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Research Article

Morphormetric Analysis of Prostate of Castrated Sprague –Dawley Rats Treated With Combined Testosterone and Estradiol

Adesanya O.A.[1], Shittu L.A.J[2], Oyesola T.O[3], Odubela O.O[1], Adesanya R.A.[4]

[1]Department of Anatomy, Olabisi Onabanjo University, Remo campus, Ogun State, Nigeria
[2]Department of Anatomy, Benue State University, College of Medicine, Markudi, Nigeria
[3]Department of Physiology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne campus, Ogun State, Nigeria
[4]Health Centre, Federal College Of Education Technical, Akoka, Nigeria

*Corresponding Author
Adesanya O.A

Abstract: The restorative effect of testosterone (t) and estradiol (e) on the prostate of castrated sprague-dawley rats was studied after four weeks of treatment using stereological and morphometric assessment method. Combined doses of t (3mg/kg bw.) and e (200µg/kg bw) administered for four weeks, caused rapid growth of the prostate. There was a significant increase in the volume density of the epithelium [vvep] by 1.8 fold in t alone treated group and 2.3 folds in combined t and e treated group. There was also a significant decrease of the volume density of acinar parenchyma [vvap] by 3.5 fold in both t alone treated group and combined t and e treated groups when compared with the castrated control. The growth of the acinar parenchyma and connective tissue especially of the epithelium were the dominant pattern recorded. This work showed a significant restorative response of the connective tissue and glandular epithelium of the prostate of castrated rats treated with combination of t and e.

Keywords: Stereologic assessment, Prostate, Castration, Testosterone and Estradiol.

INTRODUCTION

Several factors were implicated in the development and growth of a normal prostate gland. These factors include androgens, growth factors and stromo-epithelial cell interactions. Increased level of estrrogen in the aging male was also found to play important roles in the development of Benign prostatic hyperplasia (BPH) (McVary, K. T., *et al.*, 1994). Androgen was implicated in the physiology and growth of prostate gland. The circulating Androgens are testosterone and dihydro-testosterone (DHT). About 95% of the testosterone was discovered to be produced from the adrenal gland (Coffey, D. S. 1992). Conversion of testosterone to a more potent androgen (DHT) by an enzyme 5α – reductase occur in the testis and other tissue which include the prostrate. (Yokota, T. *et al.*, 2004; Adesanya O.A. *et al.*, 2007; Yokota, T., . *et al.*, 2004).

It was reported that the normal prostate did not contribute to the circulating level of DHT (Adesanya, O. A., *et al.*, 2007), although there may be a contribution by this organ in subject with benign prostatic hyperplasia (BPH) (Ghanadian, R. *et al.*, 1977). Male rats that were sexually active were found to have larger accessory sex glands-prostate, seminal vessicle (functional hypertrophy) than other male rats that were sexually inexperience (Sodersten, P. *et al.*, 1977). However, this increase was said to depend on the frequency of sexual stimulation and intensity of sexual experience. The reported increase was said to be reversible after sexual rest (Sodersten, P. *et al.*, 1977).

Complex interaction between androgen and estrogen also regulate prostate physiology and development (García-Flórez, M., *et al.*, 2005). The prostate is an androgen dependent tissue, its physiology and pathology are also influenced by estrogen. Estrogen was also said to play some role in the development of abnormally enlarge prostate (Corrales J.J *et al.*, 1981) Pelletier (Pelletier G 2002; Eero, H. *et al.*, 1982) worked on the effects of estradiol on prostate epithelial cells and Garcia – Florez *et al.*, (2005) worked on the effects of estradiol on the ventral prostate in castrated rats, they both reported that estradiol have direct have direct effect on the prostate and preventing apoptosis of prostate cells with moderate hypertrophy of the epithelium. Benign prostatic hypertrophy of the canine

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is produced by the chronic administration of androstane – diol alone or in combination with estradiol (Huttunen E, *et al.,* 1981).

