THE EFFECT OF THE BODYBUILDING PROTEIN ON MALE FERTILITY "IN VIVO TEST ON MALE RABBITS"

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ABSTRACT

Male infertility is multi-causal; there are complex environmental factors such as exposure to chemicals, as well as behavioral factors such as the use of laptops too close to the genitals, or exposure of the male fetus to Tobacco during pregnancy. Moreover, the newest of causes is the consumption of bodybuilding proteins, which was the objective of our study. We tested a muscle-building protein called "Whey Isolate 95" on male rabbits to see its effect on weight evolution on sexual maturity by calculating the maturity indices and finally the course of the spermatogenesis where we made cuts Histological from the testicles to see the seminiferous tubules. Our results showed that these proteins had an effect on weight growth; the Gonadosomatic index (GSI) was lower than that of the control group. As a result, these bodybuilding proteins can have a negative effect on sexual maturity. However, the histological sections of the seminiferous tubules revealed no abnormalities. Therefore, we concluded that these proteins are not so aggressive on spermatogenesis, but this is recorded with proteins that are extracted from the whey that are considered the purest. It is possible that with other types of bodybuilding protein the effect will be more harmful.

1. INTRODUCTION

The World Health Organization and other organizations have established normative values for parameters such as sperm volume, sperm concentration, motility, morphology and forward progression. However, many men with normal values remain barren despite no problems with their partners1.

Studies have found contradictory results with regard to the effect of exercise on hormonal status. Several studies have shown a decrease in bioavailable testosterone in various athletes, including runners, wrestlers and weightlifters.1-4

The sport of weightlifting has been attracted by a large number of young people in Algeria or on a world scale. Their consumption of protein powders also increased as never before. These powders are available in a wide variety. Now, some people believe that protein powders eventually weightlifters have a negative effect on male fertility.

In our study, we test the effect of weight proteins on male infertility using guinea pigs as sexually mature rabbits. The principle is to administer muscle proteins to rabbits and to see their effect on spermatogenesis. At the same time, we tested an androgen and an anti-androgen to compare the effect of these synthetic hormones and the effect of weight proteins on male infertility.

2. MATERIALS AND METHODS

2.1. Goal

Our experimental study is to evaluate the effect of bodybuilding proteins on infertility masculine; we used for experimentation a rabbit’s male. At the end of the experiment, we sacrificed the animals to extract their genitals; we weighed each animal and their gonads in order to calculate the indices of sexual maturity: condition factor “CF” and gonadosomatic index “GSI”. Finally, we performed histological sections of the testes to observe the different stages of spermatogenesis in order to judge the effect of bodybuilding proteins on male fertility.

2.2. MATERIALS

2.2.1. Vegetables materials

Bodybuilding proteins (why), we provided directly trade with packaging labeled indicating the name of the species directly on their labels.
“Whey Isolate 95” ultra pure protein with high biological value, it is a compound of whey protein isolate, manufactured by Olimp Sport Nutrition brand. It is many athletes and competitors use Whey. Thanks to the high concentration of essential amino acids (BCAAs and L-Glutamine), this protein promotes muscle recovery. His amino acid content is 25%.

2.2.2. Animals’ materials

Our study was done on rabbits aged about 2 months and weighing between 2kg and 3kg from the ITELVE institute of Sidi Bel Abbes (Technical Institute of Livestock). The experiment lasted six weeks (May-June 2016). After a period of adaptation of 06 days, the animals were treated in accordance with the advice on the protection and use of laboratory animals [4]. The humidity of the animal house was 47%, an average temperature of 24-17°C and a photoperiod of 13 h of light and 11 h of darkness, with free access (ad libitum) to water and food (They were fed a standard rodent food that is marketed by the West Poultry Group (GAO ORAVIO-ORAN, Algeria). The workshop was serviced every day.

