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Original Article

EFFECT OF THE CONSUMPTION OF MILK OF SOYA ON THE MALE FERTILITY OF SWISS MICE

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ABSTRACT

Objective: of this study is to estimate the consequences of the consumption of soy milk on the male fertility of mice Swiss used as experimental model.

Methods: In our study, we have used 24 male mice 4 weeks old and weighing on average (13.93±0.50) g. These animals were divided into 4 groups; mice At the end of the 90th day, and during a week, Mice are subjected has a test of fertility Just before their sacrifice by cervical dislocation, males undergo a blood test for the dosage of the testosterone, Then testicles, épididyme and seminal vesicles are taken and weighed. Sperm cells are counted, their morphology and their mobility are studied.

Results: Our results show that The mobility of sperm cells decreases very significantly ($p < 0,01$) to the animals of all the groups having consumed soy milk.

- The number of sperm cells testiculaires and épididymaires is decreased very significantly respectively at the mice of the groups 2 and 3 and at the mice of the groups 1, 2 and 3 ($p < 0,01$).

- The sérique rate of the testosterone decreases very significantly ($p < 0,01$) to the group 2 (1,08 ± 0,41ng / ml) with regard to witnesses (6,21 ± 1,54 ng / ml).

Conclusion: the obtained results indicate that the ingestion of the soy milk is not without consequence on the function of reproduction and provokes significant change of the male fertility of mice.

Keywords: Soybean, Sperm-Fertility, Endocrine Disruptors, Toxicity.

INTRODUCTION

Food-based non-native soya protein (MAP) can be recommended for the prevention of allergy or food intolerance in infants at high risk.

Milk-based industrial soy protein is the main source of phytoestrogens in humans. It is therefore important to assess the levels of intake phytoestrogens contained in soy foods can consume infants and young children. These natural chemicals that may toxicity to reproduction because they are able to stimulate, promote or inhibit hormone action where they can in theory change the physiological process under an endocrine regulation.

Numerous studies indicate a decrease in the number and quality of human male sex cells in recent years [1].

Many authors have reported adverse effects on sexual function, factors toxic substances in our environment and diet. Include gas exhaust from automobiles [2], pesticides, xenohormones [3]. However, work on the impact of soya milk on hormonal sexual function of male and fertility are very rare and not deductive.

Our goal is to study the impact of the consumption of soya milk in Swiss male mice on: the development and maturation of the sexual organs, male fertility and finally, hormonal function.

MATERIALS AND METHODS

Products and reagents

Different products and reagents from Sigma, Pharmingen, Merck and Prolabo ® and its bioMérieux (France).

The samples used in this study

Soya milk "BIOMIL ® SOYA"

Powder, obtained from FASSKA (Belgium), is complete infant formula based on soya protein enriched in L-methionine, L-carnitine and taurine. It is recommended in case of allergy to cow's milk protein or lactose intolerance.

This is a complete food free milk from birth, lactose free, sucrose and gluten-free.

Animals

The animals used in our protocols are Swiss strain mice obtained from the Pasteur Institute in Algiers. These are mice bred and acclimatized before handling in the laboratory animal physiology and nutrition security Food in housing conditions in accordance with regulations. The Experiments are carried out according to the well-being of the animal, avoiding the stress and agitation may interfere with the results.

Subchronic test for soya milk

This experiment evaluates the long-term toxicity resulting to soyamilk repeatedly ingested orally. The test is performed while fully respecting indication and dose administered in the box of baby milk. Swiss male mice (4 weeks), (13.93 ± 0.50 g), 2 groups of 6 mice from a nursing mother fed soy for 90 days of receiving a soy, and the other (control), 2 Other groups of 6 mice from a female control one receiving soy milk and the other witness.

Measurement of weight gain

Weight gain of the animals in each group is measured weekly throughout the duration of the experiment.

Fertility test

At 13 weeks, the mice are mated control females. After parturition, they take the measurement of the body weight and small size (the 7th, 14th and 28th days).

Blood samples and organs

A volume of 2 ml of blood per mouse was collected, centrifuged. Serum recovered is used for the determination of testosterone.

After sacrificing the male mice, the testicules, seminal vesicles were removed and weighed to determine the relative weight.

The left testis and seminal vesicle are fixed in a solution of 10% formalin for histological study. The right testis and epididymis law used to count spermatozoa.

Sperm count

The sperm count is performed on adult male mice. Testis and epididymis were removed, weighed and finely chopped and homogenized for 1 minute in 10ml NaCl 9 ‰ 5µl containing Triton X-100 (Merck, Germany). The number of sperm was measured using a cell Mallassez of 5 large squares, observed under an optical microscope at a magnification of 40 [4].

