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Ultrasonographic assessment of the male koala (*Phascolarctos cinereus*) reproductive tract

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ABSTRACT

Studies documenting the application of ultrasonography to depict normal and pathological changes in koalas (Phascolarctos cinereus), especially in the male, are scarce. Sixty-two wild koalas were used in this study to define ultrasonographic protocols and features for the assessment of the male koala reproductive tract. Testis, epididymis and spermatic cord were examined using a hockey stick transducer. The normal koala testis showed a homogeneous echogenicity and an obvious hyper-echoic band corresponding to the tunica albuginea. The cauda epididymis was characterised by hypo- and hyper-echoic regions and was most effectively imaged in sagittal section. The koala prostate was assessed using a micro-curved transducer positioned midline, caudal to the bladder. On transverse section, it showed distinct margins and a well-defined internal structure, although the prostatic urethra was not apparent on most scans. To image the bulbourethral glands (BGs), the hockey stick transducer was placed lateral to the cloaca. BGIII was located just below the skin, while BGII was located deeper than BGIII. BGI was too small and not sufficiently echogenic to be detected. The ultrasonographic appearance of the BGs was similar to that of the testes but with more obvious hypo-echoic stippling. This comprehensive review of the ultrasonographic appearance of normal male koala reproductive tract can be used by veterinarians and others, in zoos or those working with wild koalas, during assessment of the reproductive tract of male koalas in relation to seasonal changes in accessory gland function or for the pathological investigation of reproductive lesions and infertility problems.

KEYWORDS: ultrasound, male, koala, reproductive.

INTRODUCTION

Despite numerous published classical dissections and line drawings of female and male koala reproductive anatomy (Brown, 1987; Forbes, 1881; Handasyde, 1986; Johnston, 1998; Johnston and Holt, 2014; Lee and Carrick, 1989; Mackenzie, 1919; Matthews et al., 1995; Obendorf, 1988; Temple-Smith and Taggart, 1990; Young, 1879), studies documenting the application of ultrasonography to depict normal features and pathological changes in this species appear limited to three reports. These are: a single ultrasonographic report of polycystic disease in the female reproductive tract (Mathews et al., 1995), a dissertation later published in the Centre for Veterinary Education Control and Therapy newsletter (Stalder et al., 2016) and a validation study confirming the use of the technique in detecting structural diseases of the urogenital tract in both male and female koalas (Marschner et al., 2014). While Blanshard and Bodley (2008) have also suggested the use of ultrasonography as an effective method of detecting and grading the severity of chlamydial urogenital disease in both sexes, a systematic description of its use with corresponding methodology has yet to be published. Given that Schultz et al (2016) have recently used ultrasonography to help diagnose a case of spermatogenic granuloma and varicocoele in the koala, the availability of detailed descriptions of normal reproductive organs for comparison would be helpful. The following descriptions and ultrasonographic images were assembled as part of a larger study examining the effects of chlamydial disease on the male koala urogenital tract. The aim of the current study was to provide a systematic description of the ultrasonographic features of the normal male koala reproductive tract to assist in the identification and diagnosis of pathological changes, similar to the work recently published for humans (Lotti and Maggi, 2015).

MATERIALS AND METHODS

Animals and clinical examination

This study was conducted under the authority of The University of Queensland Animal Ethics Permit (AE02305). The organs in this study were selected to illustrate the appearance of normal tissue from sexually mature wild male koalas (n = 62) presented to Moggill Koala Hospital, Brisbane, Australia (E152.52'26.4"; S27°34'46.56") during the period extending from January 2014 to December 2016. On presentation, each animal was anaesthetised by induction with tiletamine/zolazepam (Zoletil 100, Virbac Australia Pty Limited, NSW; Allen et al 2006) and then maintained for ultrasonography on a low rate of gaseous isoflurane anaesthesia (0.5 - 1% at 1 – 1.5L per min Oxygen). Clinical assessment consisted of determination of approximate age (Gordon, 1991), body condition (Wood, 1978) and clinical signs of trauma and/or disease (Blanshard and Bodley, 2008). The 62 koalas, assessed as not being suitable for rehabilitation from a larger group of 120 koalas, were euthanased following clinical assessment by the attending veterinarian based on careful consideration of individual welfare issues (Vogelnest, 2008). Euthanasia was carried out by means of an overdose of pentobarbitone sodium (Lethabarb, Virbac Australia Pty Ltd, NSW, or Valabarb, Jurox Pty Ltd, NSW Australia).

