

ANALYSIS OF BIREFRINGENCE (OPTICAL DENSITY) OF PERIURETHRAL MUSCLES AND COLLAGEN OF FEMALE RATS UNDER EFFECT OF TESTOSTERONE.

Hypothesis / aims of study

The purpose of this study was to analyze the effect of androgen supplementation in periurethral muscles and collagens fibers of females rat Wistar using polarized light microscopy.

Study design, materials and methods

Animals, Hormones and Histological studies

Thirty adult rats (female, virgins, Wistar), aged 90 days, were randomly assigned into two groups and received the following treatment for 60 days:

Group 1C (Controls): 15 rats receiving daily doses of 0,1ml/day of subcutaneous (SC) placebo.

Group 2M (testosterone): 15 rats receiving daily doses of 0,075 mg/Kg of SC methyltestosterone.

Treatment started 30 days after the procedure and by the end of the treatment; the bladder, urethra, vagina and pelvic floor muscles were removed en bloc.

The blocs were fixed in 4% formaldehyde and imbedded in Histosec (Merck Darmstadt, Germany). The specimens were prepared in the usual manner and two cuts of 7 um traversal to the rectum were obtained.

Image analysis:

Images were obtained with a Zeiss axiophot-2 – Olympus Bx 51 microscope. These images were analyzed into Image Pro-Plus 5.1 System (Software). The parameter analyzed was optical density (OD=absorbances).

This equipment with a Neofluar 40/2 objective optovar factor 2 to analyze periurethral muscles and 40/1 objective to collagen fibers.

All the slides were evaluated by the same investigator. Each slide had four fields analyzed.

Statistic analysis were done using the Minitab-12™ software (State College, PA) – Mann Withney test p value was 0,05 (p<0,05).

Results

The first analysis made was the calculation of optical density in perineal muscles of placebo and testosterone groups. 197 calculations were made from 15 slides for control group from which the following measurements were taken: Optical density, total area and standard deviation. For testosterone group 95 calculations were made from other 15 slides, with the same parameters analyzed. (Table 1)

TABLE 1-Comparison between Optical Density values of periurethral muscles of the two groups of rats.

Group	n	Median	Standard Deviation
Placebo	197	65,42	0,0045
Testosterone	95	84,76	

The second analysis evaluated the birefringence of collagen in both groups. 555 measures were made from placebo group and 511 from testosterone group. (Table 2)

TABLE 2- Comparison between Optical Density values of periurethral collagen of the two groups of rats.

Group	n	Median	Standard Deviation
Placebo	555	95,94	0,0001
Testosterone	511	100,86	

Interpretation of results

The results of Table 1, that presents the analysis of periurethral muscles show a statistically significant difference between the two groups, with the testosterone group having birefringence levels greater than control group.

In table 2, that analyses periurethral collagen, data shows that testosterone group had an increase in frequency of higher values of optical density, with statistically significant difference when compared to control group.

These data allow us to presume a better molecular organization and a better function of these tissues.

Concluding message

Even though recent studies don't clarify the real role of androgens in tissue morphology of lower urinary tract of female mammals, our research showed that the group that received testosterone had an increase in the birefringence (optical density) of periurethral muscles and collagen, what is consistent with a better molecular organization under the effect of this hormone.

Specify source of funding or grant	none
Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	Ethical Committee for Experimental Studies of the State University of Campinas, São Paulo