2.2.3. Laboratory equipment

2.2.3.1. Inhibition Hormone

We used "Decapeptyl" for males and females. The molecule is the Triptoreline which is an analogue synthetic decapetide natural GnRH (gonadotropin releasing hormone). Studies conducted in humans and in animals have shown that after initial stimulation, prolonged Triptoreline administration leads to inhibition of gonadotropin secretion, thus removing the testicular and ovarian functions. "Following some animal studies, another mechanism of action was discussed: direct gonadal effect by decreasing the sensitivity of peripheral receptors to GnRH [8].

2.2.3.2. Stimulating Hormone

We used testosterone, whose trade name is "Testosterone capsules - Andriol (40mg)." Testosterone belongs to the class of medications called androgens (male hormones). This medication is used to replace testosterone in men with conditions caused by low testosterone levels. It is also useful in the treatment of erectile dysfunction (erection difficulties or inability to achieve or maintain an erection) and other disorders of male sexuality caused by low testosterone levels. [6-7].

2.2.3.3. PREPARATION OF HORMONAL SOLUTIONS

- The anti-androgen: “Decapeptyl” is an injectable solution. For an adult dosage is between 75 and 300 units, so we had calculated for a 3kg rabbit, about 2 units, we used syringes with small graduations.
- The androgen: testosterone, have the capsule containing a viscous solution. Generally, a daily dosage for a 70 kg adult is 120 to 160 mg divided into two doses taken 2 to 3 weeks, so for a rabbit of about 3 kg, the dose is 5.15 mg / kg. We aspirated using a syringe this viscous solution of the capsule (40mg), we homogenized in 50 ml of water and we have our rabbits fed with about 6 ml to each rabbit.

2.2.4. The distribution of prizes

The distribution of different batches is based on the purpose of blocking testicular functions of male rabbits. then proceeded to release first with synthetic hormones and secondly with the tested plants to make a comparison operation of the genitals, therefore, to judge the effect of weight proteins on the male infertility.

Four prizes to males rabbits
Batch 1: Witness male rabbits (five rabbits)
Batch 2: Inhibition (Decapeptyl) + stimulation (Testosterone) (five rabbits)
Batch 3: Inhibition (Decapeptyl) without stimulation (five rabbit)
Batch 4: Weight Protein (five rabbits)

Preparations bodybuilding proteins (why) were administered in drinking water.

2.2.5. Removal of the testes

After the sacrifice, testes were carefully collected, weighed and placed in a conical polypropylene jar 125 ml labeled and filled with formalin to 35 %.

a- Testicular evaluation

The evolution of the weight of the testicle was evaluated on the basis of the calculation of the sexual maturity indices of the rabbits experimented with the controls.

The change in the testes was appreciated from the calculate sexual maturity indices experienced rabbits with witnesses.

b- The index of sexual maturity
The maturity indices are parameters related to the physiology of the animal, and can give us indications on the state of the reproductive system, or sexual behavior. In these studies we used two indices; the condition factor "CF" and Gonadosomatic index "GSI" according to the following two equations (breeding test protocol Environment Canada, 2010) [4]:

\[
\text{CF} = \frac{\text{Animal Weight (g)}}{\text{Animal length (cm)} \times 10^3} \times 100
\]

\[
\text{GSI} \% = \frac{\text{Gonad Weight (g)}}{\text{Animal Weight (g)}} \times 100
\]

2.2.6. The stages of the histological study
We performed histological sections of the testicles of the rabbits in order to observe the seminiferous tubules and the phenomenon of spermatogenesis.

There are five steps to achieve a histological section:

- **Fixing:** this first stage starts immediately after collecting the testis, its role is the conservation of different tissue of the organ, it prevents cell autolysis, also prevents bacterial putrefaction and allows histological technique and colorings later. We used formalin 35% [3].