Necropsy and histopathology to confirm normal tissue

After euthanasia, a thorough necropsy was performed the same day, usually within the hour, and tissues were processed for histological examination. Tissues were embedded in paraffin wax and sections (5 µm) stained with haematoxylin and eosin (HE).

The criterion for identification of normal tissue was that the individual organ was histopathologically within normal limits. Presence of pathology in one or more organs of

the reproductive tract did not rule out a particular tissue being considered normal using this definition. A koala may have had normal reproductive organs throughout its tract, only some organs may have been abnormal, or all reproductive organs may have been abnormal. Presence of pathology in the urinary tract was also not considered an exclusion factor for selection of a normal reproductive organ.

Ultrasonography

Ultrasonography was conducted using a SonoScape S6 Portable Colour Doppler diagnostic ultrasound (Sonologic Pty Ltd, Brisbane, Queensland). Two transducers were used: (1) a 10l2 hockey stick 10 – 5 MHz, 25 mm (linear surgical) transducer and (2) a C611 micro-curved 8-4 MHz, R 11 mm (paediatric cardiac) transducer (Sonologic Pty Ltd, Brisbane, Queensland). The hockey stick transducer provided high resolution but low penetration (approximately 30 mm), making it ideal to image external genitalia and small, superficial organs such as the testis, epididymis and bulbourethral glands. The C611 micro-curved transducer was used to examine the prostate. In preparation for the ultrasonographic examination, koalas were placed in dorsal recumbency and, to improve contact with the transducer, the fur of the scrotum, scrotal cord, either side of the tail and a small 30 x 20 mm area between the epipubic bones was removed (Moser Arco CordlessTrimmer, Wahl Gmbh, Unterkirnach, Germany, adjustable blade equivalent to 50 to 10 blades). Since the procedures were intended to be used in koalas being rehabilitated to the wild, the area of clipped fur was kept to a minimum. All sites were cleaned with alcohol and ultrasound gel (Aquasonic 100 Parker Laboratories Inc, Fairfield, NJ USA) applied. Ultrasonographic images were annotated on the ultrasound machine, transferred to a USB and assembled using Photoshop (Adobe Photoshop CC 2015).

RESULTS

To facilitate the interpretation of the ultrasonographic descriptions provided below, the gross morphology and topography of the normal male koala reproductive tract is included as Supplementary material 1.

Testis and epididymis

The sagittal aspect of the testis and cauda epididymis (Figure 1A) were examined using the hockey stick transducer (Figure 1B). This allowed the testis to be imaged *in toto,* with portions of the corpus and caput epididymis also detectable in the same view. To obtain a clear image using the linear transducer, the small ellipsoid testes were stabilised by gently manipulating both testes to rest against the distal end of the scrotal sac, reducing their movement. At the same time the transducer was moved across the cranial surface of the scrotum and underlying testis (Figure 1B). Normal koala testicular parenchyma appeared to be homogenous in echogenicity and was encompassed by the clear, hyper-echoic boundary of the fibrous tunica albuginea (Figure 1C, 1D). While the koala testis possesses no obvious mediastinum, it was possible to define the tunica vaginalis and the vaginal cavity.

The cauda epididymis for each testis was best imaged in sagittal section (SS) (Figure 1B), since the transverse section (TS) of this region resulted in less reliable images because of the cauda's small size and proximity to the testis. The normal cauda epididymis showed hypo and hyper-echoic regions and was clearly detectable within the confines of the tunica vaginalis (Figure 1D). The smaller caput epididymis was less obvious on ultrasound than that of caudal region.

Spermatic Cord

The spermatic cord (Figure 2A) was imaged using the hockey stick transducer (Figure 2B) and the rete mirabile (pampiniform plexus) was visualised using the colour Doppler in TS (Figure 2C) and SS. The size and relative position of the blood vessels varied between individual koalas. It was not possible to clearly define the normal vas deferens within the koala spermatic cord using ultrasonography.

