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Studies of Estrogen and Progesterone on Testicular Functions in Male Wistar Rats (Rattus Novergicus)

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ABSTRACT

Oestrogen is produced in sizable quantities in the testis, as well as the brain. Progesterone enhances libido, sperm count, improves mood, and keeps weight down while increasing muscle mass. This study investigated some of the effects of oestrogen and progesterone on the testis of adult male Wistar rats. Twenty (20) adult male wistar rats were randomly assigned into four groups (A-D) (n=5) and drugs were administered to the rats as follows: Group A received 1ml of distilled water per day, group B received 5 mg/kg b.w. of Stilbestrol per day, group C received 0.5 mg/kg b.w. of Lynestrenol per day while group D received 5 mg/kg body weight of Stilbestrol and 0.5 mg/kg b.w. of Lynestrenol. Histological, testicular histomorphometry, hormonal and semen parameters were observed. Histological evaluations for group (B-D) showed elongated seminiferous tubules, degeneration of the basement membrane, severe thinning of sertoli cells, reduced number of spermatogenic cells, wide interstitial space and scanty leydig cells. Biochemical analyses revealed a significant increase ($P<0.05$) in serum follicle stimulating hormone (FSH) levels in the experimental groups (B-D) when compared to the control group. Semen analysis showed that there was a significant reduction ($P<0.05$) in Sperm Motility and Life and Death ratio (L/D) in all experimental groups while significant decrease ($P<0.05$) in sperm morphology was observed in groups B and C while no significant differences ($P>0.05$) was observed in sperm count in the treatment groups compared with control group. These findings suggest that Stilbestrol and lynestrenol administrations had deleterious effects on testicular cell morphology.

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INTRODUCTION

Oestrogen is present in very high concentrations in the semen of several species (1). Early studies reported that the primary source of oestrogen in the immature male is the Sertoli cell (2). In the adult testis, Leydig cells express aromatase and actively synthesize oestradiol at a rate much greater than that seen in the adult Sertoli cell (3). Currently, a growing body of evidence indicates that germ cells also synthesize oestrogen, and possibly serve as the major source of this steroid in the male reproductive tract (4).

Reports has shown that the synthesis and secretion of oestrogen is done by the human testis for the past three decades (5). This was subsequently confirmed and identified the male gonads as a site of oestrogen biosynthesis (6). Hess(7) and Hess *et al.,*(8) reported the presence of large quantities of oestrogen in the rete testis fluid and spermatic vein in mammals.

Two main functions of the mammalian testis are steroid synthesis and production of spermatozoa. These functions are primarily controlled by two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as testosterone and numerous locally produced factors (9). The discovery of Sertoli cells as a major source of estrogens in immature animals has led to numerous studies in search of testicular sources of estrogens other than Leydig cells (10).

The rapid membrane effect of oestrogens in the testis forms a basis for understanding the estrogenic control of spermatogenesis and evaluating the role of exposure to endocrine disruptors (xenoestrogens) during malignant transformation of testicular germ cells (11).

Progesterone enhances sperm count and libido, improves mood, and keeps weight down while increasing muscle mass. Benefits of progesterone include stronger bones and erections, prevention of hair loss and better sleep (12).

It also increases testosterone levels in the body and enhance its effects, being that it's a precursor of the mineralocorticoid aldosterone, and androstenedione which can be converted to testosterone, estrone and estradiol (13-14). Progesterone has also been

implicated in prevention of male baldness, prostatic hyperplasia, and prostate cancer as it has been reported that progesterone act as 5-alpha-reductase inhibitor, which converts testosterone into dihydrotestosterone (DHT). By blocking 5-alpha-reductase, progesterone promotes higher levels of testosterone in the body (13).

Progesterone (PR) is expressed in the uterus, mammary gland, ovary, fallopian tube (15) and placenta (16). In non-reproductive tissues, PR is found in the peripheral nervous system, (17) the immature bladder (18), lung (19), and the islet cells of the pancreas. Progesterone actions in regulating gonadotropin releasing hormone (GnRH) production and release at the hypothalamic and pituitary level are critical to its effects on the ovarian cycle and spermatogenesis (20).

