

Descriptive anatomy of the female reproductive organs in Thamin Eld's deer (*Rucervus eldii thamin*) and Fea's muntjac (*Muntiacus feae*) with utilizing of the glycerin impregnation technique for specimen preservation

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Abstract

The reproductive anatomy of rare cervid species remains insufficiently documented, limiting comparative studies and preservation efforts. This study describes the anatomical details in the female reproductive organs of Thamin Eld's deer (*Rucervus eldii thamin*) and Fea's muntjac (*Muntiacus feae*), while the application of a modified glycerin impregnation technique was achieved for specimen preservation. Dissections of female reproductive tracts revealed morphological variations that correlate with the known female reproductive characteristics of domestic and closely related species. In both species, the ovaries were characterized as small, ovoid structures, exhibiting prominent follicles or corpora lutea consistent with the stage of the estrous cycle. The oviducts presented as highly convoluted tubules. The uterus was bicornuate, with elongated horns in Thamin Eld's deer compared to the shorter horns in Fea's muntjac. The cervix was characterized by six annular folds in Thamin Eld's deer and four annular folds in Fea's muntjac, both displaying complex cervical canals. Overall, the gross anatomy of the female reproductive tract in these cervids was consistent with the typical morphology observed in small ruminants. The tissue's inherent flexibility, coloration, and anatomical integrity of both female reproductive tracts were preserved through modified glycerin impregnation. Accordingly, the resulting specimens retained exceptional anatomical detail, making them highly suitable for veterinary education, museum exhibition, and advanced research. These findings establish foundational anatomical benchmarks for Thamin Eld's deer and Fea's muntjac, advancing both comparative reproductive biology and conservation strategies. Furthermore, this study validates modified glycerin impregnation as a practical, less toxic option for the long-term preservation of delicate reproductive structures.

Keywords: anatomy, cervids, conservation, female reproductive organ, glycerin impregnation

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Introduction

Among the cervid species, Thamin Eld's deer (*Rucervus eldii thamin*) and Fea's muntjac (*Muntiacus feae*) are two rare cervid species native to Southeast Asia whose reproductive biology remains poorly documented. The Thamin Eld's deer is currently restricted to Myanmar and parts of western Thailand. While this species has been classified as 'Endangered' on the Red List of Threatened Species by the International Union for Conservation of Nature (IUCN) (Gray *et al.*, 2015), the Office of Natural Resources and Environmental Policy and Planning in Thailand elevates its status to 'Critically Endangered' on the Thailand Red Data (ONEP, 2020). Likewise, Fea's muntjac is a small, elusive deer occurring in fragmented habitats in Myanmar and southern and western Thailand. It is globally classified as Data Deficient by the IUCN (Timmins *et al.*, 2016) but is listed as Endangered in the Thailand Red Data (ONEP, 2020). While the red muntjac (*Muntiacus muntjak*) remains widely distributed, its sister species, Fea's muntjac, is currently listed as Endangered in Thailand (Supapannachart *et al.*, 2026). Notably, the genetically verified, purebred ex-situ captive population of Fea's muntjac maintained under the Zoological Park Organization of Thailand has declined to critically low numbers, with fewer than three active breeding pairs reported in their official census. This extreme bottleneck underscores the urgent need for optimized assisted reproductive technologies and advanced specimen preservation methods to safeguard the remaining genetic reservoir of this rare cervid (Supapannachart *et al.*, 2026; ZPOT, 2026). Therefore, both cervid species represent urgent conservation priorities. The persistent absence of reproductive anatomical data continues to impede effective conservation-management strategies, captive breeding protocols, and broader comparative morphological research within the Cervidae family (Monfort *et al.*, 1993; Phraluk *et al.*, 2014). Consequently, effective conservation-management strategies must be prioritized to prevent the extinction of these rare cervids. Among these strategies, captive breeding and assisted reproductive technologies (ARTs) such as artificial insemination (AI) and embryo transfer (ET) are indispensable tools for genetic management. However, the successful implementation of these advanced reproductive protocols is severely constrained by the near-total lack of gross anatomical information regarding their female reproductive tracts. In ruminants, the cervix poses a major anatomical barrier to artificial breeding due to its highly tortuous pathway and the presence of prominent annular folds. Without detailed baseline data on the number, orientation, and diameter of these cervical folds, as well as the length and curvature of the uterine horns and body.

