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International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(4):195-201

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Morphometric Comparison Analysis of the developing gubernaculum in Male and Female rats

Aymen A. Warille*
Department of Anatomy and Histology, College of Medicine, University of Hail, Kingdom of Saudi Arabia
**Corresponding author Email: aymwar@yahoo.com*

ABSTRACT

Background: *A fetal ligament connects the inferior pole of each gonad with primordia of the scrotum in male and the labia majora in female and considered as a fibrous cord connecting two structures the testis and scrotum in male ovary and labium majus in female.* ***Objective:*** *To compare between male and female in Gubernaculum during pre and postnatal development to indicate its biological growth activity and morphological statistical differences.* ***Methods:*** *The experimental animal model utilised in this study was the Sprague Dawley rats. Foetuses (n=6) at gestational days (Embryo, E17, E18, and E20) and neonates at postnatal days (P0 [at birth], P2, and P4) were included. Morphometric assessment was performed for all feotuses and neonates at different ages.* ***Results:*** *There was a difference of means between male and female Gbn and Gbc length. Between E20 and P0, in male Gbn height increased from 493.3±133.3 to 1046.6±387.3µm. However, the female counterpart showed an increase from 303.3±84.3 to 423.3±82.3. From postnatal day P0 till postnatal day P4, the male Gbn height reduced slightly from 1046.6±387.3 to 973.3±184.4µm whilst female Gbn height reduced from 423.3±82.3 to 163.3±156.6 µm. In males, Gbc length remained consistent ranging between 410.0 - 610.0 µm, between E20 until P0, female Gbc length (from 720.0±197.9 to 1043.6±295.9 µm) remained significantly greater than male Gbc length.* ***Conclusion:*** *The present study indicates that during gubernaculum development, there is remarkable morphological statistical differences between male and females Gbn and Gbc which indicates the role of gubernaculum in gonadal descent.*
Key words: *Gubernaculum, Rats, Morphometric*

Abbreviations:
SD-Sprague Dawley, Gbn-Gubernacular cone, Gbc-Cord, WD-Wolffian duct, MD-Müllerian duct

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INTRODUCTION

Morphometry enables us to describe structures in quantitative terms and in particular reveals minimal morphological differences between states of function[1]. Traditional morphometrics analyzes lengths, widths, masses, angles, ratios and areas[2].

The gubernaculum connects the gonad to the inguinoscrotal region and is involved in testis descent. It rapidly develops in the male fetus, whereas development in the female fetus is lacking. Possible factors involved in gubernaculum development are androgens, anti-Müllerian hormone, and insulin-like factor. Sexual dimorphism in gubernaculum development correlated with the mitotic activity of cells in the gubernacular bulbs from male and female fetuses. Androgen receptor expression was restricted to the mesenchymal core of the gubernacular bulb, whereas skeletal muscle was detected in its outer layer. In an organ culture system devised to further study

gubernaculum development *in vitro*, morphology of gubernacular explants grown in the presence of testes was comparable with that of gubernacula developed *in vivo*[3].

The aim of this study was to compare the statistically difference in the length of male and female gubernaculum to indicate its biological as well as growth activity and also remarkable morphological statistical differences between male and females.

MATERIALS AND METHODS

Tissue sections of pre and postnatal Sprague Dawley (SD) rat gubernaculum stained with H and E were used for the analyses. The total number of samples studied was n=6 per developmental stage (E17, E18, E20, P0, P2, and P4) for both male and female rats. In this study, morphometric analysis was utilised to determine the length of gubernacular cone and cord using micro-promar microscope with modified camera Lucida techniques[3].

Photomicrographs of selected sections were taken including the gubernaculum. A fixed distance of 50 cms between an upright paper screen and the microscope was maintained. A fixed objective magnification of X 40 and eyepiece magnification of X 10 were employed to cast distinct images of the gubernacular structure profiles on the screen. The final adjustment focusing with drawing apparatus in the plane of the tracing paper on the screen was calibrated and kept constant. A direct tracing of the micrometer was done prior to tracing the gubernacular cone (Gbn) and cord (Gbc) of all samples studied. Measurements of Gbn and Gbc were taken along with their longitudinal axes (region of internal inguinal ring). The length of the Gbn was measured from the apex of the gubernacular cone to its base while the length of the Gbc was measured from its distal Wolffian duct (WD) or Müllerian duct (MD) attachment to the apex of the Gbn. The measurements for the length of testis and ovary along with their longitudinal axes used similar methods (Fig.1 a, b).