The observation from a study using rat model with combined administration of T and E showed a significant increase in the ventral prostate and seminal vesicle (DeKlerk, D. P., & Coffey, D. S. 1978; Rompanen T.E. *et al.,* 1980). Another report, also emphasize the importance of morphological approach in the studies of spontaneous and experimentally induced hypertrophy with canine prostrate. (Rompanen T.E. *et al.,* 1980; Barstch, G., & Rohr, H.P. 1977) Castration of experimental animals can induced prostatic atrophy which can be reversed through treatment with testosterone (Eero, H, *et al.,* 1982; Huttunen, E. *et al.,* 1981). De–Clerk *et al.,* (1978) use combined biochemical and histological method to show the differences between hyperplasia and hypertrophy of the prostate in histological sections. In another study conducted by Rompannen *et al.,* (1980), histoquantitative method and analytical technique were used to examine castration atrophy of rat prostate gland.

In view of the above, this study is design to investigate the late effects of T and E on the prostate gland of castrated rats using the stereological methods. The behavior of the different tissues compartments examined was acinar parenchyma, epithelium and connective tissue stroma.

MATRIALS AND METHODS

Animal and Hormone Administration.

Twenty-five Adult Sprague –Dawley rats weighing betwee180 –200g were obtained from the animal house of the College of Medicine, University of Lagos, Nigeria. The rats were kept in the rat control room of the Department of Anatomy, College of Medicine, University of Lagos, Nigeria. They were allowed to acclimatize for two weeks before the commencement of treatments. Testosterone propionate (T) used is a product of Shangai Medicine and Health, Republic of China and Estradiol-valerate (E) used is a product of Medipharm (Pvt.), Ltd., 108 –Kotlakhpat Industrial Estate, Lahore Indian, licensed by Schering AG, Germany. The drugs were obtained from a reputable pharmacy store in Sagamu, Ogun state Nigeria. The drugs were administered subcutaneously in the inguinal region on alternate days for 28 days. The vehicle for the drugs was corn oil which was given to the two control groups (intact rats and castrated rats) at dose 2mls per animal. Testosterone was administered at 3.0mg/kg body weight in T alone treated group. In the group where both T and E were administered, they given at 2mg/kg body weight and at 2.5mg/kg body weight respectively.

HISTOLOGICAL STUDIES

The animals were decapitated under guillotine and the paired prostate dissected free and weighed on a Mettler balance. The prostate was fixed in Bouins solution for 48 hours, dehydrated, and embedded in paraffin wax. Sections were serially cut into 5µm thick. They were mounted on chromalum gelatin coated slides and deparaffinized. Every tenth section was stained with haematoxylin and eosin solution.

STEREOLOGICAL STUDIES

The haematoxylin and eosin stained slides from the two control groups and the three experimental groups were used for quantitative estimation of the different organ structures of the prostate. The counting strategy of Huttunen *et al.,* in 1981. was used to determine the volumetric fractions (Vv) of the glandular lumen (Vvlu), epithelium (Vvep), connective tissue (Vvct) and acinar parenchyma (Vvap). Analysis was done on four photographs taken at random with axiomat photomicroscope Zeiss, Oberkochen, West Germany at magnification of 40 (objective10). The Weibel multipurpose graticule with 120 points and 60 test lines was used. for each specimen. The sample size was above 600 points, which decreased the relative error below 10% (Huttunen, E., *et al.,* 1981).

RESULTS

Obtained results from this study showed that the weight of the prostate increased from 25mg to 120mg in castrated control group which were treated with Testosterone Further increase of the prostrate to 145mg were recorded in animal group which were treated with both Testosterone and Estradiol. Non administration of Testosterone at the end of the fourth week aggravated the microscopic signs of atrophy. The proportion of connective tissue stroma was also increased while acinar parenchyma decrease in the castrated control as shown in Figure -2.

