Original Article

Activin Expression in the Testis: Exploring Its Critical Relationship with Seminal Fluid Composition and Implications for Male Fertility in Rabbits

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Abstract

Background: The activin family of dimeric peptide hormones is produced in the testis and is believed to play paracrine and autocrine roles in regulating steroidogenesis. The quality, effectiveness, and parameters of sperm can be affected by changes in estrogen and plasma levels in the blood. **Objectives:** This study aimed to evaluate the immunohistochemical activity of activin in testicular tissue and its association with spermatogenesis. **Materials and Methods**: The study was conducted at Al-Nahrain University / College of Medicine from January 2024 to March 2025, involving 20 adult male rabbits. Animals were examined to exclude unhealthy individuals and were euthanized via chloroform inhalation. We monitored their behavior and measured their weights both before and after the experiment. Testes were dissected, and seminal fluid was collected for analysis. **Results:** Comparison between the two groups (A, B) revealed statistically significant differences in number and motility of sperm, the width of germ cells, and the density of Sertoli cells. Notably, activin expression and plasma levels were significantly higher in the B group compared to those in group A. **Conclusions:** The expression and levels of activin subunits correlate with sperm parameters. In this study, the impact of disruptions in activin activity throughout the spermatogenesis cycle can be observed, highlighting the essential role of activin in balancing and regulating quality parameters in seminiferous epithelial dynamics and its complex regulatory mechanisms. In this study, we highlight the multifaceted interactions of one of the complex free hormones and its role in hormonal regulation and sperm production efficiency. We also examine the profound biological effects of this hormone, both in excess and in deficiency, on male reproductive health.

Keyword: Immunohistochemical, Tunica Albuginea, Spermatogenesis.

Introduction

The reproductive organ in male gonads is the testis. It has several basic purposes: it produces testosterone, the primary male sexual hormone, and it produces sperm. These roles are critical not only for the retention of masculine features but also for species protection, as determination is conferred in this manner [1]. Anatomically, the testis is covered with scrotal and irregular, hard connective tissue. The outermost layer of the testis is tunica vaginalis (internal spermatic fascia), and underneath it is tunica albuginea. The testicular mediastinum is formed by the tunica albuginea, which is in the posterior area of the testicle tissue [2]. Spermatogenesis is the creation of sperm cells (male gametes) in the seminiferous tubular tissue. Thyroid hormones influence the spermatogenesis development [3]. Spermatid and spermatocytes are initially formed at the age of 14-15 weeks [4]. Sperm cells are discharged into the lumen region, which subsequently travels to the epididymis for maturation. Sperm will travel via the rete testis and ductuli efferentes on their journey to the epi-

didymis. The ductuli efferentes tissue is located between the testis and the epididymis [5]. The epididymis is a five-segmented epithelial tube composed of the beginning segment, head, corpus, cauda, and vas deferens. The epididymis provides nutrition for sperm development [6]. Rete testes are brief tubules in connective tissue that are shielded with simple cuboidal epithelium and connection tubule recti and ductuli efferentes [7]. The walls of ductuli efferentes (efferent ducts) remain thinner than those of tubules recti. Histology of rete testes and ductuli efferentes is rarely studied. This article will describe the histological study of the rete testis and ductuli efferentes in rabbits. McCullagh created the word "Activin" in 1932 to describe a proteinaceous gonadal factor that particularly controlled pituitary LH [8]. During the biochemical purification of activin, two new LH-modulating proteins, activin (LH-stimulating moiety) and activin-binding protein, were purified [9]. Activin and activin constitute dimeric molecules that are connected; activin is composed of a u-subunit and a P-subunit. Activin A is assumed to have a role in reproduction management [10]. According to the activin theory, hypothalamic GnRH causes the first gonadotropin surges, while declining and insignificant activin serum concentrations allow for subsequent LH surges [11]. This study aimed to evaluate the immunohistochemical activity of activin in testicular tissue and its association with spermatogenesis.

Materials and Methods

This study was conducted at the College of Medicine, University of Al-Nahrain from January 2024 to March 2025. The experimental animals were acquired from the animal house in Baghdad. A total of 20 adult male rabbits, 30-38 weeks of age and weighing (2±0.5) kg, measured by electronic balance, were used in this study. All the animals were examined by the veterinarian staff in the animal house of the biotechnology research center at the University of Al-Nahrain to evaluate their general health to exclude unhealthy animals. The animals were held in a separate standard cage measured in a central animal care facility 50*60*40 cm in dimensions; each cage contained 5 animals and was given free access to a standard rabbit diet and free access to tap water with good ventilation. The animals were left for 2 weeks in the same environment, and during this period, 10 rabbits died before starting the experimental study. In this study, 20 adult male rabbits were divided into two groups (10 rabbits in each group) according to semen analysis to determine which is better in terms of the number and movement of sperm**.**

Group A: Consists of 10 animals; this group has a sperm count ≥ 70 million.