- **Inclusion:** the principle of inclusion is to have a rigid consistency tissue, in order to achieve very fine cuts to allow the passage of light through the fabric, in order to have a good microscopic observation. The paraffin embedding includes the infiltration and coating the tissues to be examined with paraffin. We proceed before this coating of two mandatory stages. The first step is dehydration where we will spend the tissues in growing degree of alcohol baths (70 °, 80 °, 90 °, 95 °, 99 °, and finally 100 °), the interest of dehydration is to remove the fixative. In the second step, we proceed to toluene bath which is a solvent miscible with paraffin to replace the alcohol. Finally the fabric is placed in the molten paraffin, heat will accelerate the evaporation of the solvent, then placed in small molds, at room temperature, it will harden, we turn out to obtain fractions of paraffin tissue. Using a microtome cuts is performed. The instrument moves the block on a knife. The cuts are about 5 microns. All tranches will form a tape in which we find the tissue sample cups. One makes a spreading section on glass slides, heated on a hot plate the paraffin will stick to the blade [3].

- **Colorations:** The most commonly used stain is hematoxylin / eosin / saffron (HES). Haematein is a basic substance, which stains nuclei purple therefore stained nucleic acids. Eosin is an acidic substance, which stains pink cytoplasm, it is the protein staining. Saffron color the yellow collagen fibers. Prior to staining, there is a dewaxing, one passes the blades in toluene baths. Then this is rehydration: the alcohol is mixed with water and toluene, we pass the blades in alcohol baths of decreasing degree (100 ° to 70 °) [3].

- **Mounting:** The slides were dehydrated through baths in toluene and then glue glass coverslips over with synthetic resins to preserve the preparations. These slides can be stored for several years [3].

3. RESULTS AND DISCUSSION
Our study surrounded the evaluation of the effect of weight proteins on male rabbits has revealed the following findings:

3.1. Effects of weight proteins on weight changes rabbits
The first day of the receipt of the rabbits, we took their initial weight. For a period of one month we have experienced on their bodybuilding proteins like "Pure Whey Isolate 95" are proteins extracted from whey. Before the sacrifice, we weighed them again for their final weight. The results obtained are shown in (see Fig.1) So we found that the largest weight gain is that the lot where the rabbits have been bodybuilding proteins as a beverage with a 500g weight gain. Followed the lot was subjected to stimulation with androgen "Testosterone "after was treated with anti-androgen" Decapeptyl 'or rabbits were 450g more. Followed by the control group with 350g more and last batch which only undergoes inhibition with the same anti androgen with weight gain 160g only. This makes sense since these proteins can be considered food supplements including weight gain is a common manifestation of any living organism.
3.2. Condition factor "CF" different batches of rabbit experimentation

After experimenting with duration of one month, we tested the effect of weight training on protein, breeding male rabbits, we made sacrifices of animals experienced, measure lengths and weights are made for each rabbit. We calculated the CF factor requirements using the following equation:

\[
CF = \frac{\text{Rabbit weight (gr)}}{\text{Rabbit length (cm)} \times 10^3} \times 100 \quad \text{(Environnement Canada, 2010)}
\]

We found that there is no difference between batches CF, only a small decrease on the batch that undergoes stimulation of testosterone after being subjected to an inhibition in Decapeptyl (see Fig. 2).

3.3. Gonadosomatic index GSI.

After killing rabbits, in addition to measures of lengths and weights of the rabbits, we have also weighed the gonads, and we calculated the GSI using the following formula:

\[
\text{GSI} \% = \frac{\text{Gonad weight (gr)}}{\text{Rabbits weight (gr)}} \times 100 \quad \text{(Environnement Canada, 2010)}
\]

We found that the GSI does not have a remarkable difference between the control group and those who undergoes inhibition and stimulation of testosterone. However, we noticed a small decline for the lot where rabbits consumed bodybuilding proteins, where GSI is 0.2828% from an average of about 0.3500% of the other lots is perceived control (see Fig. 3).