The purpose of this study is to examine and evaluate the morphological and hormonal effects of oestrogen and progesterone on the testis of adult male Wistar rats.

Materials and methods

A total of twenty adult male rats weighing between 150g and 200g were procured and housed in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin. The Wistar rats were subjected to a period of one week of acclimatization before treatment. The body weights of the rats were taken weekly using the High Precision Electronic Analytical Weighing Balance in the Department of Anatomy, University of Ilorin.

Animal Grouping

The animals were grouped into four consisting of five rats each. The groupings were as follows;

Group A served as the control group which received 1ml of distilled water.

Group B received 5.0 mg/kg b.w. of Stilbestrol.

Group C received 0.5 mg/kg b.w. of lynestrenol.

Group D received 5.0 mg/kg b.w. of stilbestrol and 0.5 mg/kg b.w. of lynestrenol.

Animal sacrifice

After 21 days of treatment, the animals were sacrificed on the 22nd day and the caudal epididymis were immediately removed from the testes for semen analysis (sperm count, motility, viability and morphology respectively). Blood samples were immediately collected using 5 ml syringes from the apex of the heart and the blood samples were placed in heparinized bottles, centrifuged at 3,000 rpm for 15 minutes. Serum samples were used for hormonal assay. The testes removed were fixed in 10% formal saline for testicular histology to prevent the process of putrefaction or autolysis.

Hormonal Assay

Serum testosterone, follicle stimulating hormone (FSH) concentration was estimated using Accu Bind ELISA Microwell by Monobind Inc. Lake Forest, CA 92630, USA.

Semen Analysis

The epididymis was placed in normal and used for evaluation of sperm quality (i.e. sperm count, sperm motility and sperm morphology). The concentration of spermatozoa was determined using the improved Neubauer Chamber Haemocytometer (Deep 1/10 mm, LABART, Germany).

Tissue Processing for Histological Studies

The testes of all the rats were fixed in 10% formalin, dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5μm thick paraffin section of each testicular tissue was stained with hematoxylin and eosin, followed by examination under a light microscope at ×100 and ×200 magnification and micrographs taken (21).

Testicular Histomorphometry

The histomorphometry (i.e. cross section area, lumen diameter and germinal epithelium diameter) was evaluated using Image J Software (USA) from the photomicrographs of the testes.

Statistical Analysis

The data obtained were analysed statistically by one-way ANOVA followed by subsequent analysis by GraphPad Prism 5.0 with statistical significance set at $P < 0.05$.

RESULTS

Physical Observation

The animals that were administered 5.0mg/kg Stilbestrol developed swelling in the thyroid region of neck. This could be pituitary adenoma as reported by Storer *et al*., (22). Other evident observations were weight loss, loss of appetite, severe diarrhoea, alopecia, low immunity thereby exposing them to infections and eventually death. In the group administered 0.5mg/kg lynestrenol alone, the animals were noticed to be depressed and fatigued, occasionally sleeping in corners of the cages. Excessive urination with less faeces was also seen. In the group 4 i.e. the animals administered with both 5.0mg/kg stilbestrol and 0.5mg/kg Lynestrenol, aggression was noticed. The animals tend to fight each other.

Body Weight Changes

No significant difference ($P>0.05$) was observed in the body weight of experimental animals compared to the control group (fig 1).

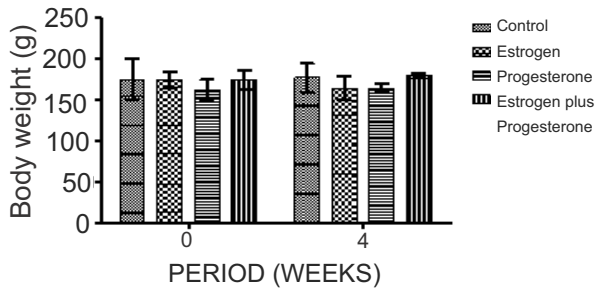

Figure 1: Chart showing body weight changes of control and treatment groups.