Sperm motility and abnormality determining

The epididymis is placed, cut in 4 ml of saline and then incubated in an oven at 37 ° C for 15 min. 20µl of the cell are deposited Mallassez [5]. La sperm motility is performed after 10 microscopic observations magnification 40. The percentage of sperm motility is defined by [6]. To assess sperm, morphology will be fixed with ethanol 95 ° stained with gentian violet and rinsed with distilled water. A minimum of 500 sperms are examined by light microscopy. Abnormal sperms are classified according to: (Abnormalities of the head, the intermediate part of the flagellum and the presence of cells others).

Testosterone dosage

The dosage of the hormone is made by the immunoassay by competition final fluorescence detection (Enzyme Linked Fluorescent Assay). The disposable cone serves both the solid phase and the pipetting device. The other reagents of the immunological reaction are ready and pre-distributed in cartridge.

Statistical methods

The results are expressed as mean ± standard error (X ± SE). The averages were compared using a Student t test for paired data and unpaired. Statistical analysis was conducted using a statistical software program named STATISTICA (5.1.2006).

Analysis of variance was performed with the ANOVA test. The significance level used is 5%.

RESULTS

Effect of soya milk on weight gain

Our results showed a very pronounced increase of body weight depending on the time. However, there was a significant decrease in body weight of mice who ingested soy milk throughout the experimental period. Soy milk induces significant decrease in the growth of mice, p <0.01 with ingesting soya milk.

Their weight has changed to 17.02 ± 0.54 40.71 ± 1.35 g g for controls, 11.97 ± 0.40 g 37.03 ± 0.91 g for mice of group 1 and 11.55 ± 0.10 37.86 ± 0.94 g in g for animals

However, group 2 mice in group 3 weight changed 15, 17 ± 0.21 39.01 ± 1.41 g (Figure 1). Weight and litter size in mice from group 3 to 7, 14 to J28 has decreased very significantly compared to the control (Figures. 2 and 3). Conversely, the weight and litter size in mice of groups 1 decreases very significantly on day 7 and J14 compared to control mice (p<0.01). A weight ranges J28 mice groups 2 reduces to a very significant and their size D7 and D28 compared to control (P <0.01).

Fertility test

The study was conducted on 18 male and female litters. The intersection of the four groups male mice with female controls led to the observation of the fertility of mice experimental ingesting soy milk compared to control mice. The results Table 1 shows the fertility index.

Effect of soya milk on the relative weight of the sex organs

The relative weights of testis, epididymis, and seminal vesicles in males only not change among groups who ingested soya milk during 90 days compared to controls (Table 02).

Effect of soy milk on sexual parameters in male mice

The objective of this part of work is to evaluate the effect of the consumption of soy milk on parameters sexual Swiss male mice by calculating the following parameters:

Sperm motility

Our results show a significant decrease in the percentage of mobility epididymal spermatozoa in groups of mice ingesting soy milk which values are respectively 37.89 ± 3.27, 44.25 ± 1.32 and 49.82 ± 4.48 compared to group witness have a value of 74.34 ± 2.13 (p <0,01). Throughout these data shows that the sperm motility are significantly decreased in males consuming soy milk compared to the control group (p <0, 01) (Figure 4).

These results confirm that the percentage of dead sperm, tend to be higher in animals ingesting soy milk especially those of groups 1 and 2, which have a nécrospermie (many dead sperm).

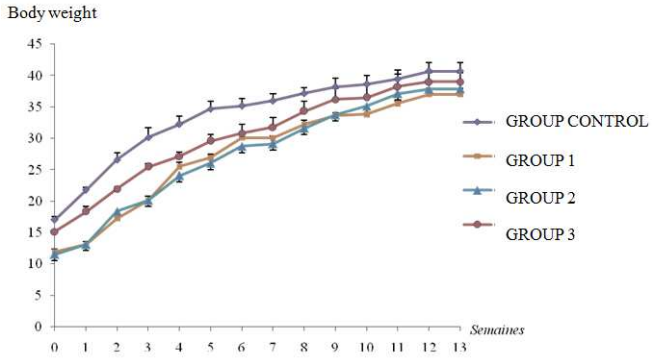


Fig. 1: Growth in weight of male mice receiving soy milk and the control group (n = 6 mice).