Accordingly, this report represents a coefficient of maturity leads us to say that bodybuilding proteins can affect the reproductive apparatus of male rabbits.
3.4. Effects of weight proteins on the histology of seminiferous tubes of male rabbits

After the period of experimentation, our rabbits of different batches have undergone a sacrifice plus weights and lengths taken the bodies of rabbits and their gonads, we conducted histological section of all gonads rabbit’s experience. The end of our study was microscopic observations of fixed slides prepared.

The microscopic study showed us the state of spermatogenesis in rabbits of different lots. In the case of the control (Fig.4), it was noted that there were seminiferous tubules of normal size, the number of tubes is usual with all lines of germ cells. The light of the seminiferous tubes is occupied by mature sperm, but we noticed that the light of some empty seminiferous tubes appears this can be explained by the fact that they are young rabbits aged two months ago, so it's early sexual maturity. For lot 2 that underwent blocking with an anti-androgen (fig.5), we noticed that there is a total absence of germ cells, the lumen of the tube is empty, there is no sperm and we notice even atrophy of the seminiferous tubules. Consequently lot three where we administered androgen after blocking (fig.6), we noticed a trigger spermatogenesis, seminiferous tubes are normal form with all the germ cells. Moreover, in the end for lot 4 is most important for our study (fig.7), where rabbits consumed bodybuilding proteins, then the results show that spermatogenesis occurs in the normal way with a full light tube sperm, results leads us to say bodybuilding proteins have no adverse effect on spermatogenesis.
FIG. 4 Cup histological gonad rabbits of the control group saw the light microscope
A: (G= 40) B: (G= 100) C: (G=400)

FIG. 5 Histological section of the gonads rabbits experienced consignments subject to inhibition with Anti-Androgen
"Decapeptyl" D: (G = 40) E: (G = 100).
**FIG. 6** Histological section gonad experienced batch rabbits subjected to stimulation with testosterone after inhibition. F: (G = 40) G: (G = 100) H: (G = 400)

**FIG. 7** Cutting histological gonad experienced batches rabbits consuming bodybuilding proteins, without inhibition. I: (G = 40) J: (G = 100) K: (M = 400)
4. CONCLUSION
Proteins are essential for our body. Athletes consume it abusively. However, is taking supplements essential? According to advertisements, these proteins are at controlled amounts not harmful to health, but it remains to meditate who want to popularize their product. Well behind, there is a body that suffers all these extras and that will eventually melt. Among the problems with these supplements, we have the problem of male infertility, which continues to increase. It is the purpose of our study; we will try to assess the effect of weight proteins on male infertility. Our experimentation occurred as following:
After a week of adaptation we divided the rabbits into different batches, the first batch is obviously the witness, the second has undergone a blocking spermatogenesis by an anti-androgen "Decapeptyl" took the third has an androgen "Testosterone" and the fourth took a bodybuilding proteins. After sacrificing the animals, we took the weight and length of the rabbits and the gonad weight to calculate the indices of sexual maturity. Last step, we performed histological section of the gonads of all experienced rabbits. The results enabled us to draw the following conclusions:
☐ Concerning weight gain rabbits during the experimental period, the results showed that the largest weight gain is one of the lot where we administered bodybuilding proteins with 500g increase;
☐ The indices of sexual maturity, We recorded CF very similar in different batches, while a GSI of 0.2828% for the lot where rabbits consumed protein is a slightly lower percentage relative to other batches;
☐ Microscopic observation of histological sections of the testes, we noticed the seminiferous tubules of normal size and number, with a satisfactory presence of sperm.
By these results, we can conclude that the weight proteins have a positive effect on weight gain rabbits. However, the maturity factor (GSI), who presented a small decrease from the other lot we can said it is proteins may have a small negative effect on sexual maturity rabbits.
Concerning the observation of the seminiferous tubules, the findings withdraw from the histological sections show that there is no real effect on the proper conduct of spermatogenesis, but it should be noted that we used protein natural extracted from whey, it is the purest of the rest of proteins found in the trade.

5. REFERENCES