Understanding the anatomy and function of the female reproductive organs is fundamental to elucidating species-specific reproductive cycles, assessing fertility, and identifying potential barriers to successful breeding. This knowledge provides a critical foundation for advancing reproductive biotechnology,

improving conservation strategies, and supporting veterinary interventions across diverse cervid species. Extensive descriptions of the female reproductive tracts have been well-documented in various domestic and wild cervids, such as the red brocket deer (Mayor *et al.*, 2012) and pampas deer (Ungerfeld *et al.*, 2008; Perez *et al.*, 2013); however, comparable gross anatomical data remain severely lacking specifically for the highly endangered Fea's muntjac. This knowledge gap is significant because both species of cervids face increasing pressures from habitat loss and hunting, emphasizing the need to conserve them. Strengthening anatomical knowledge across cervid species not only advances comparative research but also provides essential tools for reproductive management, thereby enhancing conservation strategies, supporting veterinary interventions, and enriching zoological education aimed at safeguarding both well-studied and understudied species (Clark, 2011; Korzekwa and Kotlarczyk, 2021).

Beyond anatomical data, the preservation of rare specimens is crucial for strengthening research and educational outcomes. Although formalin fixation and plastination are widely utilized for specimen preservation and have advanced anatomical knowledge, these methods are accompanied by distinct limitations. Formalin fixation, while effective in preventing tissue decomposition, is associated with a strong odor, notable toxicity, and potential health hazards, including carcinogenic risks for students, instructors, and researchers (Adamovic *et al.*, 2021). Additionally, prolonged storage in formalin frequently results in tissue hardening, discoloration, and shrinkage, which diminish the natural appearance of specimens (Likhithaswamy *et al.*, 2022). The plastination technique, though highly effective in producing durable and lifelike specimens, requires specialized equipment, considerable financial resources, and technical expertise that may not be readily available in many academic institutions (von Horst *et al.*, 2019). A replacement preservation technique that has gained increasing attention in recent years is 'glycerin impregnation', known as the Elnady technique, which involves the gradual replacement of water in tissues with pure glycerin (Elnady, 2016). This procedure offers several advantages for anatomical preservation, as specimens remain soft, keep their natural coloration, and do not emit toxic vapors, thereby enhancing both safety and educational value (Daniel and Kukor, 2024). However, while glycerin itself is non-toxic, standard laboratory safety protocols must still be observed, as the pre-treatment and dehydration stages often involve other chemical agents that require careful handling. Glycerin-preserved organs are highly suitable for teaching purposes, as they allow students to handle and examine anatomical structures with reduced chemical exposure. Furthermore, glycerin impregnation is relatively inexpensive and does not require complex equipment, making it accessible for many academic institutions with limited resources (Bernal *et al.*, 2022; Pichardo-Molinero *et al.*, 2024; Tienthai *et al.*, 2025). While the benefits of this method are well-established for

domestic species, its use in the preservation of rare cervids has yet to be explored, representing a significant gap in wildlife anatomical research.

To overcome the deficiency of anatomical information in these species, both thorough morphological analysis and improved preservation methods are required. Therefore, this study aimed to characterize the gross anatomy of the female reproductive organs of Thamin Eld's deer and Fea's muntjac and to evaluate a modified glycerin impregnation technique as an alternative method for specimen preservation.

