Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 5 (2)

Histomorphologic Effect of Castration on the Prostate Gland in Albino Wistar Rat

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ABSTRACT

10 Albino Wister rats of about the same post-pubertal age, size and weight were used in this study. 7 of them were castrated and sacrificed in succession of four days interval to observe the effects of castration on the prostate gland. The other 3 served as the control.Paraffin wax sections were made, stained with Weiggert'sHaematoxylin and Van Gieson's stain and studied under the light microscope.There was rapid loss of stereocilia, secretory granules and rapid reduction in height from Pseudostratified columnar epithelium to simple cuboidal cells. The interacinar tissue increased with reduction in size and diameter of acini. The nuclei became pyknotic. These go to show the dependence of the prostate gland on functional testes.

Key words: Accessory Reproductive, Castration, Acini

INTRODUCTION

The prostate is ahighly specialized male accessory genital gland that secrets products which protect and affect the viability and fertilizing potential of spermatozoa. They are not essential for life but are of major medical importance as frequent sites of infection, inflammation, hypertrophy and carcinoma.

The rat prostate is the largest of the male accessory organ next to the seminal vesicles. The prostate contains 30 – 50 branching tubuloalveoler or saccular glands, 16 – 32 excretory ducts, a capsule, dense stroma, blood vessels, lymphatics and nerves. The lumen of the ducts and glands usually are dilated with secretion. The adult prostrate presumably is in a continuous state of secretory activity. Each day 0.5 – 2ml of secretion are transported from the excretory ducts into the urethra via the openings on the right and left sides of the colliculusseminalis (verumontanum or prostatic utricle). The thin fibroelastic capsule surrounding the prostate gives rise to septa, which extend inward and subdivide the tissue into lobes, embedding the glands in a fibroelasticstroma.

The tubuloalveolar glands vary in size and shape in the adult. The fibroelastic stoma accounts for approximately 25% of the total volume of the gland. Smooth muscle cells and fibroblasts are the most common cell types. These cells, plus the abundant collagen and elastic fibres, impart a characteristic elastic consistency to the gland, which is useful for distinguishing normal from abnormal glands during physical examination. The epithelium is highly folded and often forms papillae, which project far into the lumen. Each fold is supported by an attenuated branch of stromal connective tissue and the epithelium rests on a thin basallamina. The elongated tubuloalveolar glands are irregular, tortuous and branching. Saccular recesses and cystic dilatations of the alveoli and ducts are common.

Within the alveoli are two types of epithelial cells. The predominant cell is a columnar secretory cell containing a moderate number of oval mitochondria, apical Golgi apparatus and densely packed secretory vacuoles[1]. The secretory vacuoles are present in the apical cytoplasm and Golgi region. Formation of multivesicular bodies seems to occur in the area of the Golgi. The rough surface endoplasmic is well developed, although less so than in the rat, and tends to extend throughout the cytoplasm. The apical surfaces of the columnar glandular cells have a moderately well developed coat of short microvilli.

According to some investigators, morphological differences between central and peripheral regions of the prostate can be seen at both the gross and microscopic levels in coronal and sagittal sections of the whole prostate gland[2,3]. The secretion of prostatic fluid is continuous in the aged. The secretion frequently contains acidophilic, lamellated, oval concretions or "corpora amylacea", which stain brightly with Eosin. Aging increases the number of corpora amylacea in the gland and establishes extreme variation in

size.However, with aging and presumably response to hormonal stimulation, extensive hypertrophy and hyperplasia occurs within the gland.

Prostatic secretions account for approximately 30% of the seminal fluid or ejaculate. During sexual excitement, certain compounds are secreted at an accelerated rate; for example, resting fluid contains a lower concentration of acid phosphatase than does stimulated secretion[4]. Human prostatic fluid contains diastase, B-glucuronidase, proteolytic enzymes, fibrinolysin, citric acid, acid phosphase, choline, cephalin, cholesterol, magnesium, and zinc[5,6]

Normally, the immature prostate is very small, is usually non palpable and does not secrete. At puberty, the hormonal secretion of the testis, activated by pituitary gland induces maturation.Castration and allied phenomenon vizhypophysectomy or androgen deficiency produce atrophy and cessation of secretion in post pubertal individuals and prevent normal growth and development of the prostate in pre-pubertal individuals. Oestrogen causes atrophy of the prostate in most post-pubertal individuals and interfere with the normal growth and development of the prostate in most male animals. Androgen replacement therapy will reverse the atrophy (post-pubertal) or restore normal maturation of the gland (pre-pubertal) in most castrated mammals, as well as those with androgen deficiencies.