Prostate

The prostate was imaged using the micro-curved transducer positioned midline, caudal to the bladder (Figure 3A). The location and the characteristic carrot-shaped SS (Temple-Smith and Taggart,1990, p48.) or ovoid TS appearance of the prostate was confirmed by inserting a gloved finger or probe into the rectum of the koala and gently pushing the prostate back and forth so that the organ's movement could be visualised (Supplementary Material 2). This procedure was particularly useful when the bladder was thickened and/or retracted due to cystitis or the prostate was small and hidden within the pelvic inlet. Locating the prostate using TS rather than SS (Figure 3B, 3C) proved more effective because the organ's margins on SS were less distinct (Figure 3D). Figure 3C, a more caudal TS view of the same prostate as seen in Figure 3B, reveals an internal structure. In koalas with a small prostate, the organ was located by elevating the pelvis with one hand, whilst angling the transducer head towards the pelvic outlet, resulting in a more oblique than TS view. The normal prostatic urethra was not usually apparent in either TS or SS. Figure 3D shows the poorly defined edges of the prostate on SS, a hyper-echoic central region but no discernible urethra.

Bulbourethral glands

Figures 4A and 4B show, respectively, the relative *in situ* and dissected topography of the bulbourethral glands (BGs) in the peri-cloacal region. The most effective procedure for imaging these glands was to place the hockey stick transducer lateral to the koala's tail, as shown in Figure 4C, and to move the tip of the probe from left to right to locate the relatively thickly encapsulated crus penis muscles, adjacent to BG III. These glands were located approximately 0.5 – 1 cm below the skin. BG II could then be located deeper to BGIII in the same field of view. BGI was too small and not sufficiently echogenic to be detected. The appearance of the normal bulbourethral glands was similar to that of the testes but with more obvious hypo-echoic stippling (Figures 4D). The bulbourethral glands had a definite capsule, much thinner than that of the crus penis muscle, and frequently a hypo-echoic peri-capsular area was seen around BGIII. The movement of the bulbourethral fluid within the gland was readily apparent, particularly in BGII.

DISCUSSION

In this study we have presented ultrasonographic protocols and corresponding images for the assessment of the male koala reproductive tract. We have demonstrated that it is possible to define normal testicular, epididymal, scrotal cord, prostatic, and bulbourethral gland structure by means of ultrasound, thereby providing a foundation for disease investigation in the male koala. While ultrasound has been used previously in koalas, primarily to assess female reproductive pathology associated with chlamydiosis (Stalder, 2003), that study was based only on three individuals, provided no reference to normal tissue and was reported in a publication difficult to access. In addition, while Marschner et al. (2014) have reported the ultrasonographic examination of 14 male koalas (23% of 62) and validated that changes seen on ultrasound were representative of

pathology, they did not include images to support their observations or describe details of the appearance of the normal organs.

The appearance of the koala testis on ultrasound is similar to that of the dog (Penninck and d'Anjou 2008; Pugh et al 1990) except that there is no mediastinum, and hyper-echoic regions relating to connective tissue were not apparent. The koala testicular capsule is well defined, as is the appearance of the epididymis, with the cauda epididymis being more easily visualised than the corpus and caput. The shape of the prostate was carrot-shaped on sagittal section and oval or circular in transverse view. As in the dog (Davidson and Baker 2009), the lumen of the prostatic urethra was not normally visible. The koala prostate can be divided into three histologically distinct regions (anterior, central, posterior), the functional significance of which have yet to be determined (Temple-Smith and Taggart, 1990). However, ultrasonography was not able to specifically differentiate these three regions

In the dog, prostate length, width, height and estimated volume obtained from ultrasound images are positively correlated with the animal's body size and age (Ruel et al, 1998) and formulae have been developed to express these relationships (Atalan et al, 1999). By contrast, Hogan et al. (2010) used ultrasound in the koala's close relative, the Southern Hairy Nosed wombat, to document seasonal changes seen in size of the wombat prostate and bulbourethral glands. Unfortunately, the measurements of the koala prostate found in the current study could not be used in a similar manner because the total number of koalas with normal prostates (as defined in Materials and Methods above) was too low (n= 3 out of 62) and the prostate *in toto* was frequently obscured within the pelvic inlet. A dissection study has demonstrated that seasonal changes in the reproductive tract of male koalas do occur (Allen et al 2010), so in the absence of easily detectable prostatic

changes, variations in the bulbourethral glands may prove more useful for evaluating reproductive status in the koala.