During hormones (Testosterone or and Estradiol) administration, the volume fraction of acinar parenchyma (Vvap) decreased from 0.22 in the castrated control to 0.063 in both Testosterone treated group and Testosterone – Estradiol treated group prostates as shown in Table 3. The volume density of the connective tissue stroma (Vvct) decreased in Testosterone treated group to 0.09 but increased slightly in Testosterone-Estradiol treated group to 0.31 relative to the castrated control which is 0.30.

The volume fraction of the glandular lumen (Vvlu) increased from 0.13 in castrated control group to 0.25 in Testosterone treated group and to 0.31 in Testosterone – Estradiol treated group. Volume fraction of epithelial cells increased from 0.20 in castrated control group to 0.38 in Testosterone treated group and 0.47 in Testosterone – Estradiol repectively.

Although, the volumetric fraction of connective tissue (Vvct) stroma was reduced in the Testosterone treated to 0.09, reverse was the case in the Testosterone – Estradiol treated group which is 0.30. In

the castrated control animals that received the corn oil (vehicle for the hormone), atrophy of the prostate were evidently shown by the weight of the gland been 25mg /kg body weight. Four weeks after treatment, when they were compared with the Testosterone treated group there was an increase to 120 mg /kg B.W and 145 mg / kg body weight in Testosterone – Estradiol treated group, which were significantly higher at P value of 0.05 by weight as shown in Table-2.

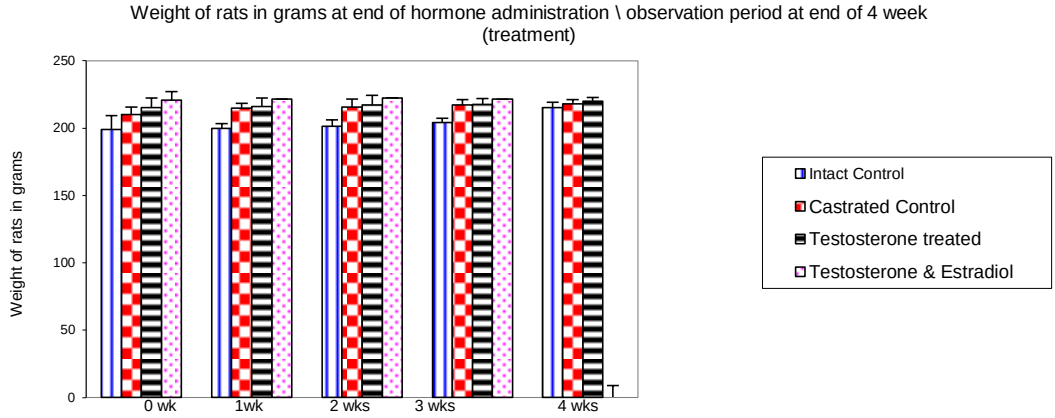

Fig.-1: Weight of rats in grams at end of hormone administration \ observation period at end of 4 week (treatment)