Group B: Consists of 10 animals; this group has a sperm count < 70 million.

Animal Dissection

The animals were euthanized by inhalation of chloroform that was soaked in a cotton piece in an airtight chamber for 3-5 min. Then, the animal was set on an anatomical stage in a dorsal position and fixed on the dissecting table by its four limbs. Although the mortality cases during the experiment were excluded, the animals' behavior was observed during the experiment. In addition, we measured the weights of animals before and after the experiment. Dissection of the rabbit was done using a scalpel and scissors. They were dissected by median abdominal incision ventrally to excise the testis from the abdominal cavity. Then, we collected seminal fluid by milking the epididymis to count the sperm and check the motility and morphology. We then measured the volume of the testis

before preserving the specimens in 10% formalin for (24-48) hours to obtain paraffin sections.

Tissue Preparation for Paraffin Section

The testis specimen was prepared for paraffin section as follows:

- **Fixation:** Tissue samples were kept separately in containers, and specimens were directly fixed in 10% neutral buffered formalin, pH: 7. The saline was prepared from Na2HPO4 (6 gm), NaH2PO4.H2O (4 gm), formalin (100 ml), and distilled water (900 ml). The tissues were fixed for 48 hours.
- **Processing:** The fixed tissues were then processed for the routine paraffin wax embedding process. This procedure includes: dehydration, clearing, infiltration, and embedding (11).
- **Dehydration:** Achieved by passing the tissue through ascending concentrations of ethanol (70%, 90%, and 100%) at various times; the tissue was saved in ethanol 70% for 24 hours, then passed to ethanol 90% for six hours, and ethanol 100% for two hours.
- **Clearing:** Accomplished by putting the tissue through the xylene (15 minutes) and using two changes of xylene to remove alcohol from tissues and ensure good tissue transparency.
- **Infiltration:** Achieved in paraffin wax (two hours) at 60°C in the hot air oven (Fisher Scientific, Model 615G); two changes were implemented, one hour for each.
- **Embedding:** Finally, embedding was achieved in paraffin wax at 60°C. The specimens were transported to be blocked in paraffin wax using a labeled tissue cassette and stainless-steel molds. An electric wax dispenser (Lips Haw, Model No. 222) was used for embedding the sections. After hardening of the blocks, they were separated from the molds and kept in a refrigerator at 4°C until they were sectioned.
- **Sectioning:** Paraffin blocks were sectioned sagittally at 5μm thickness using a microtome (Richert - Jung, 2030 MOT Biocut); the microtome works in both manual and automatic modes. Ribbons of sections were properly laid down on the surface of hot water (40°C) using a water bath. The ribbons were then collected on clean glass slides; the sections will be dyed with hematoxylin and eosin for general histological examination.
- **Hematoxylin-Harris stain:** It contained alum as a mordant and was chemically ripened with mercuric oxide; Harris is useful for general purposes hematoxylin and gives particularly clear nuclear staining (blue-black). The staining solution was prepared as follows: Hematoxylin 2.5 g, absolute alcohol 125 ml, potassium alum 50 g, distilled water 500 ml, mercuric oxide 1.25 g, and glacial acetic acid 20 ml.
- The hematoxylin was dissolved in absolute alcohol, and the alum had been dissolved in warm distilled water in a flask, each one prepared separately. The first solution was then added to the dissolved alum; the mixture was rapidly brought to the boil, and the mercuric oxide was then carefully added. The solution was rapidly cooled by plunging the flask into cold water. When the solution was cold, whene added a glacial acetic acid , the stain was ready for quick use [12].
- **Eosin staining:** Eosin is the most suitable stain used with hematoxylin as a counter stain, to show the general histological architecture and to distinguish between the cytoplasm of different cell types (red-pink). stain formulated was done by dissolving 0.5 g of Eosin-Y in 95% ethanol with 0.5 ml acetic

acid. After filtering the stain, it became ready for use [12].