In conclusion, this study represents the first comprehensive review of the ultrasonic appearance of the normal male koala reproductive tract. The information presented here can be used by veterinarians and others, in zoos or those working with wild koalas, during reproductive assessment of male koalas to detect seasonal changes in accessory gland functions or for the pathological investigation of reproductive lesions and infertility problems.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. (A) Sagittal section through the normal koala testis showing the testicular parenchyma (TP), vas deferens (VD), rete mirabile (RM), cauda epididymis (Cd) and caput epididymis (Cp); (B) position of the transducer to examine the left cauda epididymis SS; (C) ultrasound SS of normal right testis and cauda epididymis (white arrow) with colour Doppler; (D) ultrasound SS of normal left testis and caput epididymis (arrowhead) without Doppler. Note the hyper-echogenic tunica albuginea (black arrow) and tunica vaginalis (black triangle).

Figure 2. Ultrasonography of the koala spermatic cord. (A) Gross transverse section through the spermatic cord; (B) position of the hockey stick probe to examine the spermatic cord in TS; (C) ultrasonographic colour Doppler TS image of the spermatic cord.

Figure 3. Normal prostate in male koalas. (A) Position of the curvilinear transducer to image the transverse section of the koala prostate. Note the finger inserted into the cloaca so as to move the pelvic contents to aid in location of the prostate; (B) TS view of the normal prostate (*yellow dots*) showing its position relative to the urinary bladder (Bl); (C) more caudal TS view of the prostate of the same animal as (B) (*yellow dots*), showing a clear internal structure; (D) SS of the normal prostate showing the indistinct margins (*dotted line*) encountered with this view, with no visible urethra.

Figure 4. Bulbourethral glands in the male koala. *In situ* (A) and dissected (B) pericloacal region of the koala showing location of the bulbourethral glands (BGs); (C) transducer position for visualisation of the left BGIII and II; (D) ultrasound image of the right BGIII, showing the shallow depth of the gland 0.5 - 2 cm below the skin, and hypoechoic stippling, and the right BGII adjacent to and at a deeper position compared to BGIII, but similar hypoechoic stippling. BGI is not sufficiently echogenic to be visible.

Legend: BGI = bulbourethral gland I; BGII = bulbourethral gland II; BGIII = bulbourethral gland III; Pe = penis; CPMI = crus penile muscle I; CPMII = crus penile muscle II.

Figure legends supplementary material

Supplementary material 1. Sagittal dissection of the normal male koala reproductive tract showing the approximate topography of the bulbourethral gland I (BGI), bulbourethral gland II (BGII), bulbourethral gland III (BGIII), bladder (Bl), coccyx (Cx), epi-pubic bone (Ep), penis (Pe), prostate (Pr), cut pelvic floor (Pv), scrotal cord (SC), testis with the tunica vaginalis (Te), ureter (Ur) and removed crus penile muscles (CPM).

Supplementary material 2. Ultrasonography TS of the prostate. Use of a gloved finger (or in this case a rectal electroejaculation probe) into the rectum to move the prostate (yellow dots at the end of the video) cranially to aid in establishing its location caudal to the bladder (yellow dots at the commencement of the video).

