A SLUICE-LIKE STRUCTURE DRIVEN BY MUSCULAR PUMP IS RESPONSIBLE FOR THE URINARY CONTINENCE IN FEMALE CANINE

Hypothesis / aims of study
The anatomical and functional basis of urinary continence in female canine remains confusing and controversial. The urethra is directly in charge of urinary continence and, it is such a complex and dynamic organ that conducts dramatically contrasting functions—to close tightly at most of time but to open widely at voiding. This means we need to understand the morphology and physiology at different states. Given the limitations of direct study of human beings, some lab mammals are alternatives. Female canine has unique advantages for this purpose: its urethra has clear tissue layers, long proximal urethral segment with pure smooth muscle and a distal segment with strong striated muscle. Importantly, canine urethra structure represents a preliminary status of the urethra evolution because canine locates at the lower level of the mammal branch on evolution tree. This study takes advantages of canine to elucidate urethral continence mechanism by urodynamic and morphometric analysis.

Study design, materials and methods
Totally 12 adult female canines were used and urodynamic tests were conducted under pentobarbital anaesthesia. A midline abdominal incision were made with the pubis symphysis cartilage divided to get good expose of pelvic structures. A surgically installed bladder catheter was connected to the urodynamic equipment for leak point pressure (LPP) test which was conducted with manually press the bladder with the abdominal wall open. The possible movements of pelvic structures were observed directly and video-recorded and, the electromyography (EMG) of the striated urethral sphincter (SUS) and the levator ani (LA) were recorded. In 6 randomly chosen canines the urethral pressure profile (UPP) was recorded with a 7F urethral pressure catheter. Then we determined the segmental distribution of continence function along the whole urethra by the test of segmental invalidation of urethra (TSIU). This was achieved by inserting a 12F catheter in urethra lumen step by step at an interval of 1/16 of the whole urethral length (each interval is 4 to 5 mm, which was determined on basis of UPP of individual animal) and recording LPP at each step. The other 6 animals not having UPP and TSIU were used for morphological study. The whole urethra together with vagina was fixed and divided averagely into 16 segments and then embedded in paraffin. Each segment was averagely divided into 4 levels. For each level a slice of 5μm was stained by trichromatic staining, and serial three sections were immunolabeled for smooth muscle (Sm. M) and striated muscle (St. M, fast/slow) respectively. The digital images of all trichromatic stained slides were obtained on the Hamamatsu Nanozoomer Digital Pathology (NDP) System. Quantitative analysis of the different component of each 1/16 segment of urethra were performed using the Vectra imaging system according to manufacturer's protocols. Statistical analysis was performed with SPSS (version 20.0, IBM). The correlation between morphometric variables, which include submucosa, smooth muscle and striated muscle with urethral pressure at different segments of urethra was analyzed.

Results
The UPP curves displayed low pressure zones in the proximal 56.25% urethral segments and high pressure zones in the 43.75% distal segments (Fig 1 b). In TSIU, the distal 43.75% segments had a much higher LPPs indicating strong continence function, comparing to the proximal 56.25% segments that had lower LPPs indicating weak continence function. The LPPs changed sharply when the distal 2.5 to 3.0 cm of the urethra were invalidated (Fig 1 c&d). At direct observation, we noted that the distal urethral part was much thicker than the proximal part (Fig. 2 a). Also, only the distal urethra contraction (in a way like the heart beats) and only the SUS-EMG bursting occurred upon bladder pressure raised. There was no LA contraction and no LA-EMG bursting recorded.
The St. M might be further divided into two sections: one located at the most distal parts (1/16 to 3/16) with fibers distributed in a fan-like orientation and surrounded a large volume of spongy tissue with thin tubular sinusoids (TTS); another located close to the middle part (4/16 to 6/16) with fibers circularly orientated and surrounded some large cystic sinusoids (LCS) beneath the urethral mucosa. The bundles of dense connective tissue formed 3-dimensional framework in urethra to serve as foundation for other functional components. TTS mainly distributed in 1/16 to 3/16 but also appeared throughout the whole urethra, while the LCS only appeared in 3/16 to 6/16 segments, which corresponding to the peak pressure zone in UPP. These LCSs located close to urethral lumen and formed valve-like structures (Fig 2, b&c). The distribution of urethral pressure failed showing any linear correlation with any particular tissue components in different urethral segments.

Interpretation of results
The female canine urethra can be divided into two segments in terms of the urodynamic features and the histological components. The proximal 56.25% segments that lack St. M and had low UPP and LPP pressures and thus had only weak continence function. The distal 43.75% segments covered St. M and had high UPP and LPP pressures and thus had predominant continence function. The fan-like distributed St. M in the very distal segment acts like a pair of hands holding a reservoir. At stress, the St. M contract and act like muscular pump to push the blood within the spongy tissue flowing into the LCS, and the later extend to form a valve-like structure and close the urethral lumen.

Concluding message
In summary, we conclude that a sluice-like structure driven by muscular pump within the urethra is responsible for urinary continence in female canine.

Disclosures
Funding: This study was supported by National Natural Science Foundation of China (Nos: 30772290). GHD obtained funding.
Clinical Trial: No Subjects; ANIMAL Species: Canine Ethics Committee: Tongji Hospital Ethics Committee