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Melatonin Effect (Under Alternating Light and Injection) On Testicular Tissue

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Abstract: ***The research experience was designed according to the complete random sectors. The applied method was used to determine the relationship between the exposure of the alternating light period and the environment of the organism, the synthesis of melatonin by the pineal gland, and the effect on the reproductive efficiency of male laboratory animals (hamsters). Through a tissue study. The study was conducted at two stages of sexual age (before and after puberty), and two periods of alternating and neutral lighting. The effect of injection of melatonin externally under the skin, it was found that the different concentrations were 6.25µg - 12.5µg - 25µg with the concentration of the hormone melatonin internal origin produced by the pineal gland to its lower limit) at the previous two stages, on the male reproductive efficiency of this animal. It was found that exposure to long photovoltaic periods had an effect on increased body weight, especially in post-puberty sex and exposure to long periods of light, has an impact on the male reproductive system, and structure of tissue, and the broadness of the glands of sperm. And large in the cells of Sertoli, and change the form of the interstitial cells to the shape of the spindle, and increase the brightness of the channel conveying the sperm, and the thickness of the thickness of the muscle layer. The hormone melatonin injected has an inhibitory effect on body weight, proportional to the amount of concentration, and the stage of injection, as well as the hormone melatonin injected has an inhibitory effect on the size of the male reproductive system. Atrophy in the testis, lack of dimensions, weight loss, inhibitory effect of the structure of tissue. Appeared larger in the second group, and the injected hormone had an effect on the tissue structure, stenosis of tubular and epidermal ducts and ducts of the sperm. Causes of the disappearance of spermcells with n1 pigment, and the small size of the cells Sertoli and interstitial cells and increase the thickness of the muscle layer.***

Keywords: Light period, Melatonin, testicular tissue

1. Introduction

Many of the environmental factors affect the life of the organism, and light is the most important, because of its role in the organization of many of the most important functions in the body, the most important secretions pineal gland, this gland is located in the roof of the third ventricle on the dorsal face of the brain(The upper face of the animal or posterior of the human)which produces the hormone melatonin, a hormone found in all living organisms primitive and developed one installation, according to one system in secretion. It is produced at night and disappears during the day, and is considered one of the most effective substances in the human body, in his province. On the health of the body through resistance to germs Viruses, and improved sleep quality and insomnia resistance and prevention of cancer. These hormonal secretions play a role in control, especially in mammalian breeding seasons, such as the hamster, which is considered one of the best laboratory animals because of its easy breeding, small size, rapid reproduction and abundance of births, which is the case for all seasonal animals, The study of the relationship of this organism to the surrounding environment, by studying the effect of light as an environmental factor on its behavior and reproduction, through the study of the mediator, the hormone melatonin. Which in response to light affects the secretions of the pituitary gland, thus contributing to the research to maintain a link in the chain of food in the world of wildlife, and this study may receive some light and contribute to the detection of the reality of the hormone melatonin, which is conflicting views, Italian researcher Walter Pierpaoli, who said in his joint book with the American physician William Regelson, entitled The Melatonin Miracle, which was issued in 1994, where melatonin was attributed to longevity, rejuvenation, revitalization of the elderly and recovery from AIDS, according to the Oxford Journal of Reproductive Biology, article "Melatonin is the time in its glass". Since then, there is still an urgent need to study the effect of this hormone. A team of researchers, such as Professor Singer of the University of Oregon, said: "Melatonin research is still slow, What is the optimal dose? "This research may help meet some of these requirements, by studying its effect on the tissues of the male reproductive system, in terms of tissue before and after puberty, in highlighting some of the facts that may be the basis for other studies. Although studies on the effect of the light factor on the daily vital rhythms in the body and on reproduction in pre and post puberty are still few and do not give us answers to many questions. Also, very few researchers have been exposed to them, according to research procedures and methods The study did not benefit from the developments that took place in the field of research institutes and the new means of investigation, and showed studies limited to the aspects did not touch the substance of the subject, according to our knowledge, although important, so we hope that this research fills a vacuum and provide some information that may contribute to the progress of the march Scientific research and God conciliator**.**

2. The Goal of the Research

Study the effects of the hormone melatonin (internally, from the pineal gland when affected by controlling the periods of lighting - externally, and through injection rates and specific periods) with different concentrations on the tissues of the reproductive system in males of this animal, and determine the reproductive efficiency and vulnerability.