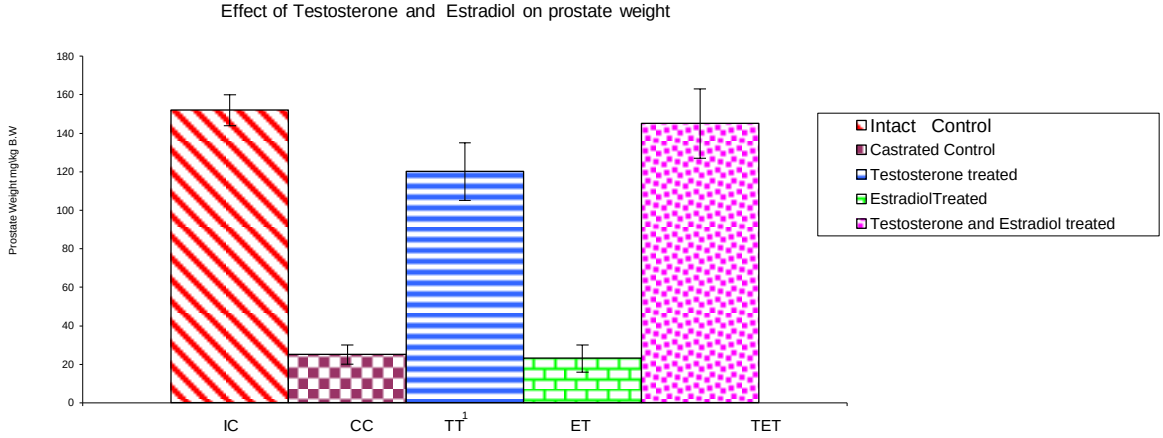

Fig.-2: Effect of Testosterone and Estradiol on prostate weight

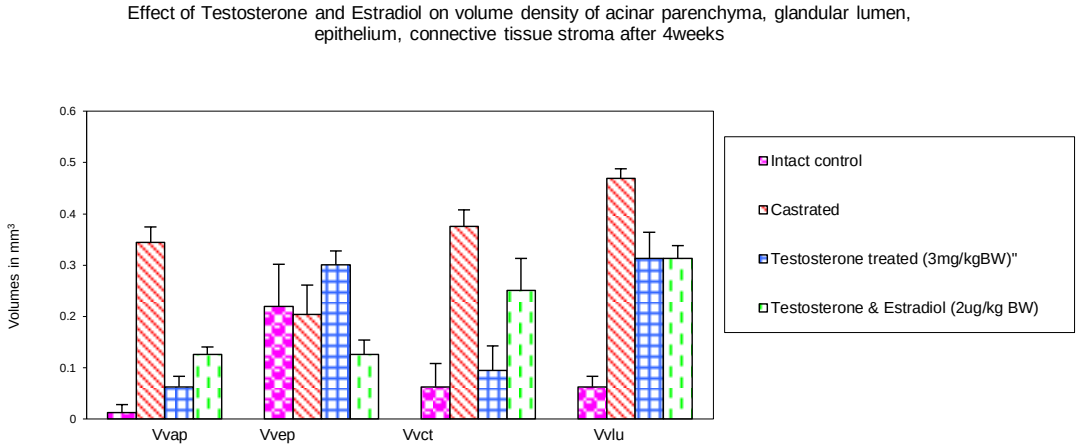

Fig.-3: Effect of Testosterone and Estradiol on volume density of acinar parenchyma, glandular lumen, epithelium, connective tissue stroma after 4weeks