Histological Observation

A normal cytoarchitecture of the testis was observed in Figure 2A, while reduced size of seminiferous tubule, degenerating basement membrane, reduced spermatozoa in the lumen and wide interstitial spaces were observed (Figure 2B). In animals that received both progesterone and progesterone+estrogen (Fig 2C and 2D), elongated seminiferous tubule with intact basement membrane and wide interstitial space with scanty leydig cells were observed.

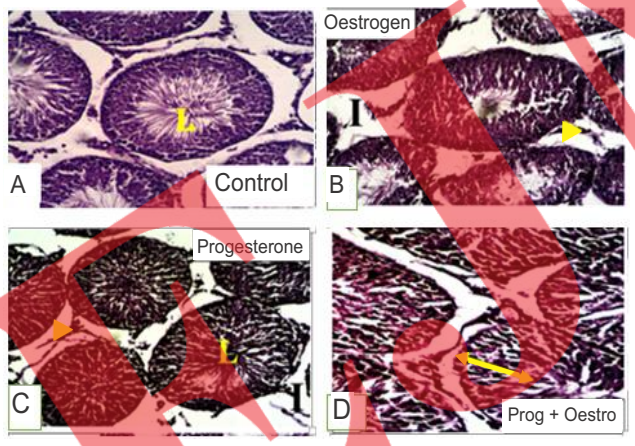

Figure 2 (A-D): Photomicrograph representative of testicular structure in the control, estrogen, progesterone and estrogen+progesterone groups. L-lumen, Arrow head- leydig cells, I-interstitial space, Spanned arrow- spermatogenic cells. Haematoxylin and Eosin x100

Semen Analysis

Sperm motility and Life and Death ratio (L/D) reduced significantly (P<0.05) in all experimental groups compared to the control group. Significant decrease (P<0.05) was observed for sperm morphology in the groups that received 5.0mg/kg of Estrogen and 0.5mg/kg of Progesterone respectively while no significant differences was observed in sperm count in the treatment groups compared with the control group (Figure 3).

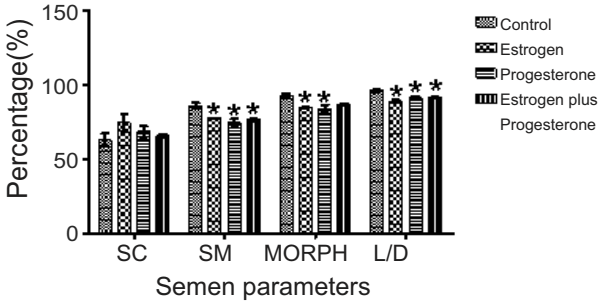

Figure 3: Showing semen analysis of rats in the treatment groups compared with the control group.(P<0.05)- significant difference.*

Hormonal Analysis

No significant difference (P<0.05) was observed in the testosterone levels between the treatment groups after administration of stilbestrol and lynestrenol when compared with the control group (Figure 4A).

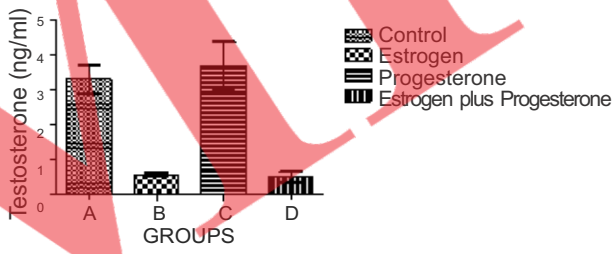

Figure 4A: Showing serum testosterone levels between treatment groups and control group.

Significant decrease (P<0.05) in luteinizing hormone (LH) was observed in the group that received 0.5 mg/kg b.w. of progesterone while no significant decrease (P>0.05) was observed in the estrogen and estrogen+progesterone groups compared to the control group (Figure 4B).