3. Research Importance

The effects of melatonin in different concentrations on the tissues of the male reproductive system have been exposed to very few researchers, according to relatively different research procedures and methods of study, which did not benefit from the developments that took place in the field of research equipment and new means of investigation. The importance of the topic to be discussed according to our knowledge, despite its importance. Therefore, we hope that this research fills a gap, and provides the students with some information that may contribute to the advancement of scientific research and God conciliator.

4. Reference Study

Although the main food of hamsters is grains, only individuals are omnivorous. Omnivorous, which feeds on vegetables, seeds, fruits and meat, and needs relatively large amounts of drinking water (d. Manla 2001). The hamster is characterized by rapid reproduction. Female births are given once a month. The gestation period ranges from 16-22 days., And females have 4 to 16 nipples for breastfeeding, Craficroma-SA, A981). The hamster is an active night animal living in burrows of several rooms with a single hole, with an average depth of about one meter. The males are single. While females live together, people fill their zebra bags with grain from the fields and move them to their dens. However, It has several benefits of insecting insects, and types of small mice such as wild mice Gerbillusdasuruswagner (Dr. Al-Ahmadi and M. Husseini 1987).

This animal is classified as follows:

Phylum: Chordata
Class: Mammalia
Order: Rodentia
Suborder: Simplicindentata
Family: Cricetidae
Sub – Family: Cricetinae
Genus: Mesocricetus
Species: M. auratus

The hamster is an example of a study of photoreceptor control in reproduction.. Reiter-RJ1980 (Pevet-p., 1988), however, there are internal and external factors that share the influence of the light period, such as the genetic variation within the same species (Dark-j., Et al., 1983) (Wood-RI &Coolen-LM, 1997; et al.), And the age at which the mother transmits light-period information to her embryos (Stetson-MH & Watson-Whitmyre-M, 1986) (Shaw-D & Goldman-BD, 1995)

Postpartum births are not affected by photodiode during the first two weeks of life, not even by melatonin (Stetson-MH, et al., 1989), while beginning to be affected since the third week of life (Rollag-MD, et al., 1982).

External factors, which share the effect of the light period on the reproductive system, are as diverse as the quality and quantity of food and drinking water.(Idell-2017) (Ibuka-N, & Fukumura-K.1997, et al)

And the physical nature of light, where the highest effectiveness of light blue then green and then yellow and red and then the near ultraviolet spectrum, and that the ultraviolet light in the physiology of the pineal gland during exposure in the middle of the sunny day. (Hut-R., et al., 2000) also knows that moonlight affects most of these animals. (Brainard-G.C, et al., 1986)

The relation of the pineal gland with the light period:

Note the diversity of light receptors.

1. Optical receptors outside the retina are associated with the brain that sends information to the actors, which is observed in invertebrates and vertebrates. (Hattenhaver-H, 1986) by mediating the pineal gland as an auxiliary structure, as if it were a third eye, or by mediating skin photoreceptors in adult individuals, reptiles and amphibians (Smith-V.C., 1977).

2. Optical receptors in the retina transmit the light of the affected organ through two hormonal neural groups, without the presence of an intermediate organ by the first-order hormonal nervous system, or through an intermediate component, the pituitary gland, that reaches part of the alert Light, from the area under the optical bed, fromthe pineal gland through the pathway under the clinical pituitary. Hypothalamus - Hypophyses System Thus, according to the neuro-nervous system of second order, as is the case in birds and mammals. (Curtis-SE, 1981) The pineal gland is the central part that interacts with the optical period, which comes from the retina and is produced by a hormonal action, through its secretion of the melatonin, which controls the vital rhythms of the vertebrate body (Stetson-MH & Watson-Whitmyre- M., 1986) and Goldman-BD, 1983. The removal of the pineal gland in the lower vertebrates leads to the disappearance of these effects except for certain species (Filadelfi-AM & Castrilicci-A, 1996). This has led to the mistaken belief that melatonin is the only hormone of the pineal gland (Wurtman-RJ & Ozaki-Y, 1878; Mullen-PE, et al., 1979; Balemans-MGM, 1973; Mc Isaac-WN, et al. (Wurtman-RJ, et al., 1964), but it was later found that eradication at salmon and rodents, such as rat and hamster, does not eliminate its presence (Ozaki-Y & Lynch-HJ, 1976). (Kenneth-DJ, et al., 1977), suggesting that there are other sources for the manufacture and secretion of this hormone, and with subsequent research it was found to be made in the retina (Alonso) - Cardinali - DP &Rosner (1996); Pvet - p., Et al., 2000; Joliffa - Carolina - O., et al. (Bowmanas-MGM, et al., 1980) and in the gut (Quay-WB, & Ma-Y. H, 1976) and in the Harderian gland The hormone directly affects the glands that make it first, such as the pineal, retinal, intestinal, and glandular glands (Merle-A, et al., 2000). (1961), and then its effect on other organs in the body, including the reproductive system (Ffynn-AK, et al. 2000; Gerlach, 1999) 1999). Although it is manufactured and excreted in several areas of the body, studies confirm that the pineal gland has the most important role. The gland possesses light-sensitive cells of a fungal nature,