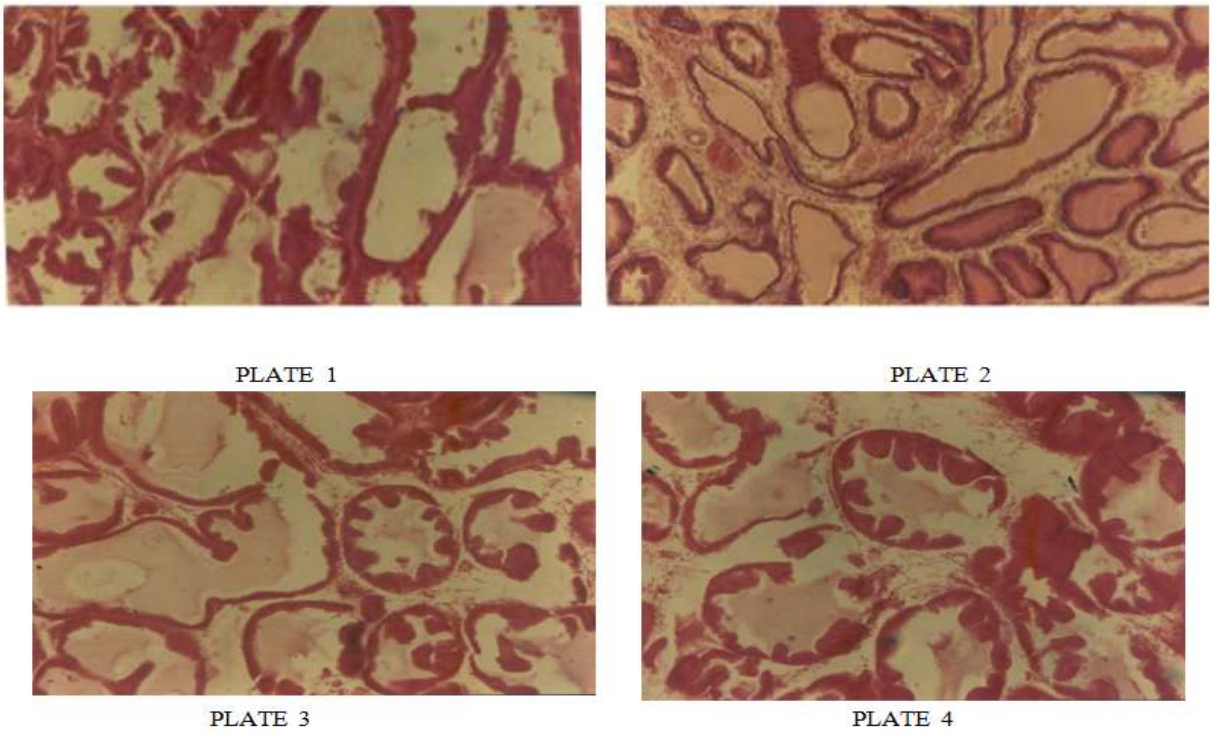

Plate 1 Photomicrograph of Intact prostate.
Plate 2 Photomicrographs of Castrated prostrate
Plate 3 Photomicrographs of Testosterone treated prostate
Plate 4 Photomicrographs of Combined Testosterone and Estradiol treated prostate.

DISCUSSION

This study provides morphometric data on castrated rat prostate treated with combined testosterone and estradiol which can be correlated to available histological information, thus contributing to quantitative cell biology for the prostate. Several recent articles have appeared on the prostate morphometry utilizing a variety of stereological approaches (Eero, H. et al., 1982) and comparing the morphometric data in castrated rats with prostate of testosterone treated they reported increase in size of acinar parenchyma and decrease in connective tissue stroma after hormone treatment. In the work of Huttunen *et al.*, (1982), the growth of the acinar parenchyma was the dominant feature. In the present work testosterone alone treated group reacted in a similar manner to Huttunen *et al.*, (1982) prostate. But the combined testosterone – estradiol treated group gave a different pattern altogether, both the acinar parenchyma and the connective tissues stroma increased in size as correlated by the morphometric data obtained in Figure-3. The present work is different from previously published morphometric data, as they did not contain much information about the action of combined testosterone and estradiol on the rat prostate as refilled by this present study. Corrales *et al.*, (1981), reported the use of combined dose of Testosterone and Estradiol but with emphasis on the effect of oestrogen, so the work did not discussed the combined action of testosterone and estradiol and no morphometric data on the prostate was provided. The present work presented morphometric data on prostate using the light microscope. There was alteration of the tissue of the testosterone treated rats alone and combined testosterone and estradiol treated prostate as shown by a decreased in the volume density of acinar parenchyma in both Testosterone alone and combined testosterone – estradiol treated prostate, thus indicating the increase in size of the acinar parenchyma per reference area.

In the T-alone treated, the acinar parenchyma shows a distinct preponderance over the connective tissue stroma but the reverse was the case in the combined T and E –treated as both the connective tissue and acinar parenchyma seems to have been influenced by the hormone administration, though not to the same degree.

This study provides morphometric data on castrated rat prostate gland with combined Testosterone – Estradiol treatments. This result can be correlated to available histological information that will contribute to quatitative cell biology for the prostate gland. Stereological approaches have been utilized in several recent activities on prostate gland morphometric (Eero, H. *et al.*, 1982; De-clerk D.P, & Coffey D.S. 1978). They have compared the morphometric data in castrated rats with prostate of of Testosterone treated rats and

they reported an increase in the size of acinar parenchyma and decrease in connective tissue stroma after hormonal treatment.