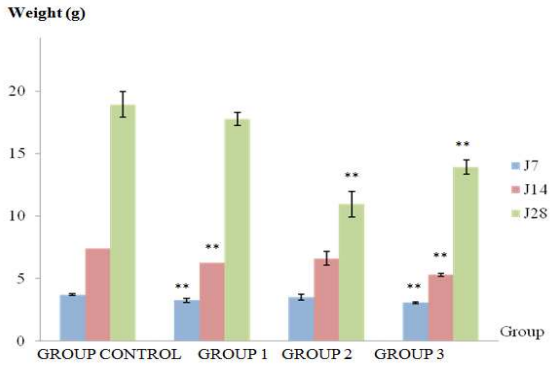


Fig. 2: The weight change of the consumption of soy milk on offspring

The values shown are averages and their standard errors (X ± SE); ** P <0.01

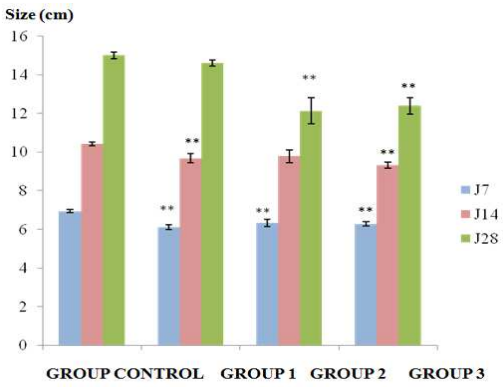


Fig. 3: The effects of soymilk on the evolution of the size of the seed

The values shown are averages and their standard errors (X ± SE); ** P <0.01

Table 1: Fertility Test

Control parameters	Control group (%)	Group1 (%)	Group 2 (%)	Group 3 (%)
Fertility index ᵅ	6/6 (100%)	4 /6 (67%)	4 /6 (67%)	5/6 (83%)
Number of newborns	56	39	18	32
Average newborn	9,33±0,56	6,5±2,11	3±1,13	5,33±1,28
Weight (g)ᵝ				
J7	3,73±0,06	3,26±0,17**	3,51±0,23	3,08±0,07**
J14	7,41±0.21	6,27±0.29**	6,61±0,54	5,30±0,12**
J28	18,93±1,02	17,76±0,52	10,96±1,01**	13,93±0,56**
Size (cm)ᵝ				
J7	6,94±0,07	6,11±0,13**	6,33±0,19**	6,27±0,11**
J14	10,42±0,1	9,68±0,24**	9,78±0,34	9,32±0,16**
J28	15,01±0,17	14,61±0,15	12,14±0,68**	12,4±0,42**

α: Number of fertile males / total number coupled.

β: Statistical study was performed on 18 carries for the weight and size.

Value ** very significant compared to control (p <0.01).

Table 2: Effect of soy milk on the relative weight of the sex organs in male mice Seminal vesicles testicles epididymis

	Testicles	Seminal Vesicles	Epididymis
Control group	0,62±0,03	0,72±0,10	0,24±0,03
Group 1	0,55±0,04	0,7 ±0,12	0,28±0,01
Group 2	0,57±0,04	0,57±0,11	0,3 ±0,01
Group 3	0,56±0,02	0,53±0,07	0,24±0,01

(n = 6 mice) for each group.

Group 1: from a nursing mother fed soy and receiving milk for 90 days soya.

Group 2: from a nursing mother fed soy and receiving for 90 days plugs and water.

Group 3: from a female witness and receiving for 90 days of soybean

Control group: received during the entire experimental period plugs and water.

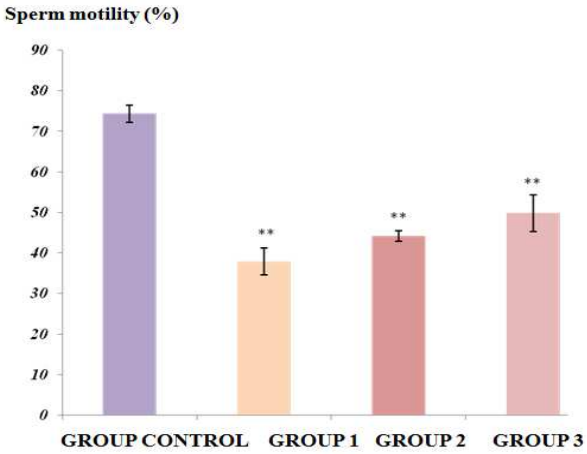


Fig. 4: The percentage of mobile epididymal sperm of mice with experimental ingested soy milk compared to the control.

The values shown are averages and their standard errors (X ± SE).

** P <0.01

Group 1: from a nursing mother fed soy for 90 days and receiving soy milk.

Group 2: from a nursing mother fed soy and receiving for 90 days and plugs water.

Group 3: from a female witness and receiving for 90 days of soybean

Control group: receives throughout the experimental period plugs and water.