producing two groups of substances, one of which is IndoleaminesThe second peptides, melatonin is manufactured from the first group. Lerner-AB, et al., 1959 and his colleagues were able to isolate it, and others were able to confirm that it was manufactured from the deep area of the pineal gland Pevet-P, et al., 1980, which is manufactured daily and after dark, This requires the installation of large amounts of proteins, evidenced by the increased metabolic efficiency of pineal gland cells, enzyme activity and the compounds needed for its manufacture (Ribelayga-C, et al., 1998). Several studies have shown that exposure to long light periods, (Pelisek-v. Vansek-J, 2000; Gauer-F, et al., 1994), when compared with the exposure of animals to a light period Neutral.(Idell, 2017) (Donham-R.S., et al., 1996; Porkka-Heiskanen-T., Et al., 1989)

3. Materials and methods of research:

The research was conducted on members of the hamster, the first laboratory animal to be used in such experiments, and was raised at the Research Laboratory at the College of Science and Arts at Qolawah. The average temperature was maintained at an average of 20-24 ° C and the food and water were adequate and free throughout the experiment, Which provided a mixture of fodder mixed uniform, ready and includes grain (wheat - barley - beans - salts and metal elements, vitamins and supplements...). Twelve glass crates specially made for the hamster were equipped during the research period. The laboratory was equipped with a controlled lighting system to ensure the alternating lighting required in each experiment cages. The animals were placed on cages at a rate of 6 replicates per cage according to age.

Research Groups:

Phase I (Applicable procedures and hormonal treatment)

Number	Duration	Treatment	Groups
6	100 Day	12 Light – 12 Darkness	First (witness)
6	100 Day*	14 Light – 10 Darkness	the second
6	100 Day**	14 Light – 10 Darkness	Third
6	100 Day ***	14 Light – 10 Darkness	Fourth
6	100 Day ****	14 Light – 10 Darkness	Fifth

*Injected daily with a physiological fluid 0.1 ml Ethyl alcohol + 10 ml saline solution 0.9%
- ** Daily injections with melatonin at a concentration of 6.25 gμ
- *** Daily injections with melatonin at a concentration of 12.5 gμ
- **** Daily injections with melatonin at a concentration of 25 gμ

Phase II (Applicable procedures and hormonal treatment)

Groups	Treatment	Duration
135 Day	12 Light – 12 Darkness	First (witness)
135 Day*	14 Light – 10 Darkness	the second
135 Day**	14 Light – 10 Darkness	Third
135 Day***	14 Light – 10 Darkness	Fourth
135 Day****	14 Light – 10 Darkness	Fifth

*Exposure to the long period of light with daily injections with the physiological fluid mentioned above.
- ** Daily injections with melatonin at a concentration of 6.25 gμ
- *** Daily injections with melatonin at a concentration of 12.5 gμ
- **** Daily injections with melatonin at a concentration of 25 gμ

Note: Melatonin injections are performed before dark for 3-4 hours, i.e., when the hormone melatonin is an internal source (extracted from the pineal gland) to its lower limit of blood

5. Research Plan

Note: In addition to the hormonal treatment described in the previous tables.

Phototherapy 14 Light - 10 darkness		Phototherapy 12 light - 12 darkness		Total Age	Stage
Darkness hour	Light hour	Darkness hour	Light hour	Day	
1000	1400	1200	1200	100	Stage1
1350	1890	1620	1620	135	Stage2

Note: In addition to the hormonal treatment described in the previous tables.