Staining Procedure

1. Dewaxing: In xylene for (20-30) minutes.
2. Rehydration: The sections were passed through descending grades of ethanol alcohol (100%, 90%, and 70%) for a total period of 3-5 minutes.
3. The slides were then washed in distilled water for five minutes.
4. impaired Slides for (1m) in hematoxylin stain, rinsed in tap water till the deepening of color develops (usually 2-3 minutes required).
5. Then stained with eosin for one minute.
6. Dehydration: Sections were dehydrated in an ascending concentration of ethyl alcohol (70%, 90%, and absolute), 3-5 minutes for each.
7. Then slides were cleared with xylene for five minutes.
8. Finally mounted in Canada balsam. Histological specimens of the testis were examined under a light microscope (Richert Chung) at powers of 10X, 40X.

Photography, Morphometric and Histological Analysis of Testis Section Examination and Photography

Photography: The prepared histological tissue slide, stained with hematoxylin & eosin staining, was examined for histological evaluation and estimation using a light microscope (LEICA DM750, Germany). The observed fields were snapshot using a Digital Microscope Camera (Model MC500) with 5 megapixels resolution, and the pictures were saved in JPEG format. All the examination procedures took place in the histology & embryology laboratories at the Department of Anatomy, College of Medicine/AL-Nahrain University.

Morphometry: Image J analysis was done using the software Image J (Java–based image processing program developed at the National Institutes of Health, USA) version 1.47p, which was already installed on a personal computer. Image J software is the keystone in the morphometric study, by which different processing and procedures can be performed that can read certain image formats, including TIFF, GIF, JPEG, and BMP. It can calculate area and pixel value statistics of a user-defined selection. Also, it can measure distance and angles. The procedure for morphometric analysis of images was done by applying the following steps:

1. The image J program was opened by double-clicking its exe. icon.
2. The file option in the menu bar was clicked, and the open command was chosen and clicked.
3. A calibration slide image (which was previously captured and saved on the personal computer) was selected. As the calibration slide image was opened, select the straight tool from the tools bar in the program panel for measuring straight lines (length) of distance, for example.
4. Applying this tool for measuring the distance between intermediate and long lines present in the image of the calibration slide (distance is equal to 50 µm).
5. Analyze option from the menu bar was clicked, and the set scale command was selected.
6. After selecting and clicking the set scale option, a window will be displayed.
7. Then, File from the menu bar was selected again, and the Open option was chosen.
8. A saved captured image was selected and opened to carry out a morphometric analysis.
9. Then, calibration for the tested image was done using the appropriate measurement tool.

Statistical Analysis

Data were analyzed BY using Statistical Package Programe for Social Sciences (SPSS) version 26. The data are presented as mean, standard deviation, and range. Categorical data presented by frequencies and percentages. Relying on t-test and fact-finding of Disagreement (ANOVA) (two-tailed) were used to compare the continuous variables accordingly. Post hoc tests (LSD) were run to confirm the differences that occurred between the study groups. It turned out that a level of P-value less than 0.05 was considered significant.

Ethical approval

The College of Medicine at the University of Al-Nahrain ethical committee approved this study's ethical approval, obtaining verbal consent from each specimen of this study.

Results

Immunohistochemistry of Testicular Tissue Immunohistochemical Staining of Group A

The **activin** expression of testicular tissue from rabbits with high sperm count showed high immunohistochemical intensity, as shown in Figure 1, with positive staining in both Sertoli cells and Leydig cells.

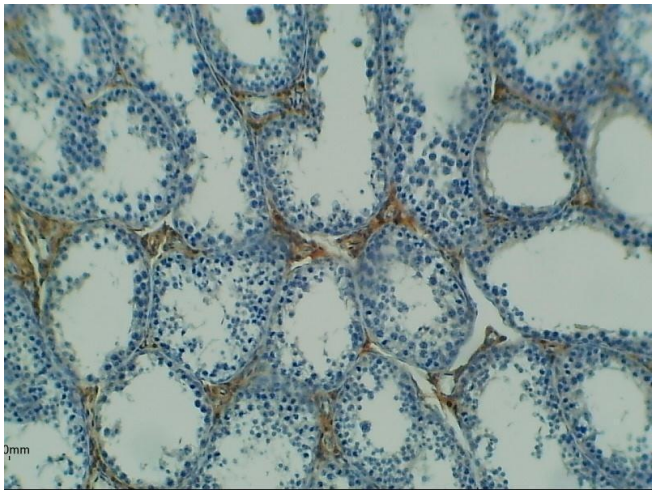

Figure 1: Immunohistochemical stain of testicular tissue from animals with high sperm count showed **activin-positive** staining in both Sertoli cells (blue arrow) and Leydig cells (green arrow). DAB stain, Hematoxylin counter stain 40X

Immunohistochemical Stain to Group B

The **activin** expression of testicular tissue from rabbits with high sperm count showed high immunohistochemical intensity Figure (2), more evident in the cytoplasm of Sertoli cells and Leydig cells.