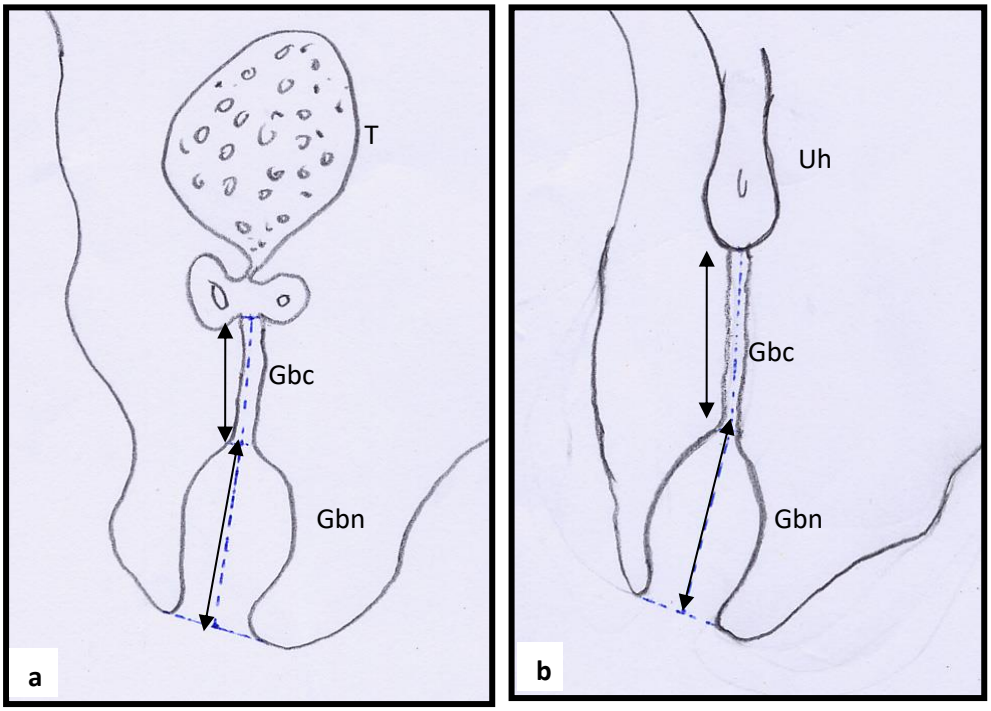

Fig. 1. Diagram showing Gbn and Gbc at E18. **(a)** Male **(b)** Female

Statistical analysis

The type of statistical test applied was the t-test, used to compare two different means; this test is applicable even with unequal variance assuming that the samples came from normally distributed population with confidence level of 95%. The p value <0.05 was considered significant. For gubernacular cone (Gbn): Null hypothesis was that there was no difference in the means between male and female Gbn length. Alternative hypothesis was that there was a difference of means between male and female Gbn length. For gubernacular cord (Gbc): Null hypothesis was that there was no difference of means between male and female Gbc length. Alternative hypothesis was that there was a difference of means between male and female Gbc length.

RESULTS

Rat foetuses at E16 were excluded from morphometric analysis because sexual orientation remained indeterminable at this stage. Analysis thus included prenatal stages such as E17, E18, E20 and postnatal stages such as P0, P2 and P4.

Gbn height in males increased slightly from E17 (326.6±83.5) to E18 (386.6±86.4) but did not differ significantly from Gbn height in females at same days (E17=306.6±37.2, E18=316.6±48). However, between E20 and P0, male Gbn demonstrated significant accelerated growth and 2 fold increase of Gbn height (from 493.3±133.3 to 1046.6±387.3) although attaining maximal height at these same days. The female counterpart failed to show such a similar dramatic increase (423.3±82.3 - 240.0±80.9). From postnatal day P0 till postnatal day P4, the male Gbn height reduced slightly (from 1046.6±387.3 to 973.3±184.4) whilst female Gbn height dropped by more than 50% (from 423.3±82.3 to 163.3±156.6). Thus, between E20 until postnatal day P4, male Gbn height remained significantly greater than female Gbn height at all corresponding days (Fig. 2, Table 1).

In males, Gbc length remained consistent ranging between 410.0 - 610.0 μm, reaching maximal length at P0. However, female Gbc length steadily increased from E17 (426.6 μm), demonstrating a more than 2 fold increase at postnatal P0 (1043.6 μm) and finally an almost 5 fold increase (2136.6 μm) by P4. Thus, between E20 until P4, female Gbc length remained significantly greater than male Gbc length at all corresponding days (Fig.3, Table 1).

DISCUSSION

The gubernaculum at prenatal (E17, E18 and E20) and postnatal (P0) animals was analysed for dimensional changes via morphometry of light microscopy photomicrograph to document detailed alterations in Gb tissue morphology.

At E17, male and females gonads were clearly distinguishable by LM with the testis located just below the inferior pole of the kidney and the ovary located lateral to the kidney. Similar findings were reported in rats[4], in which the ovary remained in close proximity to the caudo-lateral pole of the developing kidney.