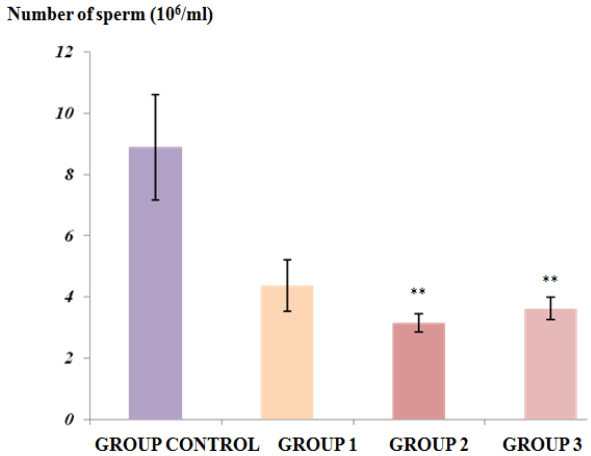


Fig. 5: Number of sperm (106/ml) in the testes in the control mice males and experimental mice ingesting soy milk (n = 6 mice).

The values shown are averages and their standard errors (X ± SE).

* p <0.05, ** p <0.01, ** p <0.001.

Group 1: from a nursing mother fed soy for 90 days and receiving soy milk.

Group 2: from a nursing mother fed soy and receiving for 90 days and plugs water.

Group 3: from a female witness and receiving for 90 days of soybean
Control group: receives throughout the experimental period plugs and water.

Counting sperm in the testis and epididymis

The results show a significant and very significant number of testicular and epididymal sperm in mice groups who ingested milk soybean (p <0.01). Mice ingested soymilk groups 2 and 3 have a oligospermia (the presence of abnormally low sperm quantity) compared to control mice. The number of testicular sperm pass (8.9 ± 1.72) x 106 / g in witnesses to (3.17 ± 0.30) and x106/ml (3.63 ± 0.36) x106/ml mice in groups 2 and 3 respectively (Figure 5). Epididymal sperm count is decreased very significantly in Groups 1, 2 and 3 having ingested soy milk whose values are respectively 7.43 ± 0.41 and 5.27 ± 0,53,6,5 ± 0.59 sperm compared to control mice 14.53 ± 1.43 (p <0.01) (Figure 6).

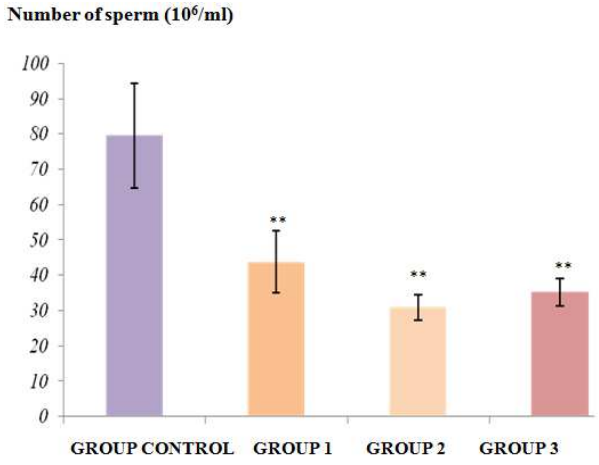


Fig. 6: Number of sperm (106/ml) in the epididymal in the control mice males and experimental mice ingesting soy milk (n = 6 mice).

The values shown are averages and their standard errors (X ± SE).

* p <0.05, ** p <0.01, ** p <0.001.

Group 1: from a nursing mother fed soy for 90 days and receiving soy milk.

Group 2: from a nursing mother fed soy and receiving for 90 days and plugs water.

Group 3: from a female witness and receiving for 90 days of soybean

Control group: receives throughout the experimental period plugs and water.

Sperm morphology

Morphology, last parameter analyzed the semen does not seem to escape adverse effects of soymilk. The results of the figure *(Figure 7)* show that the percentage the teratozoospermia (abnormal sperm morphology) is greater in mice ingested soy milk compared to the control group. The percentage of abnormalities abnormal sperm morphology is more common in mice ingested soymilk groups 1 and 2 respectively have values ranging from 16.3 ± 2.41% and 19 ± 2.57% compared to the value of the control group is 7.67 ± 0.60%. Morphology sperm is analyzed according to the different forms abnormal level of the head (Microcephalic, macrocephaly, head headless and irregular), and flagellum piece Intermediair (Coiled, short, with handle, double) observed with an optical microscope at different face (Figure 7.8.and 9).