Materials and Methods

Collecting specimens and anatomical analysis: The female reproductive tracts of the Thamin Eld's deer ($n = 1$) and the Fea's muntjac ($n = 1$), both species from the captive population at Khao Kheow Open Zoo in Chonburi Province, Thailand, were examined after their natural deaths. Historically, the Thamin Eld's deer population at this facility originated from founder lineages established through international conservation exchange programs with the Smithsonian's National Zoological Park, USA, and the Union of Myanmar. Conversely, the Fea's muntjac specimens were derived from rescued wild-born individuals originating from the Tenasserim Range and mountainous regions of Western/Southern Thailand, subsequently managed under the ZPOT's captive conservation program. Detailed biological background, including age and body weight, was documented for reference. Following notification of the deaths, zoo veterinarians promptly transported the carcasses to the pathology laboratory for necropsy. To prevent post-mortem autolysis, the carcasses were kept in an insulated icebox container at 4°C immediately after death and transported under refrigerated conditions to the laboratory within 30 minutes post-mortem. The female reproductive tract was carefully isolated and excised in its entirety. To prevent mechanical damage, tension-induced stretching, or distortion of the pelvic tubular organs (specifically the cervix and vagina), an osteotomy was performed to open the narrow pelvic canal of the cervids. Using the obturator foramina as key anatomical landmarks, the pubis and ischium were bilaterally sectioned utilizing a bone-cutting saw and bone shears. Once the pelvic floor was opened and reflected, the entire reproductive tract extending from the ovaries to the vulva was exposed. The tract was then meticulously dissected and removed as a single, intact unit using standard surgical scissors and anatomical forceps, ensuring all anatomical structures remained preserved in their natural relations for subsequent gross measurements. All quantitative gross anatomical measurements were obtained from the fresh specimens immediately following extraction, prior to any chemical fixation or glycerin impregnation, to eliminate the risk of tissue shrinkage or morphological distortion. Measurements were conducted using a digital Vernier caliper and organ weights were recorded using a digital weighing scale. To ensure accuracy and minimize human error, all

measurements were performed in triplicate by a single experienced observer, and the mean values were calculated and recorded for analysis. Internal examination was performed by making longitudinal incisions along the dorsal midline of the vulva, vestibule, vagina, uterine cervix, uterine body, and uterine horns. Morphological features of each internal structure were described and recorded. The presence of ovarian follicles and corpora lutea was noted to assess reproductive status. Photographs of the reproductive organs were taken with a digital camera to provide visual documentation. In this study, anatomical terminology was derived from *Nomina Anatomica Veterinaria* (World Association of Veterinary Anatomists, 2017) and *The Viscera of the Domestic Mammals* (Nickel *et al.*, 1979), a widely recognized reference in veterinary anatomical education.

Modified glycerin impregnation technique: Because of the small size of the female reproductive organs from Thamin Eld's deer and Fea's muntjac, all preservation procedures were slightly modified from those previously established by Tienthai *et al.* (2025). The modifications included shorter durations for fixation, dehydration, and glycerin impregnation, as well as maintaining the dehydration step at -5 °C, as outlined below.

Fixation step: After anatomical exploration, the female reproductive tracts of both species were initially immersed in plastic containers filled with a specific fixative solution. Each liter of this solution consisted of 700 mL tap water, 100 mL of 37% formalin, 100 mL of 95% alcohol, 90 mL of glycerin, and 10 mL of phenol. The solution was stirred for 5 minutes to ensure homogeneity, and the volume of fixative used was about five times that of the specimen to allow complete immersion. To prevent floating, the reproductive organs were covered with cotton cloths and kept submerged. The containers were tightly sealed and stored at approximately 4°C in a household refrigerator for 14 days. After fixation, the specimens were removed and rinsed under running tap water for 48 hours.

Dehydration step: This step was carried out to remove residual water and lipids from the tissues of female reproductive organs. Following fixation, the reproductive organs were sequentially placed in acetone solutions of increasing concentration: 80% acetone at -5°C for 2 weeks, 90% acetone at -5°C for 2 weeks, and finally 100% acetone at room temperature for 2 weeks. The volume of acetone used was approximately five times the volume of each specimen to ensure complete immersion.