Dramatic changes are noted in growth and involutional patterns with fetal development, puberty and old age. Involutional atrophic changes occur again with advancing age- usually during the fifth decade- and affect different parts of the gland in a non-uniform pattern. Carcinoma mainly originates in the posterior lobe or purely male region i.e. the part sensitive to androgens[7]. Most age-associated changes, however, have been noted in male rodents. A comparable set of alterations have not been reported in elderly men. In older men however, there is no depletion of acid phosphatase, and the gland often increases in size and shows little or no impairment of its ability to take up androgenic hormones.

Extensive work has been done with the light microscope in the investigation of the effects or consequences of castration on the ventral prostate gland and is well documented. The early workers in this field are[8,9,10,11]. The testis produces testosterone, the body's major androgen on which the accessory reproductive organs are dependent for growth, development and maintenance.

Castration cause a decrease in serum testosterone level in the body and the level in castrated rats drops only slowly and at 45 days is till 20% of normal[12]. The effects of castration on the prostate – prostatic atrophy, can be reversed with testosterone treatment[8,13,1] Histoquantitative means have also been developed to precisely describe the morphologic events in the prostate tissue and[14] have developed a method especially suitable for electron microscopic assessment of cellular alterations in the prostate.

Moore *et al.,*[8] reported that few days after castration the prostatic epithelium decreases, the glandular acini shrink and the proportion of the stroma appear to increase.In the earlier works, the histological investigations had been descriptive or qualitative in nature hence the difficulty in evaluating the changes of epithelium and stroma during tissue atrophy excepting Arvola's study[15] on hormonal control of the amount of prostatic tissue components. But now new stereological, morphometric or histoquantitative methods have been successfully applied to the studies on the prostate gland.The epithelium of the prostate used to be the only or main target of androgenic hormones with special reference to testosterone, and possibly its only target[11]. Later much attention was being paid to the role of the stroma in the regulating the differentiation of the prostatic epithelium[16,17,18].

Ahistoquantitative method[19] showed the effects of castration on the various stereologic and morphometric parameters of the rat prostate with the intention of achieving a deeper quantitative insight into the involvement of the epithelial and stromal tissue compartments in hormone induced tissue atrophy. The action of testosterone on the ventral prostate lobe of castrated rats as assessed with stereologic - morphometric method, was studied byHuttunen*et al.,*[20] in which attempts were made to clarify the response of the ventral prostate tissue compartments, the acinar parenchyma, and inter-acinar tissue to testosterone treatment initiated 30 days after the animals were castrated, and when the glands are completely atrophied. Also reported is reduction in size and number of smooth muscle and epithelial cells[21]. Testosterone replacement therapy in castrated rats usually completely reverses these responses mentioned above[22].

The present study attempts to establish the effects of post-castration atrophy on the ventral prostate and make histoquantitative morphological analysis. The changes that follow castration, whether sudden, or gradual and if maintained, increased or decreased with increasing number of days post castration, is also noted.

MATERIALS AND METHODS

A total of 10 albino rats of about the same post-pubertal age and size, and weight that fall within the range of 280 ± 20gm, were put indesiccators and anaesthetized with ether. The hair overlying the scrotal area was shaved. The skin was cleaned with an antiseptic (dettol in water). An incision was made through the skin of the scrotum and the testis on one side carefully separated from the surrounding tissue by blunt dissection. A second incision was then made through the transparent tunica vaginalis, and then retracted exposing the testis. A ligature of catgut suture was made around the tunica vaginalis and spermatic cord thus cutting off blood