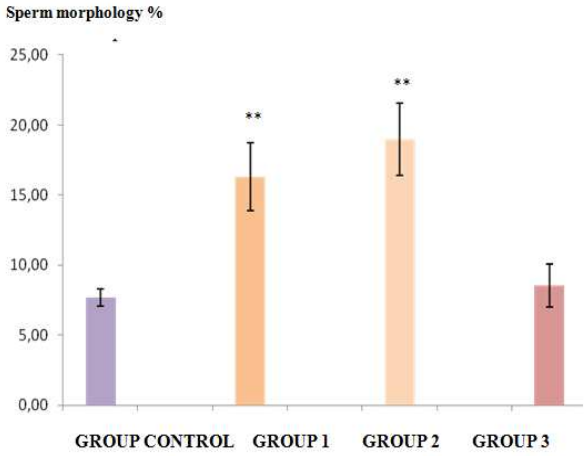


Fig. 7: Percentage of abnormal sperm in animals of different groups.

The values shown are averages and their standard errors (X ± SE).

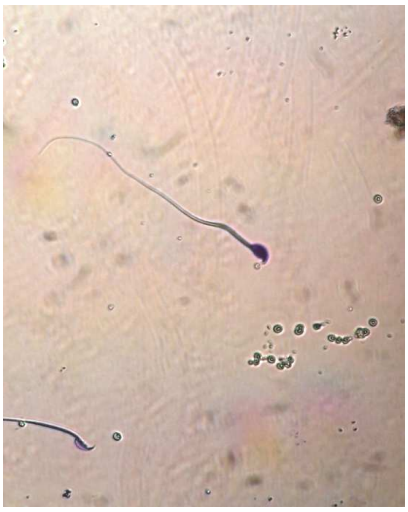
* p <0.05, ** p <0.01, *** p <0.001.

Group 1: from a nursing mother fed soy for 90 days and receiving soy milk.

Group 2: from a nursing mother fed soy and receiving for 90 days and plugs water.

Group 3: from a female witness and receiving for 90 days of soybean Control group: receives throughout the experimental period plugs and water.

(a)



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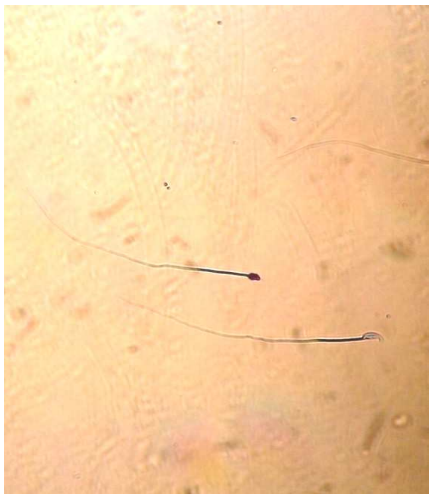
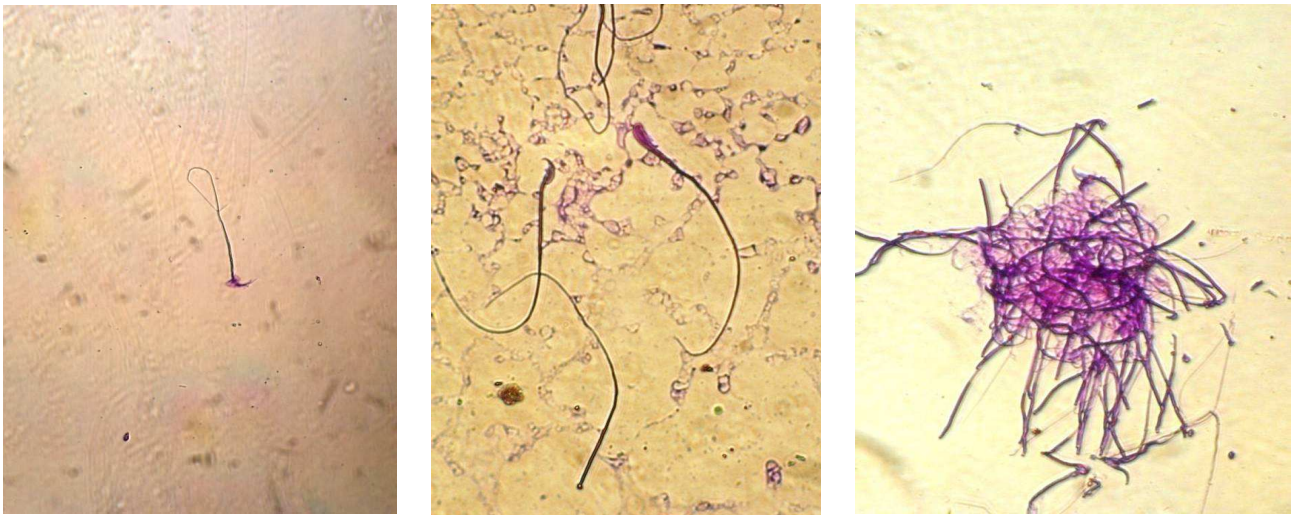


(c)



(d) **(e)** **(f)**

Fig. 8: **Optical microscope observation of the various abnormalities of sperm head stained with gentian violet in [magnification ×400].**

[a] Amorphous. [b] macrocephalous. [c] microcephalic. [d] head irregular. [e] Acéphale. [f] Grouping into clusters.