Glycerin impregnation step: Following completion of the dehydration step, specimens were rinsed to remove residual acetone and subsequently immersed in tightly sealed containers filled with pure glycerin. The volume of glycerin used was approximately five times the specimen volume, ensuring complete immersion.

Samples were maintained at room temperature for 6 weeks. To prevent floating, each reproductive organ was gently pressed and secured with cotton cloths during immersion.

Curing step: This step contained the draining and finishing processes. After saturation with pure glycerin, the specimens were removed from the containers and placed on racks to drain for a minimum of 3 days at room temperature. Residual glycerin was thoroughly wiped away using absorbent paper or sponges. The finishing process involved storing the specimens in cornstarch to facilitate curing. To prevent direct contact, each female reproductive organ was placed in a sealed cotton bag before being embedded in cornstarch. The containers were maintained at room temperature for at least 2 weeks, during which glycerin exudation was monitored. During this period, specimens were occasionally turned and gently massaged within the bags to promote uniform curing. Cornstarch that became drenched and clumped due to excess glycerin was replaced with fresh powder as needed.

Storage step: After completion of the curing process, the female reproductive specimens of both animals were carefully cleaned using soft brushes and then stored in appropriately sized transparent acrylic boxes at room temperature.

Results

Female reproductive organ in Thamin Eld's deer: The female Thamin Eld's deer specimen was salvaged from a nulliparous individual estimated to be 25 years of age, weighing approximately 33 kg, following its natural death on September 5, 2025. The cause of death was confirmed to be natural and was unrelated to any diseases affecting the reproductive tract. During necropsy, the female genital tract was incomplete due

to a veterinary oversight; consequently, the vulva and vestibule were not collected. In the descriptive anatomy, the ovaries, oviducts, uterine horns, uterine body, uterine cervix, and vagina were documented as major parts of the female reproductive tract of the Thamin Eld's deer (Fig. 1a). Both ovaries were relatively small, oval, and positioned at the cranial end of the reproductive tract (Figs. 1b and 1c). The left ovary measured 1.2×0.9 cm (length $\times$ width) and showed a dominant follicle measuring 0.4 cm in diameter (Fig. 1b). In contrast, the right ovary measured 1.0×0.3 cm (length $\times$ width) and displayed a smooth surface with several small follicles (Fig. 1c). To maintain the precise anatomical orientation of the ovaries relative to the reproductive tract, both ovaries of the Thamin Eld's deer were retained in situ during the examination. The oviducts were thin, highly tortuous tubes that were difficult to distinguish during necropsy (Figs. 1b and 1c). The total length of the oviducts measured 10.3 cm on the left and 10.1 cm on the right. The uterus consisted of two uterine horns, a single uterine body, and one uterine cervix. The uterine horns were of the bicornuate type, tapering toward their tubal extremities and displaying the characteristic curvature typical of this morphology (Fig. 1a). The left uterine horn measured 3.5 cm, and the right horn measured 4.0 cm along the greater curve. The uterine body measured about 3.0 cm in length. Upon longitudinal opening of the uterine horns and body, the caruncles were scarcely visible. The uterine cervix measured about 4.8 cm in length, and it was thick-walled with a tortuous cervical canal formed by six annular folds. The annular folds closest to the vagina were relatively large, while the subsequent folds progressively decreased in size toward the uterine body. The terminal annular fold projected into the vaginal lumen, clearly forming the intravaginal part of cervix. The vagina was a muscular tube about 5.1 cm in length, characterized by a wide lumen and a mucosal lining with delicate longitudinal folds.