The work of Huttumen *et al.*, in (1982), showed that the growth of the acinar parenchyma was the dominant feature. Similarly this present study showed a pattern reported by Huttumen *et al.*, in (1982), about the prostate gland in Testosterone treated rats. But in combined Testosterone - Estradiol treated rats a departure from the reports of Huttumen *et al.*, was observed, in both the acinar parenchyma and the connective tissue stroma increase in size as correlated by the morphometric data obtained in Figure-3. This work is different from previous published morphometric data, as they do not contain much information about the action of combined Tesosterone and Estradiol on the rat prostate gland Corralles *et al.*, in (1981), reported the use 0f combined doses of Testosterone and Estradiol with emphasis on the effects of oestrogen without the combined action of Testoestrone and Estradiol and no mophometric data were provided on the prostate gland. But the present work presented a morphometric data on the prostate gland using the light microscope. Observed more alteration of the tissue of the Testosterone treated rats and combine Testosterone – Estradiol treated prostate gland and showed a decrease in the volume density of acinar parenchyna in both Testosterone and combined Testosterone – Estradiol treated prostate gland, thus indicating increase in size of the acinar parenchyma per reference area. Acinar parenchyma in testosterone alone treated postate gland showed a distinct pre ponderance over the connective tissue stroma but the reverse was the case in the combined testosterone – estradiol treated prostate gland as the connective tissue and acinar parenchyma seems to have been influenced by the hormone administration though not to the same extent.

By selecting high dose in this study, we expect strong androgenic action and a rapid growth of the prostate with substantial changes in the tissue compartments that could be assessed with stereologic morphometric method (Rompanen *et al.*, 1980; Huttunen *et al.*, (1981).

The hormone treatment was initiated four – weeks after castration when the prostate atrophy was completed, in this way, we could minimize the effect of early reaction to castration (Rompanen T.E, *et al.*, 1980). Previous investigators have used similar doses of testosterone propionate T [nw 12, nw 11 16, 17] while some others have employed larger doses (Rompanen T.E. *et al.*, 1980; Huttunen, E., *et al.*, 1981; De-clerk D.P, & Coffey D.S.1978; Brandes, H., *et al.*, 1978).

The connective tissue has been considered to provide a suitable microenvironment that permits androgenic response (Pelletier, G. 2002; Rompanen T.E, *et al.*, 1980; Cunha G.R, & Lung B 1980). In conclusion, we observed an increase in the amount of connective, epithelium, and acinar parenchyma size in the combined Testosterone - Estradiol treated prostate gland which was almost similar to what was obtained in Testosterone alone treated prostate glands, but showed slightly higher figures which are not significant when the two were statistically compared. The mechanism by which Estradiol induced increased epithelial size and large connective tissue stroma require further investigation.