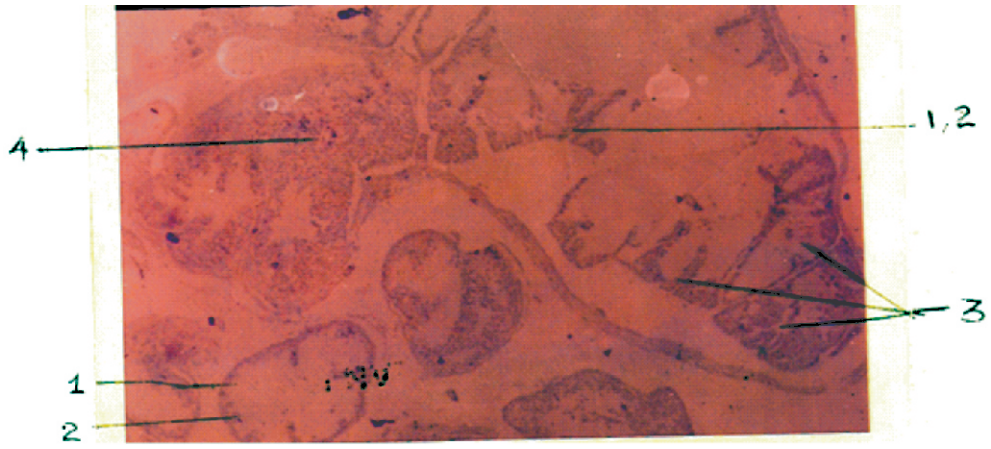
supply and maintaining haemostatis. The testis is removed by a cut just distal to the ligature. The second testis is then removed in the same manner and the skin incision edges approximated and closed with two or three silk sutures. Care was taken not to interfere with the function of the rectum and anus. Procaine penicillin was sprinkled on the wound before the actual suturing was done. The rats remained under post-surgical care which involved daily cleaning of the wound with sulphonamide powder.

Finally, the rats were sacrificed, 3 on day zero (i.e. day of surgery) to serve as controls, then the rest which served as test groups were sacrificed on days 4, 8,12,16, 20, 26 and 32. The prostate glands were carefully dissected free and fixed in 10% formalin at 25°C (room temperature) for 48 hours and processed by routine histological techniques for paraffin wax. From paraffin embed, 5 µm tissue sections were cut and subsequently stained with Weiggert'shaemotoxylin and Von Gieson's stain according to Von Gieson's method for collagen fibers[23] , cleared with Xylene and mounted in resin medium (DPX).They were visualized with a light microscope; using both low power (x10) and high power (x40) magnification for histological study of both test and control tissues. Photomicrographs were taken using a Leitz-Dialux 20 EB microscope with attached photographic camera.

One of the sections of each prostate gland was cut at about the level of largest circumference of the lobe of the gland. About 10 slides of such sections were representative of the whole gland. The cyto-architecture of the each specimen mounted under the light microscope was studied in details taking note of changes noticed in the epithelium, stroma, alveoli, position of the nucleus, aggregation of nuclei, smooth muscle cells and its lamina propria. The number of alveoli on each slide was counted; and with the same magnification for the varying number of slides got after castration, the number of alveoli in the field of view were counted and recorded.

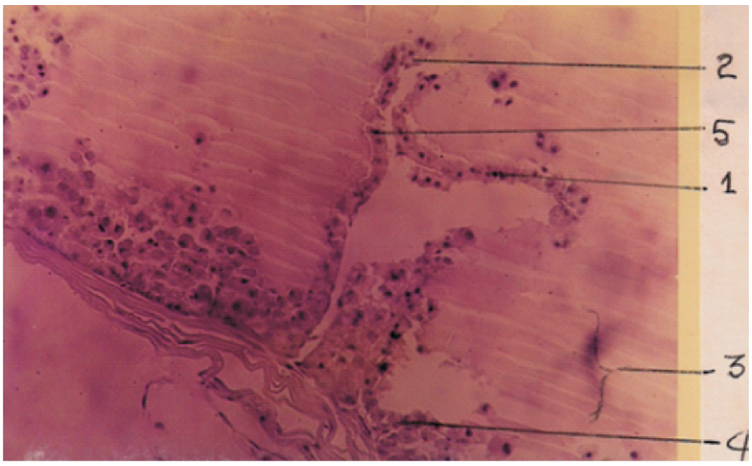
RESULTS

Control Specimen: At the end of the experiment, the controls showed no significant Gross Anatomical changes. There was also no significant increase or decrease in the glandular mass of the gland. A view of the control specimen under the microscope revealed alveoli that were varied in size and had no defined shape. The lumen of each acini was irregular in shape and large because of folds of epithelial cells seen projecting into the alveolar lumen.