Morphometric analysis of Gb at this stage carried out to compare Gb dimensions revealed no statistically significant differences between the length of male and female Gbn and Gbc ($p>0.05$).

The morphometric analysis of the Gbn indicated the male Gbn at E18 showed slight increase in the length compared to female Gbn but, it was not significant ($p>0.05$). The length of the male Gbc was reduced compared to female Gbc but again this was not statistically significant ($p> 0.05$).

By E20, the size of the Gbn in males was maximal which was comparable to the finding [5] who stated that the dynamic development of the Gbn in rats before testicular descent had an important role in the activity. Morphometric analysis revealed that the male Gbn length at E20 was greater compared to female Gbn, whereas, the Gbc length showed an inverse relationship to female counterparts. Statistical analysis of Gbn and Gbc lengths resulted in significant ($p<0.05$) differences between the male and female Gbn and Gbc. In support of these findings, the Gbc was noted to become shorter in male rats, while the Gbn lengthened.

At birth (P0), in the male, the oval testis was demonstrated to possess the epididymis interposed between this and the internal inguinal ring. In the female, the elongated ovary containing distinct primary follicles remained positioned at the inferio-lateral pole of the kidney. The region of the internal inguinal ring was a useful landmark to locate the presence of Gbn.

In the present study, it was observed that male Gbn was involuted at this stage and consisted of distinct skeletal muscle fibres at the periphery and vacuolation within the core. Another study reported that in rats at P0, the Gb starts inversion as the first step towards the formation of the muscular cremaster sac [4]. This finding was confirmed by our SEM study, which revealed invagination of the Gbn and shortening of the Gbc. The female Gbn had reduced cellularity and no sign of Gbn inversion compared to male Gbn and Gbc appeared slender and much elongated compared to male counterparts. This was confirmed by morphometric study of male Gbn ($p<0.05$) and female Gbc ($p<0.05$).

In this study, the Gbn structure at P2 was altered, exhibiting reduction in size and regression at this stage. Whereas, in females, the Gb also appeared to contain reduced cellularity and comprised fibro-fatty tissue. This finding was in agreement with in which the male Gbn core decreased dramatically in volume and almost completely disappeared by postnatal day P3 [6].

At P4, the epididymis began to enter the inguinal canal followed by the testis. The invaginating Gbn was accompanied by a marked invagination of the mesothelium lining of the Gbn along with a medial and inferior direction within the inguinal region. In female gubernacular tissue, the Gbn appeared much reduced with an increasingly fibrous and slender Gbc forming the round ligament precursor.

Morphometric analysis of male and female Gbn and Gbc length indicated that Gbn in males increased in length compared to female Gbn. However, the length of male Gbc was much reduced ($p<0.05$) when compared to the elongated female Gbc. This finding was similar to the findings who reported swelling and shortening of the Gb in males, whilst in females [7], the Gb persisted as a long and thin ligament to form the round ligament of the uterus. One study reported that rat Gb revealed minimal changes in its appearance from P3 to the time of testicular descent at P21 [8]. Attah and Hutson reported that in the human female, the Gb remained small and thin after the fourth month of uterine life [9]. The Gb finally differentiated to form the suspensory ligament of the ovary and the round ligament of the uterus. The Gb was attached to the uterus near the origin of the uterine tube. The cranial part of the Gb became the ovarian ligament; the caudal part formed the round ligament of the uterus.

Gubernaculum during the development showed remarkable morphological statistical differences between male and females in this study. This concluded that further investigation is required in elucidating this mysterious gubernaculum tissue in both sexes [10].

CONCLUSION

The present study indicates that during pre- and postnatal gubernaculum development showed remarkable morphological statistical differences between male and females.

ACKNOWLEDGMENT

Thanks to Dr. Normadiah M. Kassim, Professor, Department of Anatomy, Faculty of Medicine, University of Malaya, Malaysia and Dr. Lakshmi Selvarantnam, Associate Professor, Department of Anatomy, Faculty of Medicine, University of Monash for their guidance in the preparation of this paper and also to Dr. Fatima Badawi for statistical analysis, College of Medicine, University of Hail, KSA.