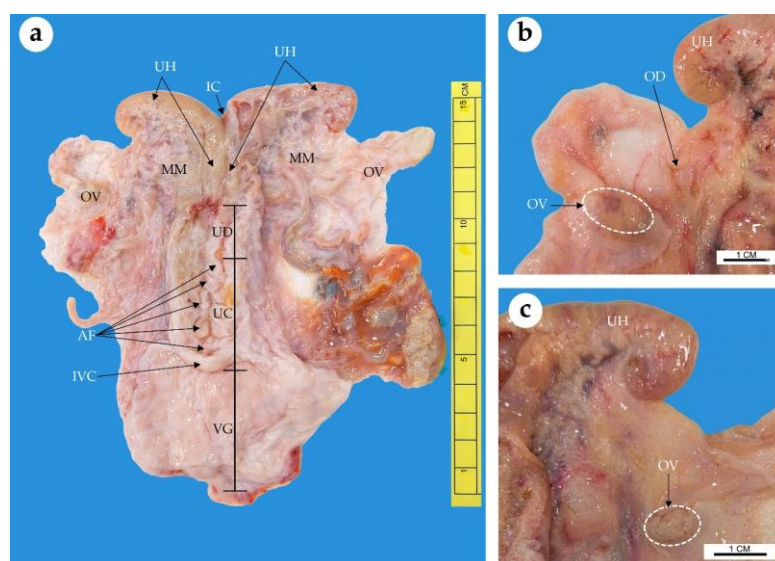


Figure 1 Overview of female reproductive tract of the Thamin Eld's deer (*Rucervus eldii thamin*). (a) Dorsal aspect after partial longitudinal incision during autopsy; (b) anatomical position of the left ovary; (c) anatomical position of the right ovary. UH = uterine horn, IC = intercornual ligament, MM = mesometrium, OV = ovary, UD = uterine body, UC = uterine cervix, AF = annular fold, IVC = intravaginal part of cervix, VG = vagina, and OD = oviduct.

Female genital tract in Fea's muntjac: The anatomical evaluation was performed on a female Fea's muntjac that died naturally on September 1, 2025. At the time of death, the animal was 19 years of age and had a body weight of approximately 25.2 kg. All parts of the female reproductive organs were intact after removal from the pelvic cavity. The ovaries, oviducts, uterine horns, uterine body, uterine cervix, vagina, vestibule and vulva were observed as the anatomical structures of the Fea's muntjac (Fig. 2a). At necropsy, the external morphology of the female reproductive tract in the Fea's muntjac appeared normal. After longitudinal sectioning, however, purulent material was observed within the caudal lumen of the uterine horn adjacent to the uterine body, accompanied by moderate endometritis affecting both the uterine body and horns (Fig. 2b). The bilateral endometritis and purulent uterine contents were incidental post-mortem pathological findings discovered during necropsy, rather than being related to active reproductive or breeding activities. Both ovaries were small and oval, characterized by an uneven surface topography (Figs. 2c-2e). The left ovary measured 1.2×0.5 cm (length $\times$ width), while the size was 1.8×0.7 cm (length $\times$ width) in the right ovary (Fig. 2c). On longitudinal section, the left ovary contained two distinct follicles within its parenchyma, measuring 0.3 cm and 0.4 cm in

diameter, respectively (Fig. 2d). In contrast, a corpus luteum (CL) measuring 0.8 cm in diameter, dark red in color, and a follicle 0.4 cm in diameter were observed in the right ovary (Fig. 2e). The oviducts were difficult to examine in the fresh specimens. The uterus consisted of two uterine horns, one uterine body, and one uterine cervix. The uterine horns were of the bicornuate type, characterized by being shorter yet more curved than those of the Thamin Eld's deer (Fig. 2a). The left uterine horn measured 2.7 cm, the right horn measured 3.5 cm along the greater curvature, and the uterine body measured 1.8 cm in length. Following longitudinal opening of both uterine horns and body, the luminal surface was shallow mucosal folds, and caruncles were inconspicuous and lacked distinct protrusion. The cervix measured 3.6 cm in length, with thick walls and a tortuous cervical canal formed by four annular folds. The last annular fold was enlarged and projected into the vaginal lumen, forming the intravaginal part of cervix. The vagina was approximately 6.0 cm in length, with a broad lumen and longitudinal mucosal folds. The external urethral opening was located at the vagino-vestibular junction. The vestibule appeared as a pouch measuring about 4.1 cm in length. The vulva was composed of left and right labia that were relatively small and externally covered with hair.