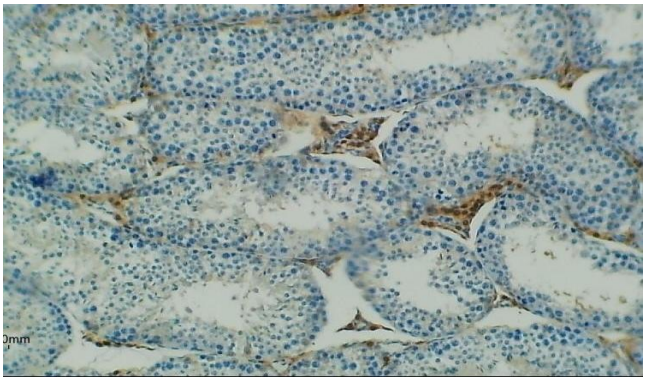

Figure 2: Immunohistochemical stain of testicular tissue from animals with low sperm count showed high expression of **activin** in the cytoplasm of Sertoli cells (black arrow) and Leydig cells (red arrow). DAB stain, Hematoxylin counter stain 40X.

Correlation Coefficient

Correlation between immunohistochemical expression of **activin** in the study of groups as shown in (Table 1).

Table 1: Correlation coefficient between immune-histochemical expressions of **activin** with (sperm count, motility, germinal layer width) in the study groups.

Test	Parameters	Correlation coefficient-r
		Activing
Intensity (IHC)	Count	0.70 *
		0.89 **
	Motility	0.62 *
		0.77 **
	Germinal layer	0.47 *
		0.61 **
(P≤0.01).		

*= value for group A

** = value for group B

Correlation between activin levels in the study of groups by using ELISA, as shown in Table 2.

Table 2: Correlation coefficient between **activin** levels and (sperm count, motility, and width of germinal layer) in groups

Test	Parameters	Correlation coefficient -r
		Activing
ELISA	Count	0.78 *
		0.73 **
	Motility	0.66 *
		0.68 **
	Germinal layer	0.73 *
		0.42 **
(P≤0.01).		

*= value for group A
** = value for group B

Discussion

Correlation between activin immune-histochemical expression and plasma level with sperm count

In this study, it was shown that **activin** hurts sperm count. When the strong expression and plasma level of **activin** are associated with high sperm count by both the IHC test and ELISA. Previous studies showed that serum **activin** in infertile men was strongly correlated with sperm count [13]. Additionally, there is a clear inverse relationship between serum **activin** and LH level in adults [14]. Serum levels of activin exhibit a robust positive correlation with both testicular volume and sperm count; among individuals experiencing infertility, this relationship is particularly pronounced, **activin** decreases and LH increases. In general, there is a very good correlation with the degree of spermatogenic damage [13]. Furthermore, neither sperm motility nor morphology was significantly related to **activin** level in any group. In all patients, the sperm concentration was positively correlated with **activin** concentrations (r = 0.70, P > 0.01). It was clearly stated by the current study that **activin** B may be a marker of exocrine testicular function and could offer improved diagnosis and treatment modalities for male infertility [15].

Correlation between Activin Immuno-histochemical Expression and Plasma Level with Sperm Motility

When studying the motility of sperm and the changes that occur when the **activin** expression and plasma levels rise, a positive correlation between sperm motility and high expression of **activin** was observed [16]. The LH hormone is the main supporter of sperm vitality and reproduction during all stages of sperm formation and spermatogenesis. This result was agreeable with previous study results [17]. This relationship can be illustrated by understanding the negative feedback effect of activin on luteinizing hormone (LH) secretion from the pituitary gland, which subsequently weakens or inhibits the entire sperm production and synthesis process. This can be observed during sperm quality tests. This complex feedback mechanism modifies and rebalances this process, maintaining the precise functioning of the male reproductive system.

Correlation between Activin Immuno-histochemical Expression and Plasma Level with Germinal Layer Width

In this study, it was seen that the high plasma level of **activin** and immunohistochemical expression had significant sequences and effects on the histological structure of the testicle. This might be associated with changes in the LH hormone level due to the negative feedback association between the **activin** expression and pituitary LH hormonal secretion status. Expected levels of LH would decelerate the proliferation of spermatocytes, ultimately leading to low count, and even low motility and vitality index

of sperm. This study showed a negative correlation between high plasma levels and immunohistochemical expression of **activin** and germinal layer width. This relationship between serum **activin** levels and spermatogenesis may be since **activin** B in adult men is possibly a joint product of Sertoli cells and germ cells, including the stages from pachytene spermatocytes to early spermatids [18].