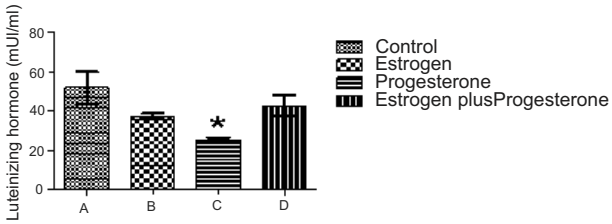

*Figure 4B: Showing luteinizing hormone levels between the treatment groups and control group. *=P<0.05*

Significant increase (P<0.05) in follicle stimulating hormone (FSH) was observed in the group that received 5 mg/kg b.w. of estrogen while no significant increase (P>0.05) was observed in the progesterone and estrogen + progesterone groups compared to the control group (Figure 4C).

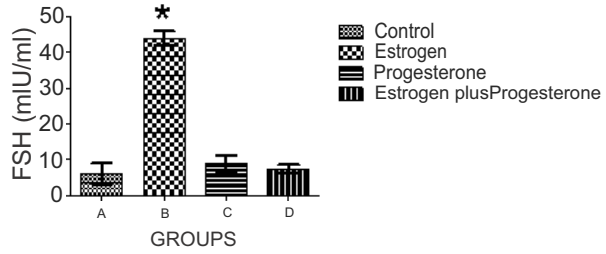

Figure 4C: Showing follicle stimulating hormone (FSH) levels between the treatment groups and control group. *= (P<0.05).

Testicular Histomorphometry

Johnsen's score showed a significant reduction (P<0.0001) in group B compared to control animals (figure 5A).

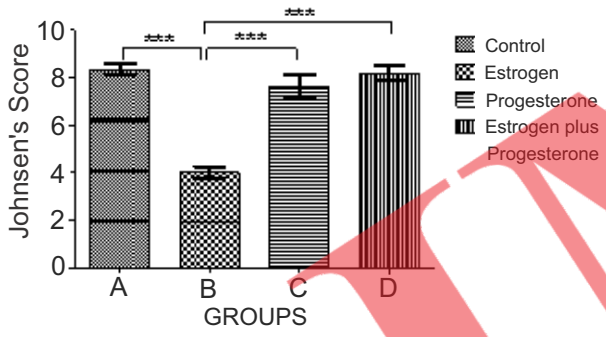

Figure 5A: Showing Johnsen's score between treatment groups and the control group. ***P<0.0001

Cross section area (CSA) of the seminiferous tubule showed an insignificant decrease (P>0.05) in the Estrogen and Progesterone groups that received 5.0mg/kg and 0.5mg/kg respectively while a non-significant increase (P>0.05) was observed in the estrogen + progesterone group compared with the control group (figure 5B).

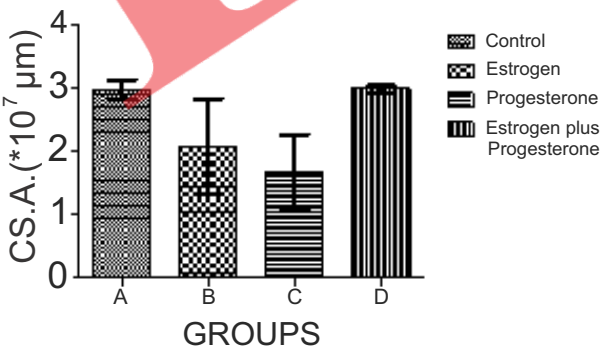

Figure 5B: Chart showing cross section area of the seminiferous tubule between treatment groups and the control group.

Lumen diameter (LD) of the seminiferous tubule showed no significant differences between the treatment and control groups (figure 5C).

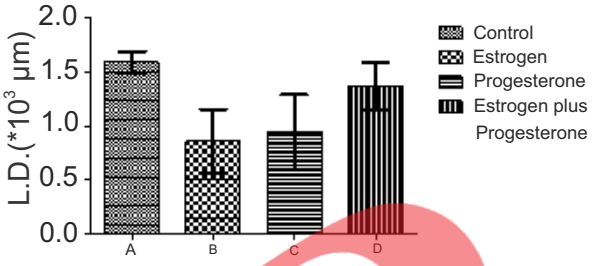

Figure 5C: Showing lumen diameter of the seminiferous tubule between treatment groups and the control group.