Photoplate I: Transverse Section of Normal (Day Zero) Prostate Gland (x40)

1. Pseudostratified epithelium 2. Stereocilia3.Irregularly shaped acini4.Fibromuscular stroma



Photoplate II: Transverse Section of Normal (Day Zero) Prostate Gland (x250)
1.Epithelium of an acinus 2. Centrally placed nucleus 3.Bands of Collagen fibers in acinar lumen
4. Distinct uninuclear columnar cells 5.Distinct cell membrane lining each cell.

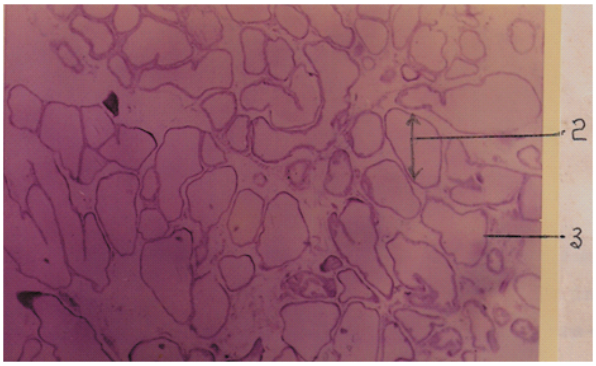
Within the lumen of some of the acini were prostatic concretions called "corpora amylacea" – they are formed by concentric layers of coagulated prostatic secretions. A look at the high power (x250) reveals an epithelium made up of simple tall columnar cells with some basal cells in-between giving it a pseudostratified epithelium appearance. Their nuclei were seen to be basally located, while the tall columnar cells had the presence of stereocilia at their apical regions. The prostate gland showed a distinct fibromuscularstroma which in the periphery is less coarse in texture and less prominent than in the central regions, and the ducts do not branch as they do in the central zone.

Test Specimen
The prostate gland is small in size which posed some difficulty in appreciating the gross anatomical changes. However, the shape was maintained but with a gradual decrease in size and glandular mass as days passed after castration. The diameter of sections made on the slides seen with the naked eye revealed a significant decrease in the diameter following the days after castration.

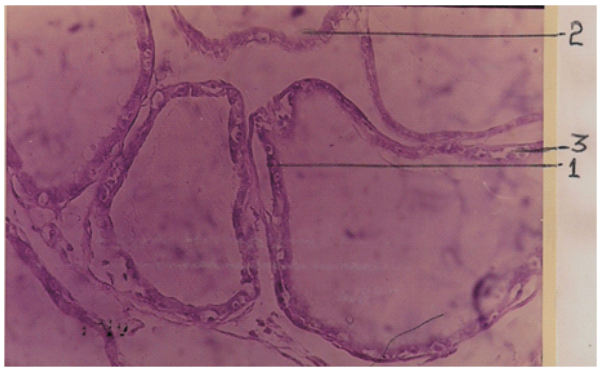
Under the microscope at x40 magnification, significant effects of the castration were seen and better appreciated. There was an increase in the number of alveoli seen in a microscopic field (magnification of x40) as outlined:

Day (Post Castration)	No. of Aveoli
Zero (Control)	7
4	20
8	25
12	20
16	12 (But most of them were coalesced together)
20	24
26	26
32	29

This reveals a rapid increase in the number of alveoli on the 4th day which is maintained with only a slight increase on the days following to the end of the experiment.

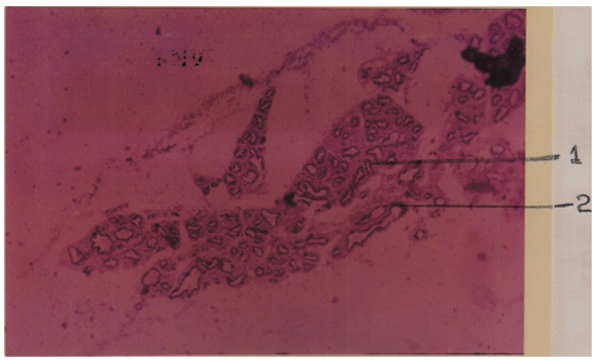


Photoplate III: Transverse Section of Prostate Gland 4 Days Post Castration (x40)
1. Increased number of acini visible in one field of view 2.Reduction in diameter of each acinus 3.Reduced height of epithelial tissues.