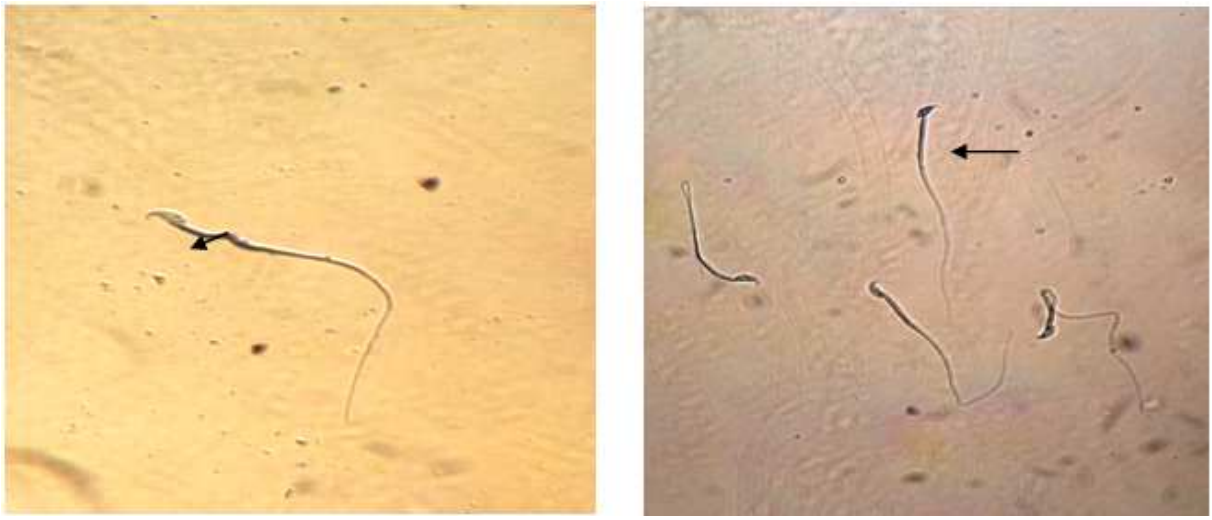


Fig. 9: **Observation under an optical microscope abnormalities of the intermediate (angled) of sperm stained with gentian violet in [magnification ×400].**

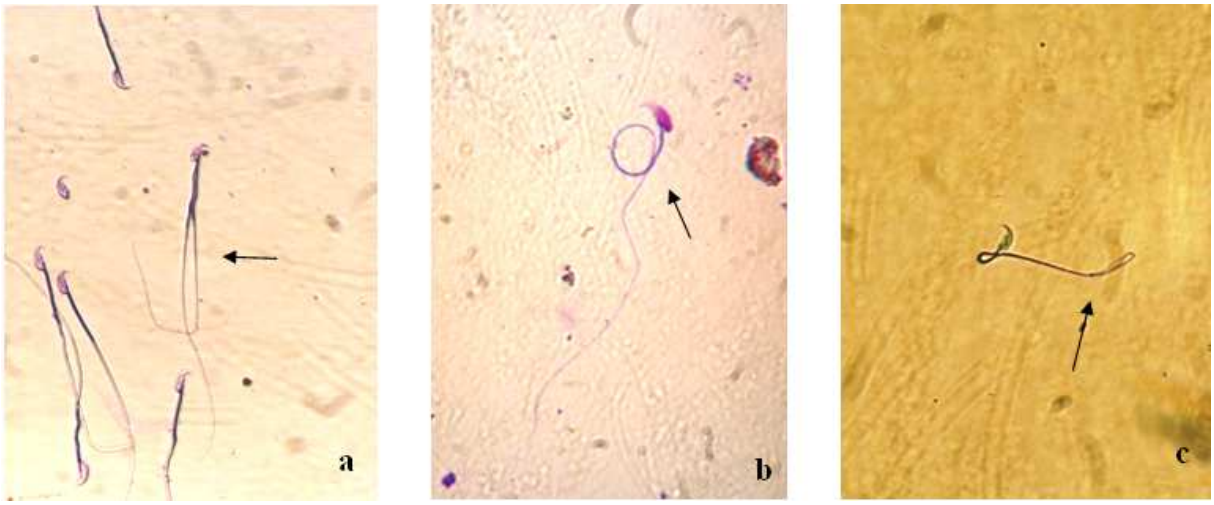


Fig. 10: Optical microscope observation of different anomalies flagellum sperm stained with gentian violet in [magnification ×400]. [a] Double. [b] and. [c] Coiled.

