.

(Table 4) Correlation Coefficient between Activin hormone concentrations According to the Test Weights

	Value	Weight1	Weight2
T1	R Value	0.857**	
	P-Value	0.001	
	N	11	
T2	R Value		0.602*
	P-Value		0.050
	N		11

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

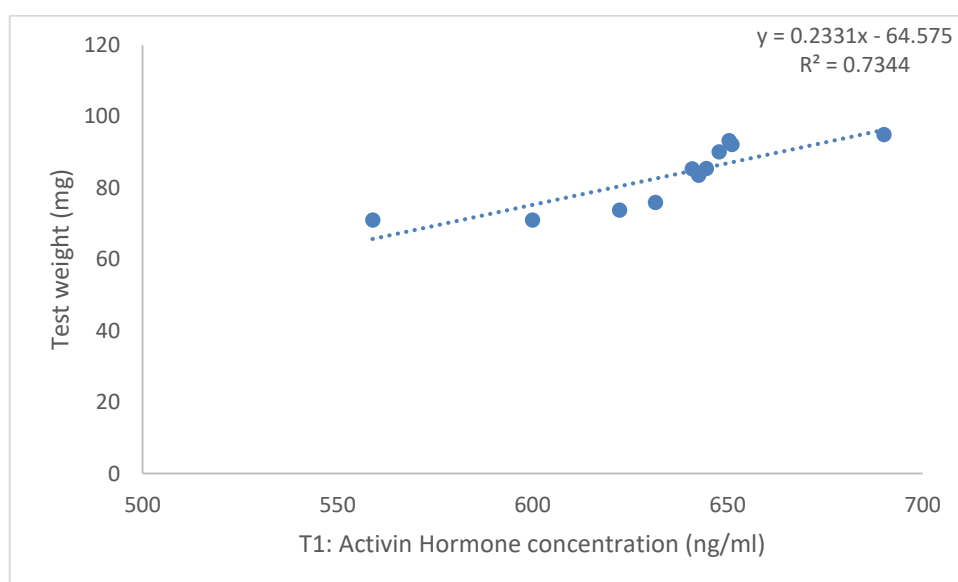


Figure 1: correlation between activin hormone and testes weight in mice treated with PVC 50 mg /kg b.w.

(Figure 1) show A linear regression established that Test weight could statistically significantly predict Activin hormone concentration, $F(1, 9) = 24.89$, $p = 0.001$ and testes weight accounted for 70.5% of the explained variability in Activin hormone concentration.

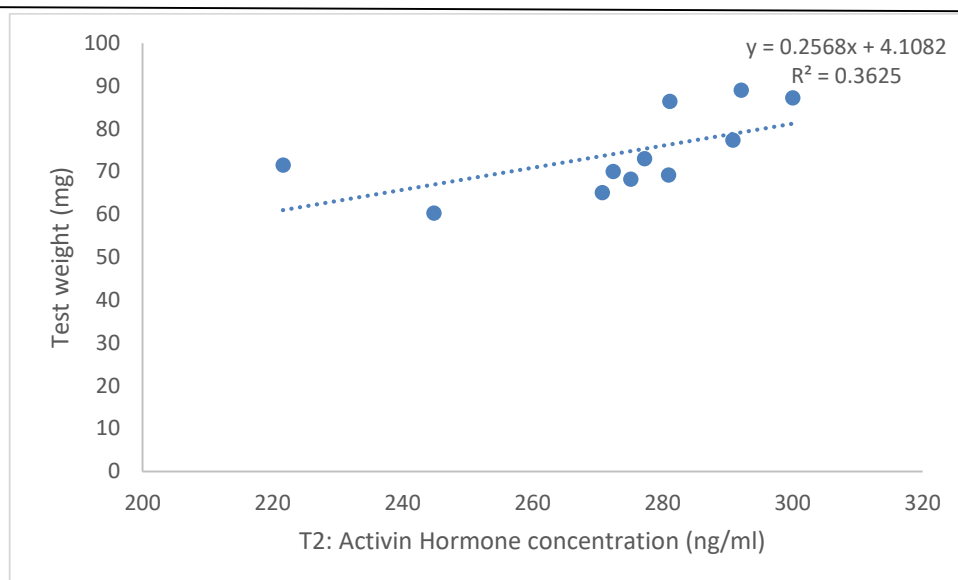


Figure 2: correlation between activin hormone and testes weight in mice treated with PVC 100 mg /kg b.w.