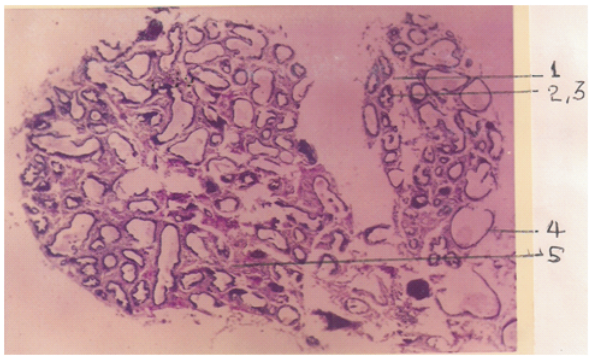
Photoplate IV: Transverse Section of Prostate Gland 4 Days Post Castration (x250)
1. Simple cuboidal cells comprising the epithelium2.Indistinct sterocilia3.Indistinct secretory granules.

Also there is a progressive decrease in the size of the lumen with days. The change in the height of the epithelium was very rapid, the tall columnar cells rapidly decreasing in height to cuboidal epithelial cells just 4 days post-castration and the decrease in height was gradual subsequently. The secretory granules andstereocilia disappeared on the 4th day post-castration. The nuclei of the gland became apparently more numerous and appeared to clump together. The nuclei are generally pyknotic and centrally located. From the 12th post-castration day, the cell membranes were no longer distinct and this gave the epithelium a multinucleated appearance.

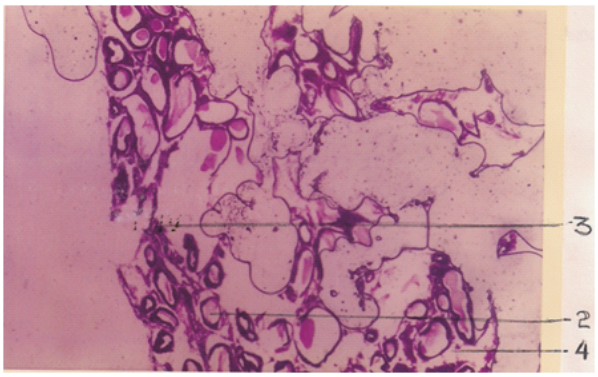


Photoplate V: Transverse Section of the Prostate Gland 16 Days Post Castration (x40)
1. Nuclear proliferation 2. Nuclear aggregation at apex of cells, with nuclei lining lumen of acinar.

On the 20th day post-castration, the cytoplasm was highly reduced and aggregates of nuclei surrounded the lumen of their acini which formed intraluminal papillae without distinct cell membrane. The connective tissue became mixed up and course in all directions. Though the elastic and collagenous fibers became distinct, there was a decrease in their individual size and mass.



Photoplate VI: Transverse Section of the Prostate gland 26 Days Post-castration (x40)
1. Increased interacinar tissue 2.Pyknotic nuclei 3. Nuclear aggregation toward lumen 4. Loss of stereocilia and secretory granules 5. Much reduced smooth muscle cells.



Photoplate VII: Transverse Section of the Prostate Gland 32 Days Post-castration (x40)
1. Atrophy of gland2. Prostatic concretion (corpora amylaceae) 3. Loss in smooth muscle fibres 4. Loss in connective tissue.

DISCUSSION:

This study was aimed at investigating the effects of castration on the prostate gland. It is important to mention the prerequisites for the use of histoquantitative methods:

1. The stereologic model of the tissue must be outlined. This is of importance as different tissue compartments in the sections and their boundaries, as well as arrangement in the reference space and geometrical shape has to be determined.

2. The degree of orientation must be defined. The section should be cut in one direction only; the surfaces of the structures have no favourite direction or orientation axis.

3. The distribution of tissue structure within the reference volume is a matter of importance. Also variations of parameters between different sections and microscopic fields must be determined. This knowledge gives an insight into the necessary sample size and shape to keep the method within the acceptable limits of error.