Dosage hormonal

The results in Figure 11 show a significant decrease in the concentration of plasma testosterone in mice from group 2 who ingested soymilk by through breastfeeding ($p < 0.01$). The serum concentration of testosterone from the value 6.21 ± 1.54 ng / ml in the control mice and 1.08 ± 0.41 ng / ml in the mice of group2.

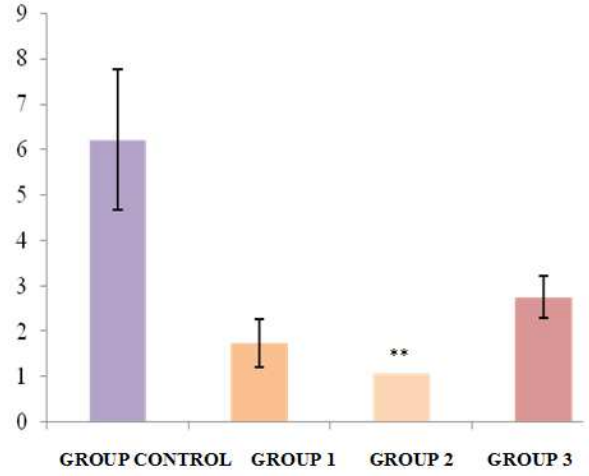


Fig. 11: the concentration of plasma testosterone (ng /ml) in animals of different groups.

DISCUSSION

Fertility in mammals is very sensitive to disturbances in the body by exogenous agents. Numerous studies indicate a decrease in the number and quality of human male sex cells in recent years [1]. It seems that disturbances of the sexual apparatus multiply human male.

Several exogenous compounds including pesticides, drugs, organic solvents, tobacco [7], xenohormones [3]. Although the biochemical mechanisms of toxicity are not well understood, they are considered true toxic agents affecting fertility [8,9,10]. Few recent studies show that phytoestrogens may have deleterious effects in the animal, in particular on the development and maturation of sex organs, and on the fertility [11]. Food soya are the main source of phytoestrogens humans, it is important to assess the levels of Phyto-estrogen intake contained in soy foods can consume infants and young children, and to consider the possible risks. Fed infants exclusively with infant formula based soya protein.

Today is the subgroup of the population most exposed to Phyto-estrogens. This milk-based industrial soya protein is the main source of phytoestrogens in humans. These natural chemicals are likely to have a toxicity to reproduction because they are able to stimulate, promote or inhibit hormone action where they can in theory change the physiological process under an endocrine regulation [11,12,13] , that is why this work was undertaken.

In the first part of our work we determined the effects of soya milk on body weight in mice. A significant decrease in body weight mice ingested soy milk compared to control mice. This decrease is probably due to the anorectic effect of soy isoflavones. The results consistent with those of [14, 15] in rabbits and rats treated with isoflavones.

The association with food intake observed in rabbits in the same study also been reported in rats fed diets containing genistein, suggesting a be anorexigenic of endocrine disruptor (PE) on the central nervous system, similar to that of endogenous estrogens. [17]However, this effect seems to be associated with a chronic treatment with large amounts of soy isoflavones. Exhibitions and pre- postnatal isoflavones in the study [18] have mounted similar declines parameter. For cons, the work by [19] showed that chronic administration and subchronic genistein in Wistar rats for 4 weeks and 13 weeks did not induce any difference in body weight.

The second part of our work has been devoted to the fertility test, the results Our experiments show that the control females mated with males ingested soy milk have a pregnancy rate (index of fertility) reduced compared to females mated with control mice, and reduced weight and size scope of the various experimental groups who ingested soya milk.

Also in another study [20] showed that the exposure of the genistein and vinclozolin gestation to adult male Wistar rats causes a reduced rate of pregnant females mated with males exposed and compared to witness.

Work [21] showed that injection of zearalenone or α-zearalenol led to a reduction in fertility and reproductive potential in male mice adults. A pregnancy rate is significantly reduced in female mice mated with males treated with Zearalenone and α-zearalenol. Also a decrease in the number of birth is probably due to poor sperm quality of males Zearalenone and α zearalenol. Similarly, it was reported by [24, 23, 22] for the Phyto-estrogen analogues estrogen, genistein did various birds and mammals led to a reduced fertility thereof. In the 3rd part of our work, we evaluated the relative organ weights of sexual Swiss mice, it showed no change in the relative weights of the testis, epididymis and seminal vesicles groups who ingested soya milk compared the control group.

These results agree with those of [25, 26] that the relative weights of sexual organs of rats did not change.