6. Results

Studying the histological structure of the male reproductive system and the effect of alternating lighting periods.

Specification of the fabric structure at the witness group:

Which have been subjected to periods of neutral lighting of light and dark. The study of the sections in the testis showed the circular shape of the spermatozoa, which were approximately 0.23 mm - 0.25 mm in diameter, were contiguous in the ocean, separated from each other as they approached the center, surrounded by connective tissues, capillaries, and nerves in abundance. In addition to the muscle cells on the fibrous layer and connective tissue, and then followed by the basement membrane, and observed Sertoli cells and seminal tissues in their differentiated as they headed towards the glands of sperm tubes, and note the primary sperm cells with large nuclei, and then followed by secondary sperm cells with smaller nuclei, A large number of sperm followed by sperm concentrated in the glands of the central tubes, and the number of sperm tubes was estimated at 620-630. The interstitial cells were formed in circular clusters between

the spermatozoa. It was noted that the larger testis was the most productive sperm and appeared to be more active.

Histological study of the witness in the second stage:

It was noted that the number of sperm tubes that did not contain sperm cells and closed with epithelial cells were lower, compared with the first, and the cells are interstitial here more distinct and several, and believed to be due to the difference in age. Especially that the interstitial cells become the production of testosterone with near the stage of sexual maturity, The effect of the neutralized days is a disincentive to the growth of the testicle, which is believed to be due to the lack of prolactin hormone, which confirms research on its relationship with melatonin hormone Sexual puberty also said Almeida-o.f. & Lincolin-G.A., 1984.

The effect of the long period of alternating lighting on the histological structure of the testicle.

1 - the use of injections physiologic fluid in the pre-puberty with long rotating light.

Compared to the control group, the proximity of sperm tubes was observed, especially in the ocean. The mean diameter was 0.26-0.28 mm. The greater the number of Sertoli cells, the higher the number of sperm cells, the more interstitial cells were observed, This is due to the expansion of the diameter of the tubes, which can be attributed to the increase of the LH-FSH concentrations affecting the surface of the plasmodium membranes of the Sertoli cells, as mentioned in Shaw-D & Goldman-BD, 1995. This activity of the testicle resulted in early sexual maturity, Long-range photovoltaic light During the first two weeks of age on the growth of the reproductive system, the malebegins to respond beginning from the third week.

2 - The use of injections physiological fluid in the post - puberty sexual with long alternating light.

The presence of spermatozoa towards the center of the spermatozoa and its number compared with the control group was observed, consistent with the results of Recio-j, et al., 1998 Who pointed out that the testicles are large and sexually active.

---- The effect of neutral alternating light on epidermal tissue in male hamsters.

1- The first stage.

This group is the group of control or witness, which will compare with the results of the other groups in the same stage. The tissue in the cross section is composed of connective tissue, smooth muscle fibers, and appeared epididymal channels in different forms, and appeared epithelial lining consists of two main cell types, cells and basal cells, and sperm contain large numbers, and this structure of the cypress is the prevailing structure and known, similar to the structure of other mammals (Idell, 2015) Calvo-A., et. al., 1997

2. Phase II

This group is also the group of control or witness, which will compare with the results of other groups in the same stage, and showed here the channels of the epididymis is more distinct and distinct and growth is developed, and we believe that the reason is here for the difference in age which is up to five weeks, and confirm the quantitative results in the tables 5 and 6 when comparing quantitative data.

- Effect of long alternating light on epidermal tissue in male hamsters.

1 - Phase I (treatment, injection of physiological fluid in the stage before puberty).
The epidermal channels appeared to be more extensive, closer to each other, and the primary epithelial cells appeared to be more ciliated, lower, and there was no change in the basal cells. We believe that the reason is due to the activity of the male hormone testosterone in this period.

-- Study of the effect on the channel of the sperm

When exposed to neutral alternating light

1- The first stage. This group is the group of control or witness, which will compare the results of other groups at the same stage.