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Fig. 2: Length of gubernacular cone in male and female rats (n=6)

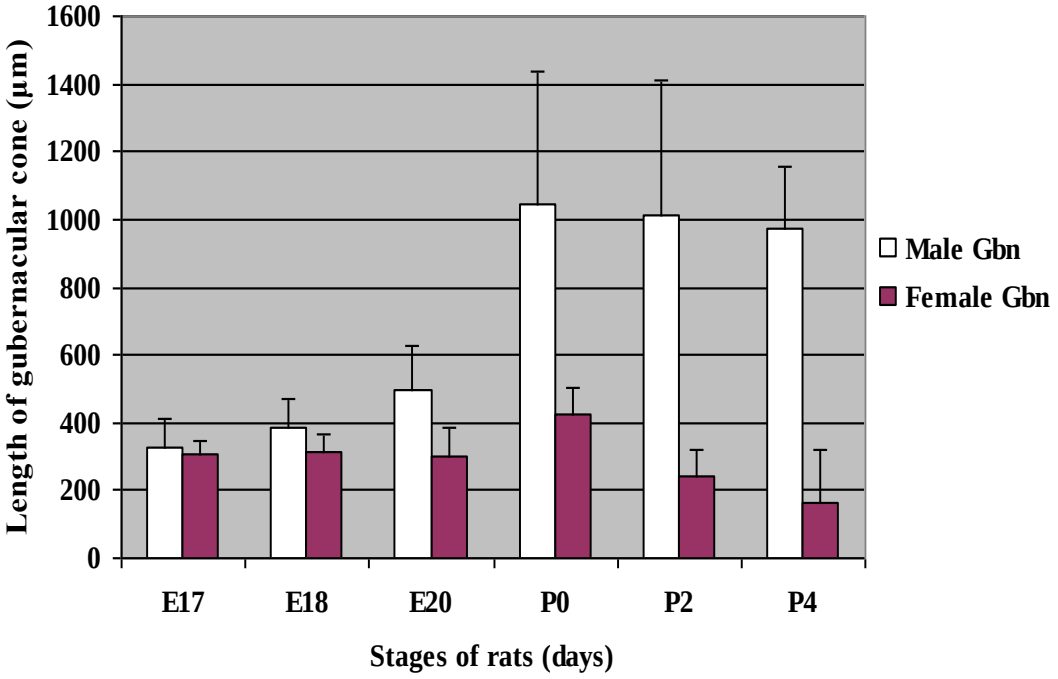

Fig. 3: Length of gubernacular cord in male and female rats (n=6)

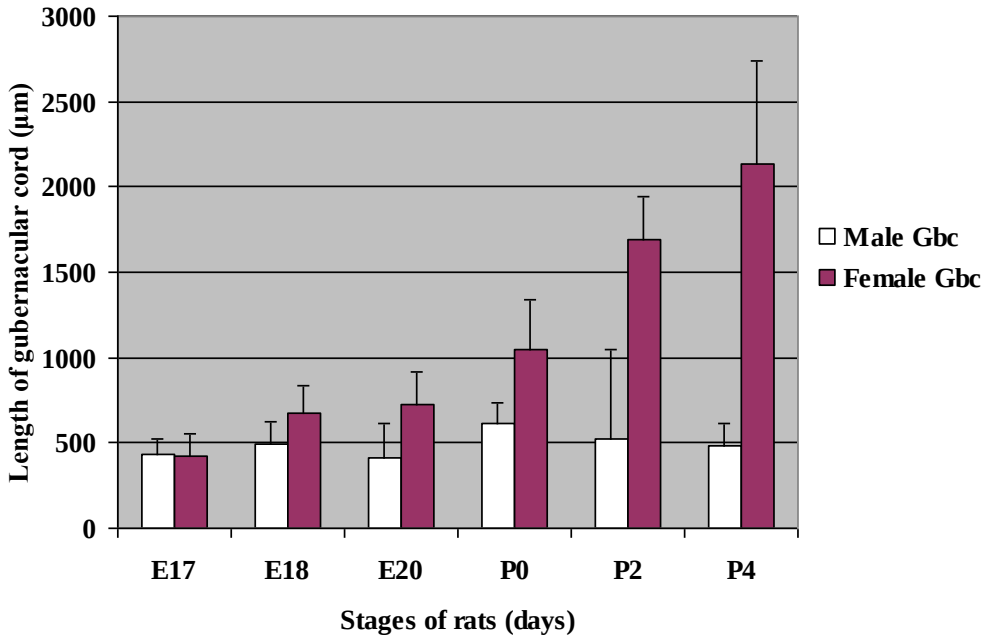

Table 1: Means and SD of Gbn length and Gbc length

Stages (Days)	**Male Gbn (µm ± SD)**	**Female Gbn (µm ± SD)**	**Male Gbc (µm ± SD)**	**Female Gbc (µm ± SD)**
E17	326.6± 83.5	306.6±37.2	433.3±87.3	426.6±130.6
E18	386.6±86.4	316.6±48	490.0±137.2	676.6±154.0
E20	493.3±133.3	303.3±84.3	410.0±204.6	720.0±197.9
P0	1046.6±387.3	423.3±82.3	610.0±123.1	1043.6±295.9
P2	1010.0±402.1	240.0±80.9	523.3±523.3	1690±253.8
P4	973.3±184.4	163.3±156.6	482.0±135.8	2136.6±597.7