4. Histochemical techniques and the effects of technical artifacts in the discussed values due to tissue processing must be reduced to minimum. This requires strict standardization.

Previous studies using histoquantitative methods have shown that castration induced prostatic atrophy.[15] Observed luminal decrease from 70% to 18% and four times increase in the proportion of stromain the prostatic tissue, during a period of 270 days, utilizing a linear measurement method.Deklerk[24] reported a 92% decrease in total epithelial cell number and 85% decrease in the epithelial cell rise, with a reduction in stromal cell number by only 32% and size by 23%, 17 days after castration.Huttenen, Romppanen and Helminen[20] used improved histoquantitative and stereologicmorphometrical parameters of the rat ventral

prostate, to confirm the process of prostatic tissue atrophyat close interval after castration.

There is considerable progressive reduction in size of each glandular mass which became more distinct in the 2nd, 3rd and 4th weeks of the experiment. This could be as a result of the deficiency of testosterone in the maintenance of the prostate.A reduction in the diameter of each glandular mass implies a reduction in diameter of individualacini. Transverse sections of glands also illustrate the progressive reduction in diameter following the days of castration.

When the test and control specimen of the prostate gland were compared microscopically, there were striking differences. There was nuclear "proliferation" in the prostate and the nuclei became characteristically pyknotic. This is the most striking feature of glandular atrophy due to reduction in testosterone level in the circulating plasma. The increased interacinar tissue corroborated with the findings ofArvola[15] and Deklerk and Coffey[25].

The speed with which the epithelial cells change from tall columnar to cuboidal cells is an index of their proneness to testosterone deprivation. This result is in line with earlier reports that epithelial cells react rapidly to lack of androgenic hormones[26,1,11]. However, it is also possible that the interacinar tissue which is especially abundant in smooth muscle cells and fibroblasts[27], would immediately react to lack of androgen hormones.

The pyknotic nuclei and atrophy of principal columnar cells of the epithelium was associated with increased lysosomal activity in the prostate.The cytoplasm of the epithelial cells were greatly reduced which resulted in their changing from tall columnar cells to cuboidal cells. The stereocilia at the apices of control specimen were no longer seen from the 4th day post–castration. The secretory granules disappeared, and the apical cells were flattened which is associated with little secretory activity tallying with Riva *et al.,*[28] findings.The reduction in size and number of epithelial cells signifies a decreased secretory activity in the prostate, which concurs with the findings of Orlandinin[21].

CONCLUSION:

This study has used a histoquantitative approach to study tissue alterationsin the prostate gland over a period of 32 days post-castration. The columnar epithelial cells of the prostate weresignificantly affected by testosterone withdrawal. The interacinar tissue,stroma, lumina of glandular acini reacted more slowly. The thickness of interactinar tissue almostdoubled after castration, but there was conspicuous diminution of the diameter of the prostate tubules.Also, there was reduction in the size and number of the smooth muscle andepithelial cells. The nucleus werepyknotic and apical cells flattened indicating atrophy of the prostate.

REFERENCES:

1. Brandes, D. Male Accessory Sex Organs. New York Academic Press Inc, 1974.
2. McNeal, J. E. "The Prostate and Prostatic Urethra: A Morphologic Synthesis." J. Urol. 1972; 197:1008,
3. Blacklock, N. J. "The Morphology of the Parenchyma of the Prostate, "Urol. Res.1977; 5:155.
4. Eliason, R, Lindholmer, C. "Functions of Male Accessory Genital Organs", in Human Semen andFertility Regulation in Man. E. S. E. Hafez, Ed. (St. Louis: C. V. Mosby) 1976.
5. Fransworth, W. E. "Physiology and Biochemistry" in Scientific Foundations of Urology. D. I. Williams and G. D. Chisholm, Eds. Chicago: William Heinemann Medical Publishers, 1976.
6. Spring-Mills, E and Hafez, E. S. K "Male Accessory Sexual Organs". In: HumanReproduction: Conception and Contraception, E. S. E. Hafez and T. W. Evans Eds. New York: Harper and Row, Publishers, Inc, 1979.
7. Mostofi, G. P, Price, E. B Atlas of Tumour Pathology. Tumours of the Male Genital System (Washington D. C. Armed Forces Institute of Pathology) 1973.
8. Moore, C. R, Price, D, Gallagher, T. F Tar – Prostate Cytology as a Testis – Hormone Indicator and Prevention of Castration Changes by Testis Extract Injections. American Journal of Anatomy 1930; 45: 71 – 102,
9. Korenchersky, V, Dennison, M. "Histological Changes in the Organs Injected with Oestrone alone or Simultaneously with Oestrone and Testicular Hormone. Journal of Pathology and Bacteriology 41, 323 – 337.
10. Price, D, H. G. Williams – Ashman, H. G. "The Accessory Reproductive Glands of Mammals." In: Sex and Internal Secretions. Vol. I. W. C. Young, Ed. (Baltimore: Williams and Wilkins, Co.) 1961; Pp 336 – 448.
11. Aumuller, G. In Prostate Gland and Seminal Vesicles, Berlin: Springer – Verlag 1979; Pp 3 – 182.
12. Amatayakul et al., 1971
13. Mann, T Biochemistry of Semen and of the Male Reproductive Tract (New York: John Wiley &Sons, Inc., 1964
14. Bertsch, G, Rohr, H. P. Ultrastructural Stereology. A New Approach to the Study of Prostatic Function.Investigative Urology 14, 301 – 306.
15. Arvola, I The Hormonal Control of the Amounts of Tissue Components of the Prostate: A Histoquantitative Investigation Performed on Rats. AnnalesChirurgiae et al., GynaecologieFenniaeSuppl.1961; 102: 1 – 120,
16. Franks, L. M, Riddle, P. N, Carbonella, A. W, Gey, G. O. A Comparative Study of the Ultrastructure and Lack of Growth Capacity of Adult Human Prostate Epithelium Machinery Separated from its Stroma. Journal of Pathology 1970;100: 113 – 119.
17. Deklerk, D. P, Scott, W. W. Prostatic Maldevelopment in the Prune Belly Syndrome: A Defect in Prostatic Stromal Epithelial Interaction. Journal of Urology 1978; 120: 341 – 344.
18. Muntzing, J, Liljekvist, J, Murphy, G. P. "Chalones and Stroma as Possible Growth Limiting Factorsin the Rat Ventral Prostate". Investigative Urology 1979;16: 339 – 402.
19. Romppanen, T, Huttunen, E, Helminen, H. J "An Improved Light Microscopical Histoquantitative Method for the Stereological Analysis of the Rat Ventral Prostate Lobe. Investigative Urology 1980;18: 59 – 65.
20. Huttunen, E, Romppanen, T, Helminen "A Histoquantitative Study on the Effects of Castration on the Rat Ventral Prostate Lobe. J. Anat. 1981;132 (3): 357

– 370,
21. Orlandini, G. E. Ultrastructure of the Human Normal and Neoplastic. Lab. Invest. 1964; 13: 1541-1560.
22. Cavazos, L. E. "Fine Structure and Functional Correlates of Male Accessory Sex Glands of Rodents": In Handbook of Physiology, Section 7, Endocrinology Vol. 5, E. B. Astwood and R. O. Greep, Eds. Bethesda, M. D: The American Physiological Society, 1975.
23. Luna, L. G Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edition. Pages 1968; 106-110.
24. Derklerk Quantitative Determination of Prostatic Epithelial and StromalHyperplasia by a New Technique. Biomorphometrics. Investigative Urology 1978;16: 240 – 245
25. Deklerk, D. P, Coffey, D. S. Quantitative Determination of Prostatic Epithelial and StromalHyperplasia by a New Technique. Biomorphometrics. Investigative Urology1978; 16: 240 – 245.
26. Harkins, J. C. The Histochemistry of the Prostate in Normal and in Castrated and Concerning the Fine Structure of the Epithelium of the Coagulating Gland. Exp. Cell Res. 1957; 3: 683-685.
27. Flicklinger, C. J. The Fine Structures of the Interstitial Tissue of the Rat Prostate. American Journal of Anatomy. 1972; 134:107-126.
28. Riva, A, Peter, B. K and Johnson, K Fine Structures of Human Seminal Vesicle Epithelium. Journal of Anatomy. 1967;102:71-86.