The serous layer is rich in blood vessels, followed by a thick muscle layer and then a rich connective tissue of elastic fibers, which separates from the epithelial layer ending with cilia. It was observed that the duct was filled with mature sperm, which corresponds to the results of Limanowski-A, et al., 1991

2 - The second stage: This group is the control group or witness, which will compare with the results of other groups in the same stage. At this stage, a slight increase in the diameter of the duct is observed, compared to the first stage, and a slight increase in the muscle layer was observed.

When exposed to long alternating light:

1 - The first stage: the stage of injections physiological fluid and before puberty,

The length of the length of the long light was not limited to the testis and the epididymis, but also to affect the channel of the vector also, This may be due to the increased concentration of GnRH secreted from the hypothalamus region, which affects the secretion of the pituitary gland from the hormone FSH, LH, which in turn stimulates the testicle and stimulate the secretion of testosterone.

The second stage was observed after puberty and only with physiologic injection. The same changes were observed in the first stage, compared to the patient. The difference in age of 5 weeks, which is believed to be affected by the

hypothalamus and increased secretion Hormone-stimulating hormone and testosterone secreted from the testicle.

Effect of injection of melatonin hormone during exposure to long alternating light on the male reproductive system in hamsters

Here, the long-exposure group was subjected to the longitudinal group. The effect on the tissues in the testis and the epididymis was studied. And the channel of the sperm, at several concentrations of melatonin injected at each stage, concentrations are

6.25 -12.5 - 25 µg

- Effect of melatonin injected at 6.25µg concentration on testicular tissue

The first phase is before puberty: It was observed from the study of the sections, the spacing of the sperm tubes from each other, especially in the center, where it reached 19mm -20 mm, and was noted the presence of primary and secondary sperm cells only, and disappeared sperm, that is a severe delay In the structure of the testis, and that hormonal injections stopped their development and development of spermatozoa as well. This can be explained by the fact that melatonin inhibited the secretion of FSH by the pituitary gland, thus decreasing the cyrtoli cells and inhibiting secretion of LH from the pituitary gland, Interstitial cells and reduced concentration of testosterone and prolactin.

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2 - The second stage, which is after sexual puberty:

In the study of the testicular sections, sporadic and empty spherical nanoparticles, especially the spacing of the center, were observed. The diameter of the tubes was almost half the witness's width. It was 0.18mm. Small size cyrtoli, primary and secondary, In the form of small and distant clusters. Compared to the first group, the number of secondary sperm cells was lower, the sphincter spines were larger, and the testicular atrophy resembled that of the pituitary gland in animals (Buzzell-GR, et al., 1992).

- 12.5µg injection effect on the histological structure of the testicle

1- The first phase was observed: the spacing of the tubules was observed here, and it was evident that it was due to the lack of diameter. The development was observed until the stage of the secondary cells only. Interference between the primary and secondary sperm cells was observed. Smaller cyrtoli cells appeared, as did the interstitial cells Sperm.

2 - Phase II: which is the study of the effect on testicular tissue, at the injection after puberty at a concentration of 12.5µg. The presence of secondary sperm cells was rare. Sertoli cells were smaller. The decrease in testicular tissue was greater, compared with the first group. Note the obvious effect of melatonin on maturity in these adult animals. The higher the concentrations of melatonin injected into the same group, the greater the effect of the hormone melatonin, which can be easily observed.

- Effect on testicular tissue at injection of melatonin at 25µg concentration.

1- The absence of sperm and sperm was noticed. Only some of the sperm cells were observed. The presence of a large number of closed and undeveloped sperm germs was noted, especially in the first stage. The absence of secondary sperm cells was observed, and this may be due to the increased concentration of the hormone injected melatonin, which led to the acceleration of laxity in the testis tissue, that is, melatonin has an inhibitory effect that increases the concentration of the hormone.

2 - Stage II: Note here, a decline in testicular and atrophy in the tissues large. Of the increase in the spacing of sperm tubes and the small diameter of these tubes, up to 12mm, and observed the decline of the stage of primary sperm cells, and the lack of size and surface for cyrtoli cells, The direct effect of melatonin injected with a concentration of 25 µg on the sperm cells, and testicular atrophy with the reduction of structural brown tissue, was observed.

Influencing the structure of the matrix of the melatonin

1. The first phase: 6.25 µg injection in animals in pre-puberty stage: The narrow structure of the epidermis was observed in the epidermal duct. The spines were observed spaced apart, and the internal diameter and growth of the connective tissue were observed. The absence of sperm in the epidermal duct and the presence of sperm cells incomplete differentiation, and we see here that the atrophy that has afflicted the testis has affected the epididymis as well.

