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## Histochemical and stereological analysis of the levator ani (pubocaudal) muscle in nulliparous and multiparous beagles

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### Abstract

Fiber-type composition and several stereological parameters of the levator ani (pubocaudal) muscle were evaluated in five nulliparous and five multiparous beagles using myosin ATPase-histochemistry and systematically selected muscle cross-sections. With respect to the narrow canine pelvic cavity, this study was also undertaken to determine whether vaginal birth of at least seven litters causes similar neuromuscular changes in the canine levator ani (pubocaudal) muscle analogous to those seen in the pelvic floor muscles of women after vaginal delivery.

The canine pubocaudal muscle is comprised of approximately equal amounts of slow twitch type I and fast twitch type II (IIA, IIS) fibers. The muscles of both the nulliparous and multiparous beagles did not display any signs indicative of denervation or myopathology. The multiparous dogs exhibited significantly increased mean absolute muscle ( $1720 \text{ mm}^3$ ) and total fiber-type I volumes ( $850 \text{ mm}^3$ ) as well as relevantly increased mean diameter of type I fibers ( $72.0 \mu\text{m}$ ) when compared with the nulliparous group.

The canine levator ani (pubocaudal) muscle is not pathologically affected by vaginal deliveries and seems to adapt to numerous successive pregnancies and births through fiber-type I hypertrophy.

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## 1. Introduction

The M. levator ani, together with the coccygeal muscle and pelvic fascia, forms the pelvic diaphragm, which defines the canine pelvic cavity caudolaterally and caudally [1]. The obturator nerve demarcates the levator ani muscle into the pubocaudal (pubococcygeal and puborectal) and iliocaudal (iliococcygeal) muscles. The levator ani is innervated by the ventral branches of the second and third sacral nerves [2]. When activated and contracted, the levator ani muscle narrows the caudal portion of female canine pelvic cavity. This, in turn, exerts pressure on the urethra and thus contributes to the complex closure mechanism of the urethra, providing urinary continence [3,4]. The pubocaudal muscle arises from floor of the pelvis (ossa pubis and ischiadicum) along the pelvic symphysis and is inserted on the fourth to seventh caudal vertebrae by means of a short tendon. The dorsocaudal part of the muscle also attaches to the lateral aspect of the rectum (puborectal muscle).

In women, pelvic floor muscles, fascia, and an intact nervous system play critical roles in urinary continence [5,6]. Vaginal birth can cause damage to the levator ani muscle and pose a major risk for the development of pelvic floor dysfunction and persistent urinary incontinence [7–9]. Pelvic floor dysfunction is the result of neuromuscular pathophysiology (denervation) as previously documented by neurophysiological and histomorphological studies of the pelvic floor [6,10–12]. The neuromuscular damage is observed as alterations in neuromuscular activity pattern and pathological muscle fibers as well as post reinnervation-muscle fiber-type grouping.

In light of the comparatively narrow canine pelvic cavity, this study was undertaken to determine whether vaginal birth of at least seven litters causes similar neuromuscular changes in the canine levator ani muscle to those seen in the pelvic floor muscles of women following vaginal delivery. For this purpose, histomorphology, fiber-type composition, and stereological parameters (absolute muscle volume, type I and II fiber volume fractions, mean diameter, and absolute fiber numbers) were compared between two homogenous groups of nulliparous and multiparous beagles.

## 2. Material and methods

### 2.1. Animals

Two homogenous groups of five healthy nulliparous and five multiparous beagles in anestrus or late metestrus weighing between 8.7 and 11.2 kg were used for this study. The animals were cared for according to principles of laboratory animal care proscribed by Swiss National Law for the protection of animals. The nulliparous bitches were between 2 and 3 years of age; the multiparous bitches were between 8 and 9 years old and had given birth to at least seven litters. The animals were sacrificed with an overdose of pentobarbital and blood serum levels of progesterone and estradiol  $17\beta$  were determined in order to assess reproductive cycle stage.

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