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REFERENCES

1. McVary, K. T., Razzaq, A., Lee, C., Venegas, M. F., Rademaker, A., & McKenna, K. E. (1994). Growth of the rat prostate gland is facilitated by the autonomic nervous system. *Biology of reproduction*, *51*(1), 99-107.
2. Coffey, D. S. (1992). The molecular biology, endocrinology, and physiology of the prostate and seminal vesicles. *Campbell's urology*, *1*, 221-266.
3. Yokota, T., Honda, K., Tsuruya, Y., Nomiya, M., Yamaguchi, O., Gotanda, K., & Constantinou, C. E. (2004). Functional and anatomical effects of hormonally induced experimental prostate growth: a urodynamic model of benign prostatic hyperplasia (BPH) in the beagle. *The Prostate*, *58*(2), 156-163.
4. Adesanya, O. A., Oluyemi, K. A., Dare, N. W., Shittu, L. A., Oyesola, O. A., & Okanlawon, A. O. (2007). Sex steroid induced changes on the morphology of prostate of sprague-dawley rats. *Scientific Research and Essays*, *2*(8), 309-314.
5. Toorians, A. W., Kelleher, S., Gooren, L. J., Jimenez, M., & Handelsman, D. J. (2003). Estimating the contribution of the prostate to blood dihydrotestosterone. *The Journal of Clinical Endocrinology & Metabolism*, *88*(11), 5207-5211.
6. Ghanadian, R., Lewis, J. G., Chisholm, G. D., & O'DONOGHUE, E. P. N. (1977). Serum dihydrotestosterone in patients with benign prostatic hypertrophy. *British journal of urology*, *49*(6), 541-544.
7. Sodersten, P., Damassa, D.A., & Smith, E.R. (1977). Sexual behavior in developing male rats Horm Behave, (8), 320- 341.
8. García-Flórez, M., Oliveira, C. A., & Carvalho, H. F. (2005). Early effects of estrogen on the rat ventral prostate. *Brazilian journal of medical and biological research*, *38*(4), 487-497.
9. Corrales, J. J., Høisaeter, P. A., Kadohama, N., Murphy, G. P., & Sandberg, A. A. (1981). A model

for studies on the response of the ventral prostate to oestrogens. *Acta endocrinologica*, *97*(1), 125-136.
10. Pelletier, G. (2002). Effects of estradiol on prostate epithelial cells in the castrated rat. *Journal of Histochemistry & Cytochemistry*, *50*(11), 1517-1523.
11. Huttunen, E., Romppanen, T., & Helminen, H. J. (1982). Testosterone action on the ventral prostate lobe of the castrated rat as assessed with a stereologic morphometric method. *American Journal of Anatomy*, *165*(2), 199-209.
12. Huttunen, E., Romppanen, T., & Helminen, H. J. (1981). A histoquantitative study on the effects of castration on the rat ventral prostate lobe. *Journal of Anatomy*, *132*(Pt 3), 357.
13. Walsh, P. C., & Wilson, J. D. (1976). The induction of prostatic hypertrophy in the dog with androstanediol. *The Journal of clinical investigation*, *57*(4), 1093-1097.
14. DeKlerk, D. P., & Coffey, D. S. (1978). Quantitative determination of prostatic epithelial and stromal hyperplasia by a new technique. Biomorphometrics. *Investigative urology*, *16*(3), 240-245.
15. Romppanen, T., Huttunen, E., & Helminen, H. J. (1980). An improved light microscopical histoquantitative method for the stereological analysis of the rat ventral prostate lobe. *Investigative urology*, *18*(1), 59-65.
16. Barstch, G., & Rohr, H.P. (1977). Ultrsturctural stereology. New approach to the study of Prostatic function. Invest. Urol, (14), 301- 306.
17. Lesser, B., & Bruchovsky, N. (1974). Effect of duration of the period after castration on the response of the rat ventral prostate to androgens. *Biochemical Journal*, *142*(2), 429-431.
18. Yamanaka, H., SHIMAZAKI, J., IMAI, K., SUGIYAMA, Y., & SHIDA, K. (1975). Effect of estrogen administration on activities of testosterone 5α-reductase, alkaline phosphatase and arginase in the ventral and the dorsolateral prostates of rats. *Endocrinologia japonica*, *22*(4), 297-302.
19. Touhimaa, P. (1980). : Control of cell proliferation in male accessory sex glands E Spring-Mills and E.S.E Hafez, eds Elsevier / North Holland Amsterdam,131- 153.
20. Brandes. H., Schmidt R.A., & Tanagho, E.A. (1978). : Hormonal regulation of fine structure in male accessory sex organs academic press, New York, 182- 222.
21. Cunha, G. R., & Lung, B. (1980). Experimental analysis of male accessory sex gland development. *Male Accessory Sex Glands. Elsevier/North Holland, Amsterdam*, 39-59.