Germinal epithelia diameter (GED) of the seminiferous tubule showed a non-significant increase (P>0.05) in group D when compared to the control group (Figure 5D).

Figure 5D: Chart showing germinal epithelia diameter of the seminiferous tubule between treatment groups and control group.

DISCUSSION

The non-significant difference observed between the treatment and control groups might be due to the fact that progesterone reduces estrogen and antagonizes estrogenic side effects like a pooch stomach and weight gain. (13-14)

A normal cytoarchitecture of the testis was observed in figure 2A, while reduced size of seminiferous tubule, degenerating basement membrane, reduced spermatozoa in the lumen and wide interstitial spaces were observed (Figure 2B). In animals that received both progesterone and progesterone+estrogen (Fig 2C and 2D), elongated seminiferous tubule with intact basement membrane and wide interstitial space with scanty leydig cells were observed.

This results agrees with Francis *et al.* (23) studies who reported an increase in the total volume of the seminiferous epithelium and estradiol doses resulted in an abundance of mature spermatozoa in the epididymis.

The defects in the structure and germinal epithelia component of the seminiferous tubule noticed were complimented by significant reduction (P<0.0001) in Johnsen's score noticed in group B treated with estrogen compared to the control group.

No significant differences was observed in the testicular histomorphmetry between treatment groups and control group. This brings to the fore the actions of progesterone in regulating gonadotropin releasing hormone (GnRH) production and release at the hypothalamic and pituitary level are critical to its effects on the ovarian cycle and spermatogenesis (20). Single or combined administration of estrogen and progesterone resulted in significant differences (P<0.05) in semen quality (motility, morphology and life/dead ratio) of treated groups compared to control group while no significant difference was observed in sperm count (Figure 3). This corroborated with Anawalt *et al.* (24) who reported that large doses of levonorgestrel suppressed sperm count alone.

Due to the fact that progesterone works a lot like testosterone, scientists are referring to progesterone as second male sex hormone. In a study, published in the book, *The Hidden Structure of Interaction*, researchers found that progesterone and testosterone levels rise and fall together in weekly and monthly cycles. Both testosterone and progesterone levels rise together in response to increased sexual activity in men (14).

Non-significant increase (Figure 4A) in testosterone levels was observed in group C that received only progesterone compared with the control group. This was in agreement with Hala *et al.* (25) who reported a rise in testosterone level in animals given a synthetic progestin compared to the animals given estrogen and a combination of estrogen and progesterone.

The significant decrease in LH concentration observed in group C (Figure 4B) showed that the positive feedback mechanism has occurred to stabilize the production of testosterone through the leydig cells, which acts locally to drive sperm production (26). This was evident by the non-significant differences in serum testosterone levels in this study.

The significant drop in LH concentration in animals given only progesterone might be due to a rise in testosterone concentration, and a rise in testosterone serves as a negative feedback signal in male to reduce or halt the secretion of LH (27).

Significant increase (P<0.05) in follicle stimulating hormone (FSH) was observed in the group that received 5 mg/kg b.w. of estrogen (figure 4C). FSH in male stimulates Sertoli cells to produce androgen-binding protein (ABP; i.e. proteins that bind with testosterone), thereby stimulating spermatogenesis. It also stimulates Sertoli cells to produce inhibin, which provides negative feedback to the anterior pituitary to decrease FSH secretion (28). This correlates with the studies done by Francis *et al.* (23) who reported that circulating FSH concentrations were significantly elevated by treatment of estradiol in *hpg* mouse.

CONCLUSION

Most studies suggest progesterone in men has a similar effect to the male hormone testosterone. This study corroborated the previous researches. However, this current study pushed the boundaries of previous knowledge by investigating the effect of estrogen and progesterone on testes. Parameters introduced in this study such as hormonal assay and histopathological viewing further revealed mild effect of estrogen and ameliorative properties of progesterone on testes.

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