Conclusion

The expression and levels of activin subunits correlate with sperm parameters. In this study, the impact of disruptions in activin activity throughout the spermatogenesis cycle can be observed, highlighting the essential role of activin in balancing and regulating quality parameters in seminiferous epithelial dynamics and its complex regulatory mechanisms. In this study, we highlight the multifaceted interactions of one of the complex free hormones and its role in hormonal regulation and sperm production efficiency. We also examine the profound biological effects of this hormone, both in excess and in deficiency, on male reproductive health.

Interest Conflicts

None**.**

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References

[1] Das PK, Mukherjee J, Banerjee D. Functional morphology of the male reproductive system. Textbook of veterinary physiology: Springer; 2023. p. 441-76.

[2] Gurung P, Yetiskul E, Jialal I. Physiology, male reproductive system. StatPearls [Internet]: Statpearls publishing; 2023.

[3] Houda A, Nyaz S, Sobhy BM, Bosilah AH, Romeo M, Michael JP, et al. Seminiferous tubules and spermatogenesis. Male reproductive anatomy: IntechOpen; 2021.

[4] Jahnukainen K, Chrysis D, Hou M, Parvinen M, Eksborg S, Söder O. Increased apoptosis occurring during the first wave of spermatogenesis is stage-specific and primarily affects midpachytene spermatocytes in the rat testis. Biology of reproduction. 2004;70(2):290-6.

[5] Nordhoff V, Wistuba J. Physiology of sperm maturation and fertilization. Andrology: Male Reproductive Health and Dysfunction: Springer; 2023. p. 55-75.

[6] Zeng J, Gao W, Tang Y, Wang Y, Liu X, Yin J, et al. Hypoxia-sensitive cells trigger NK cell activation via the KLF4-ASH1L-ICAM-1 axis, contributing to impairment in the rat epididymis. Cell Reports. 2023;42(11).

[7] ducts Delicate E. Endocrinology and pathology of rete testis and efferent ductules. Encyclopedia of reproduction. 2018:279.

[8] Woodruff TK, Mather JP. Inhibin, activin and the female reproductive axis. Annual review of physiology. 1995;57(1):219-44.

[9] Cappa M, Cianfarani S, Ghizzoni L, Loche S, Maghnie M. Therapeutic neuroendocrine agonist and antagonist analogs of hypothalamic neuropeptides as modulators of the hypothalamic-pituitary-gonadal axis. Advanced Therapies in Pediatric Endocrinology and Diabetology. 2015;30: 106-29.

[10] Mann RJ. Consequences of elevated LH on oocyte, embryo, and maternal reproductive health: Case Western Reserve University (Health Sciences); 1999.

[11] Alismail KH. Cytokine Level in Serum and Follicular Fluid in Women Undergoing

ICSI Treatment and Their Impact on ICSI Outcome: Alfaisal University (Saudi Arabia); 2022.

[12] Mendham J, Denney R, Barnes J. Textbook of Quantit A Tive Chemical Analysis. Pearson; 2016.

[13] Wijayarathna R, Hedger MP. New aspects of activin biology in epididymal function and immunopathology. Andrology. 2024; 12(5):964-72.

[14] Bloise E, Ciarmela P, Dela Cruz C, Luisi S, Petraglia F, Reis FM. Activin A in mammalian physiology. Physiological reviews. 2019; 99(1):739-80.

[15] Wijayarathna Rd, De Kretser D. Activins in reproductive biology and beyond. Human reproduction update. 2016;22(3):342-57.

[16] Kadivar A, Khoei HH, Hassanpour H, Golestanfar A, Ghanaei H. Correlation of adiponectin mRNA abundance and its receptors with quantitative parameters of sperm motility in rams. International journal of fertility & sterility. 2016; 10(1):127.

[17] Sengupta P, Arafa M, Elbardisi H. Hormonal regulation of spermatogenesis. Molecular signaling in spermatogenesis and male infertility: CRC Press; 2019. p. 41-9.

[18] Kauerhof AC. Involvement of Activin A in the Development of Chronic Testicular Inflammation and Fibrosis: Dissertation, Gießen, Justus-Liebig-Universität, 2019; 2019.