Another study that has been made by [27]did not observe any changes in rabbits testis weight.

Moreover, according to [28] exposure to genistein and diethylstilbestrol induces a significant reduction in the relative weights of these organs.

The work undertaken by [18] showed that pre-and postnatal exposure to isoflavones in rats induces an increase in testicular weight compared to the group control, however, no change was observed in the epididymides. These results are consistent with the results of the reports [30, 29]. However, a increase in testicular weight was observed in perinatal exposure of mink to genistein [31] and pre-and postnatal mice treated with genistein [23]. In contrast, some researchers have reported that the administration of exogenous estrogen or anti androgens reduces the weight of the testis and epididymis [32, 33, 34].

Routine method to assess the potential fertility of a male is the realization

of semen during which quantitative semen parameters (number of sperm) and qualitative (mobility and sperm morphological abnormalities (Abnormal forms) are analyzed. Decreased mobility and number of sperm in testicular and epididymal we observed in mice who ingested soy milk is probably due to the effect of estrogen Soy Phyto-on different control levels of spermatogenesis.

Our results show a significant and very significant percentage of epididymal sperm motility in mice groups having ingested soy milk compared to controls.

In the study [29]. The percentages of sperm motility in all animals exposed to zearalenone and its derivatives-zearalenol at all doses were significantly lower than control. These results are consistent with that of [20] have shown that exposure to genistein and vinclozolin low dose of gestation to adult male Wistar rats induced a decrease in mobility sperm. By cons, [35] have shown that in the rabbit, genistein caused increasing sperm motility.

Our results also show a significant and very significantnumber of testicular and epididymal sperm that is observed in mice groups who ingested soya. In this context, the work by [36] has cited no change in rats ingesting oral genistein confirming the results of [15, 37] however [18] shows that there was no significant change in the number of epididymal sperm in rats exposed to isoflavones.

Our results also showed that the percentage of teratozoospermia (anomaly sperm morphology) is greater in mice ingested milk soya.

These results agree with those of [29], the percentage of sperm abnormal increased in male mice exposed to zearalenone and its derivatives-zearalenol. Furthermore, no significant differences in percentage of abnormal forms of sperm were observed in rats exposed to genistein for 4 weeks [38].

Our results also revealed a significant decrease of serum testosterone in mice ingesting soya milk during exposure during the breastfeeding compared to controls.

Our results are consistent with those reported in the literature [40, 39, 23], the doses of high genistein males reduced testosterone levels. In addition, the study by [41]showed that genistein was administered to rats of adult males for 3 months, and the study of [42] obtained a vitro decreased testosterone production after exposure to isoflavones.

In contrast, exposure to long-term genistein in adult rats did not change the level of serum testosterone [41]. The same observation was made in studies [45, 44, 43, 23]. In another study [18] no significant difference serum testosterone was observed in rats exposed to genistein.The last part of our work has enabled us to assess the impact of soya milk on the histopathology of the testes and seminal vesicles.

Microscopic examination of histological sections made in the testicles mice ingesting soy milk, showed a decrease in sperm light of the seminiferous tubules of mice ingesting soy milk.

CONCLUSION

Endocrine disruptors in the environment are increasingly challenged to explain changes in male reproductive function, including in humans.

However, their mechanisms of action on reproductive function are poorly understood and place of causality in humans has not been demonstrated. Among the experimental studies reported, the conditions of exposure are often far removed from the environmental conditions (short exposure period).

We are interested in studying the effect of consumption of milk soyabeans (SOYA BIOMIL ®) on the male reproductive Swiss mice used as an experimental model.

Soya milk is a dietary product without lactose, sucrose, gluten and cow's milk proteins. It is enriched with methionine, carnitine, iron and zinc. Such milk industrially based on soya protein is the main source of phytoestrogens in humans.

These natural chemicals are likely to have toxicity function in reproduction, because they are able to stimulate, promote or inhibit hormone action where they can theoretically change the physiological process subject to endocrine regulation. This work was used to evaluate experimentally some toxic effects of consumption Subchronic soymilk male Swiss mice.

The study focused on weight gain, on the relative weight of sexual organs (testes, epididymis and seminalvesicles), mobility, count and sperm morphology and to test fertility (litter size and weight at D7, D14 and D28 and finally has to make a determination of serum testosterone hormone.

Our results show that body weight decreased very significantly in all experimental groups who ingested soya milk. However, no change relative weight of the male sex organs was observed.

In addition, we observed a decrease in sperm motility and their testicular and epididymal number, and an increase in the percentage of forms of abnormal sperm groups who ingested soya milk.

On the other hand, the serum testosterone decreased in the group who ingested milk soya beans during the lactation period.

ACKNOWLEDGEMENT

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