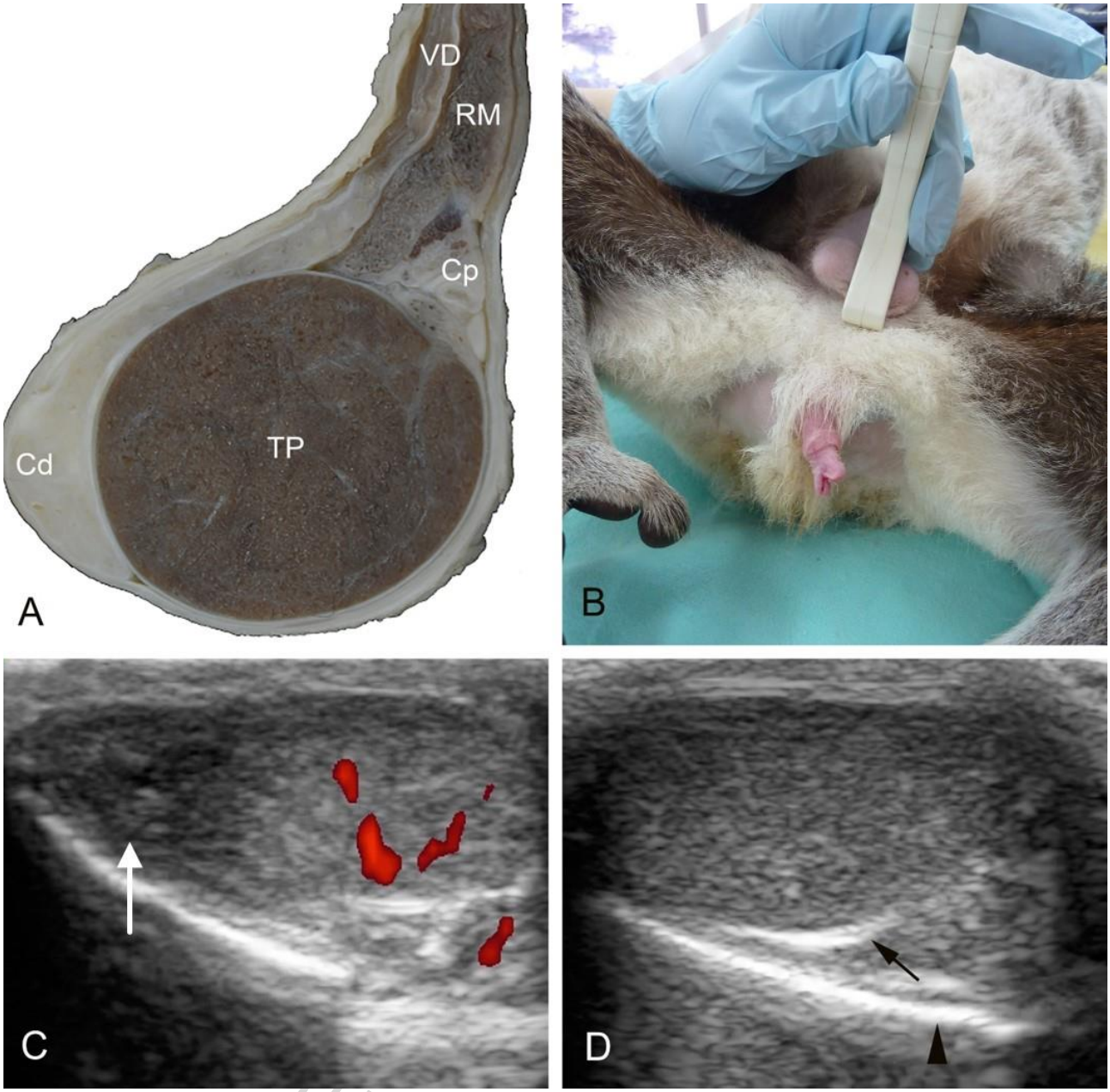

Fig. 1

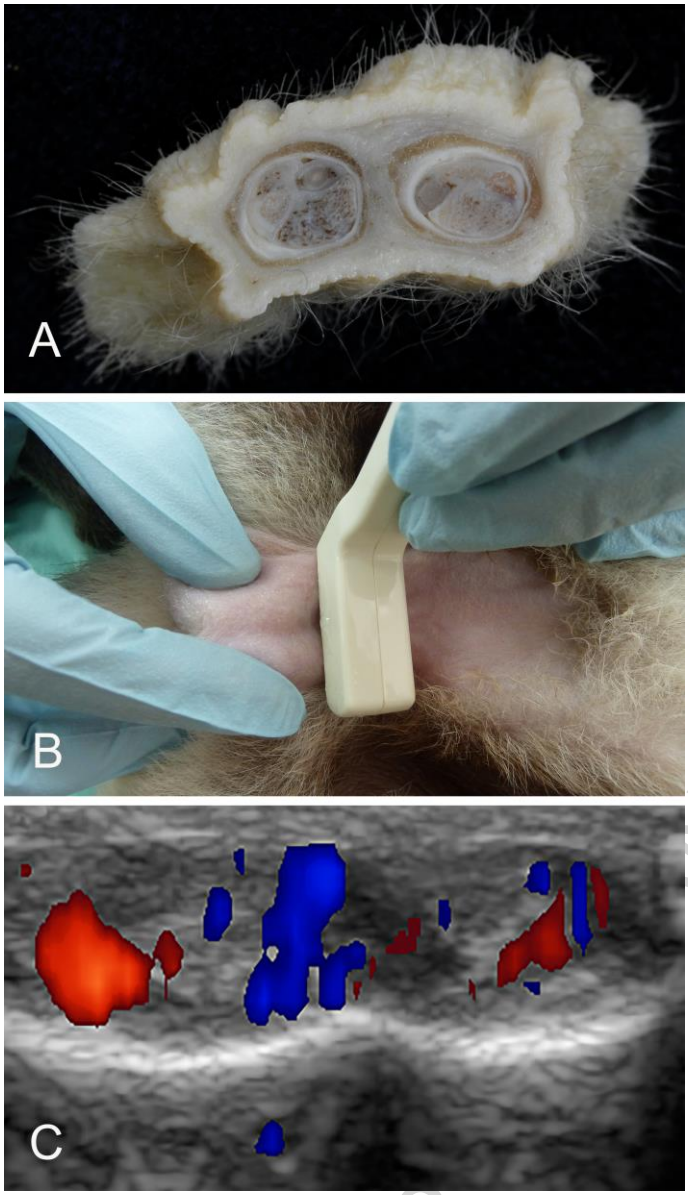

Fig. 2

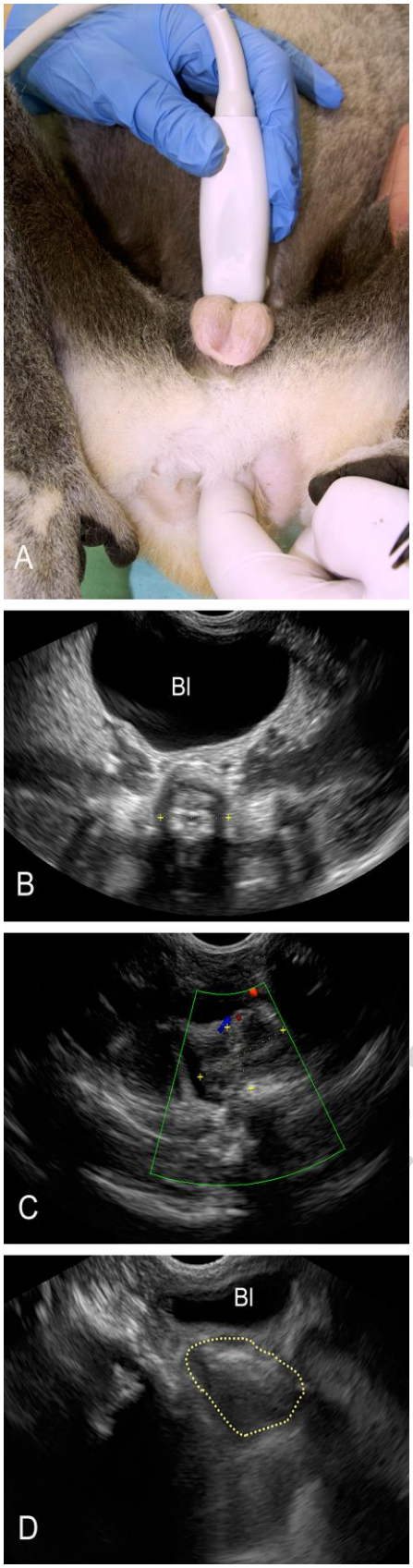

Fig. 3

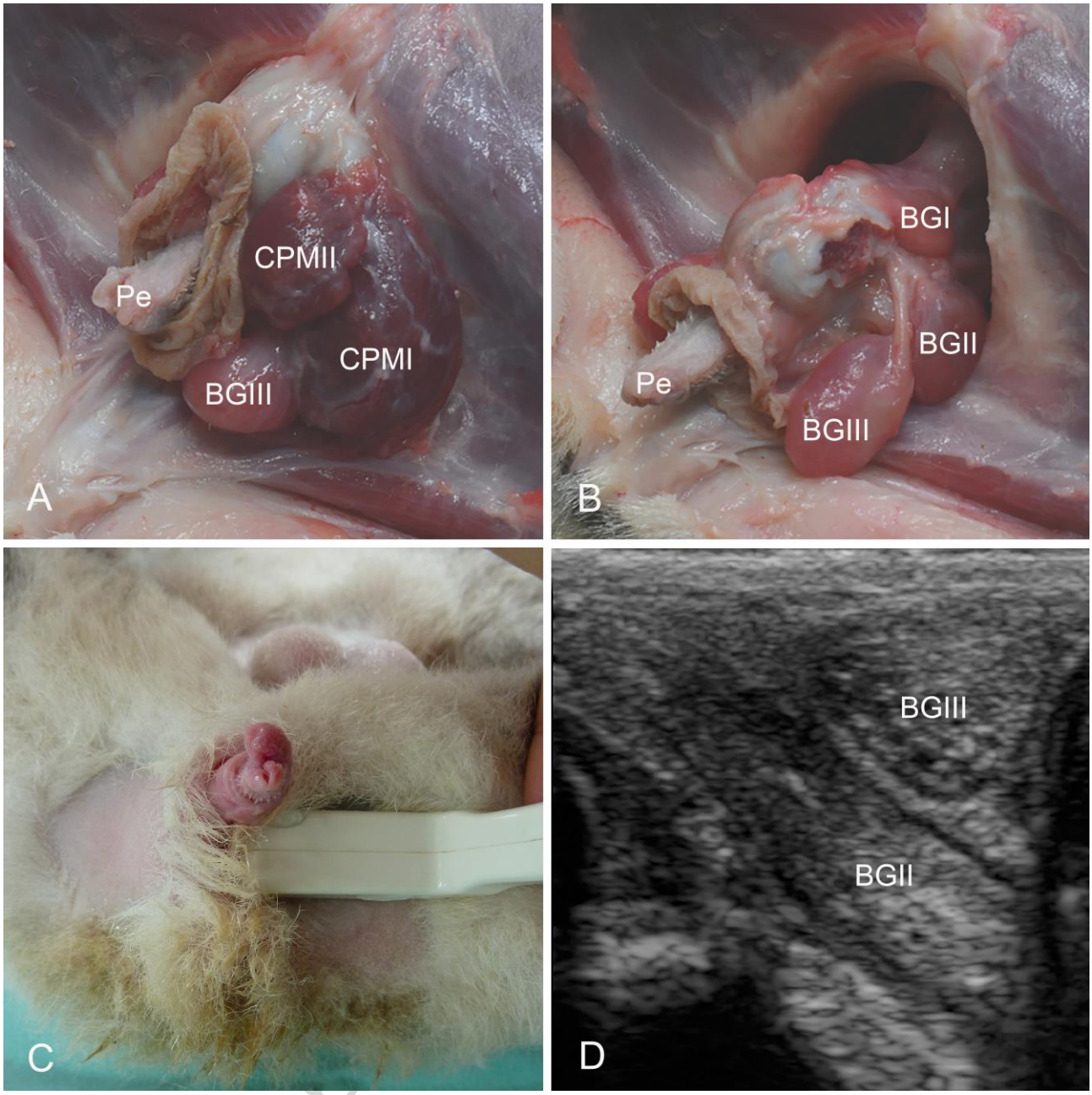

Fig. 4

Highlights

- A review of the normal male koala reproductive tract on ultrasound is provided
- Hickey stick or micro-curved transducers can be used.
- Testis, epididymis, prostate and bulbourethral glands can be succesfully imaged.
- Useful visual description for normal or pathological investigations of the reproductive tract