(Figure 2) show A linear regression established that Test weight could statistically significantly predict Activin hormone concentration, $F(1, 9) = 5.17$, $p = 0.05$ and testes weight accounted for 29.2% of the explained variability in Activin hormone concentration.

Table 5: Descriptive Statistics of Activin hormone concentration According to the Test Weights

Variable	Mean	Std. Deviation
Testes Wiegth1	83.35	9.04
T1(activin con.)	634.56	33.24
Testes Wiegth2	74.29	9.54
T2(activin con.)	273.34	22.37

DISCUSSION

The discrepancy in the concentration of Activin results from the effect of PVC on the pituitary gland and therefore on many hormones in the body. These toxic substances that are spread in the ocean affect the HP axis, which leads to a change in the functions of the endocrine glands and many pathological effects in the body, such as effects on the genetic and non-genetic nuclear pathways, steroid receptors, and ion channels. This may be associated with the development of some infections in the body and is an important cause of Activin disorder. (18)

PVC also causes a change in the liver tissue, a decrease in the effectiveness of oxidative enzymes such as glutathion peroxidase, glutathion catalase, and others, and the occurrence of necrosis in liver cells (19). The mechanism by which activin works to control the weight of the testis has been addressed by recent studies (20), where activin acts directly on the promoter of Sertoli cells in culture media (21). Recent studies have agreed that the first source of activin in the testes is peritubular myoid cells (PMT). (22)

These results confirmed the concept that a defect in activin signaling leads to limited development of Sertoli cells at critical stages of development, which leads to a reduction in testis weight in puberty. and a weakness in the sperm formation process (21).

The results also showed a difference in the concentration of activin when exposed to PVC between males and females, as studies confirmed the effect of activin on the differentiation of gonads in the early stages of the formation of follicles in female embryos, not males.

And the reduction of fertility in mice when the concentration of activin was disturbed in the body. It was observed that the weight of the testes decreased in mice after the seventh day of birth. (22), and this is consistent with the current study.

The decrease in the concentration of the activin hormone occurs in several cases, including the role of inhibin in reducing the secretion of activin and increasing the production of androgens by blocking the suppressive effect of activin A and B, as activin inhibits the formation of androgens. (23) Activin is also decreased during differentiation of adipocytes, where activin signaling is inhibited during adipogenesis in humans and decreased significantly in the group that suffered from azoospermia. (24)

Studies confirmed the disappearance of activin in the plasma after surgeries that prevent sperm production. (25)

The reason for its rise may be as a reaction to the deterioration caused by PVC to the vesicles and reproductive cells, as activin, when elevated, increases reproductive cells and increases the division of granular cells, thus increasing the primary vesicles formed from nests of embryonic cells. (26) This is consistent with the results of the many current studies, where the results showed that there were cases of inflammation in the tissues, an accumulation of inflammatory cells, and activin increased in cases of inflammation of lining cells and Sertoli cells. (27)

High activin levels are also associated with complications in the heart and increased oxidative stress. (28) and its high level is associated with glucose degradation. (29), which raises doubts about the possibility of PVC affecting the heart and pancreas.

CONCLUSIONS

- Polyvinyl chloride is considered to have an effect on the fertility of mice through its effect on reproductive hormones, directly or indirectly.
- The effect of the substance under study had a differential effect between the sexes, and this may be due to many physiological differences between the sexes.
- The activin hormone is considered to have a significant effect on the disturbance of the reproductive functions in the event of a change in its concentration, especially in the embryonic stages.

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