2 - Phase II: The spacing of the epidermal channels was observed, and we believe the reason is the lack of diameter. The growth of the tissue was observed between them, and the absence of cilia epithelial cells and the absence of sperm and the lack of distinct sperm cells were observed.

Effect of injection at a concentration of 12.5µg of melatonin on the tissue structure of the berth:

1 - The first stage which is before puberty.

The presence of primary and secondary sperm cells in the epidermal glands of the epididymis was observed without the presence and absence of sperm. This result was consistent with atrophy in tissues with atrophy of the epididymis. Concentration of injected hormone.

2 - the second stage after puberty: It was observed that the spacing of the epididymis channels appeared more, and the absence of cells and sperm, and appeared empty channels have some secretions, and likely to be due to the lack of level of testosterone, and thus the lack of protein and sugary, In line with the quantitative study significantly. And observation of atrophy associated with atrophy of the epididymis.

- Effect of injection of melatonin at a concentration of 25µg on the tissue structure of the berth:

1- Stage 1: Before puberty. The absence of cilia and the absence of sperm, and the presence of some primary sperm cells in the lumen of the epididymis, which is associated with atrophy of the testicles and poor reproductive capacity and sexual behavior Also, which is due to lack of secretion of testosterone to affect the mulch and therefore pituitary as well.

2 - Stage II: represents the stage of injection after puberty. There was a decrease in the diameter of the epididymis channels, and therefore their spacing and the growth of the connective tissue between them, and here was very large and clear, and there was an increase in the epithelial layers, the absence of sperm and sperm cells. The structural changes were great and the epididymis was very clear, referred to earlier.

- The effect of injection of melatonin on the tissue structure of the channel of the sperm

The injection is 6.25µg

1 - Phase 1: Flexions were observed in the epithelial layer with few cilia, and flexion in the basal plate. The epithelium appeared to be corneal and multilayered; the presence of primary and secondary sperm cells, absence of sperm, and increased thickness of the muscle layer.

2- The second stage: The internal flexions of the epithelial layer and the lack of cilia, the absence of sperm and the scarcity of primary and secondary sperm cells within the secretions were observed. The muscle layer in the channel glands was clearly thick, 0.22mm - 0.24 mm, while the average thickness in the control group was 0.09 mm, And in the first group 0.12mm 0.14 mm, the tissue regression here was more pronounced. This confirms the inhibitory effect of melatonin hormone and corresponds to quantitative study results.

12.5µg injections
1- The first stage:

The channel here has become narrower and completely devoid of sperm, containing primary and secondary cells, and thickness was observed in the muscle layer reaching 0.18 mm

2. Phase II:

The reduction in the tissue structure was greater. The narrowing of the duct canal and the increasing thickness of the muscle layer was observed. It was 0.26mm - 0.28 mm. This led to the epithelial layer and narrowing of the duct. This is associated with atrophy of the testicle and the epididymis.

25µg injections and an impact on the tissue structure of the sperm duct.

1- The first stage:

Similar, but larger, tissue changes were observed, the narrowness of the canal seemed narrower, the number of epithelial cells increased, their rows increased, their association was less, their cilia was absent, sperm was absent, and some primary sperm cells existed.

2. Phase II:

The thickness of the muscle layer was about 0.30 mm. None of the primary or primary sperm cells were observed, and the testicular and cytoplasmic atrophy was observed in the quantitative study referred to previously. It was observed that the injection of melatonin after puberty it had a greater effect if injected before puberty.

7. Conclusions

1 - Exposure to long periods of light has an impact on the male reproductive system and tissue structure, in increasing the weight and size of testes and size of the epididymal and the size of the epididymis and the amplitude of sperm springs, and large in the cells of Sy6rtoli and change the form of interstitial cells to the shape of spin, and increase the brightness of the channel conveying sperm and the thickness of the thickness of the muscle layer.

2 - The injected hormone has an effect on the structure of the tissue. The narrowing of the tubes and the epidermis and the channel of the sperm, and caused the disappearance of sperm cells with n1 pigment formula and small size of cytoli cells and interstitial cells and increase the thickness of the muscle layer.

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