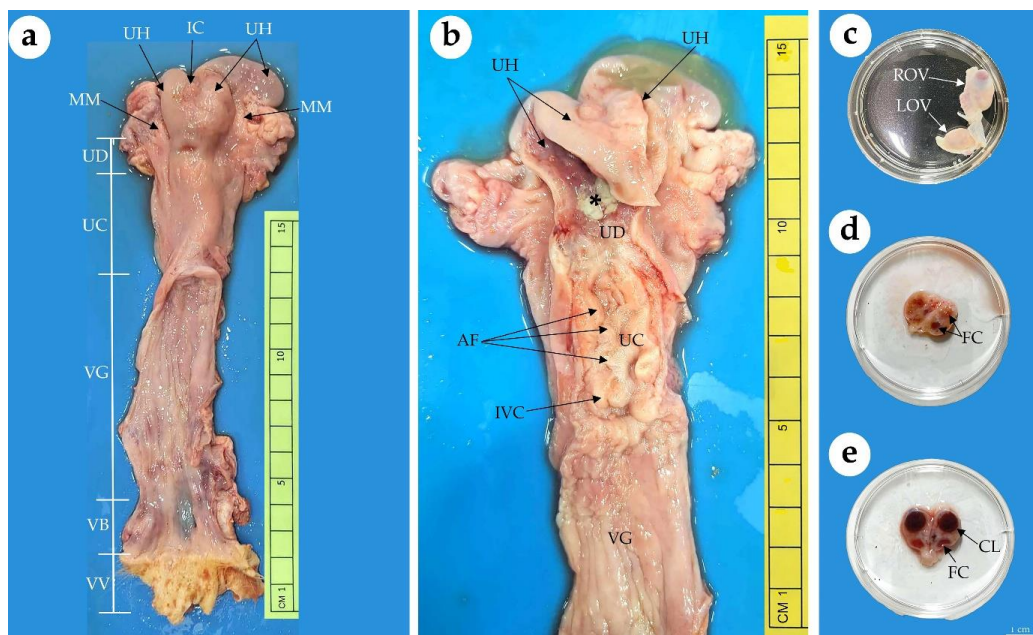


Figure 2 Overview of female reproductive tract of the Fea's muntjac (*Muntiacus feae*). (a) Dorsal aspect presenting the intact uterus, with longitudinal sectioning of the vagina and vestibule; (b) dorsal aspect following longitudinal incision, revealing the lumen of the uterine horns, uterine body, and uterine cervix; (c) external anatomical features of the intact left and right ovaries following removal from the genital tract; (d) internal anatomical appearances of the left ovary and (e) the right ovary after longitudinal incision. UH = uterine horn, IC = intercornual ligament, MM = mesometrium, UD = uterine body, UC = uterine cervix, VG = vagina, VB = vestibule, VV = vulva, AF = annular fold, IVC = intravaginal part of cervix, ROV = right ovary, LOV = left ovary, FC = follicle, CL = corpus luteum, * = purulent accumulation.

Preservation specimens by modified glycerin impregnation: Specimens of the female genital tracts from Thamin Eld's deer (Fig. 3) and Fea's muntjac (Fig. 4) were preserved using a modified glycerin impregnation technique intended for educational and museum purposes. Following preservation, qualitative handling and observation indicated that the female

genital tracts retained their macroscopic anatomical configuration, with the vagina, uterine cervix, uterine body, and uterine horns remaining clearly distinguishable. In the Thamin Eld's deer, the ovaries and oviducts remained intact, although these small structures were difficult to identify macroscopically in the preserved specimen (Fig. 3). Notably, the annular

folds and cervical canal of the uterine cervix in both species appeared more distinct and easier to detect than during the initial necropsy of the fresh specimens. The preservation method also maintained the three-dimensional morphology of delicate structures, such as the mucosal folds of the vagina and the intravaginal part of the cervix (Figs. 3 and 4). The protocol was slightly modified by reducing the duration of fixation, dehydration, and glycerin impregnation steps to accommodate the small specimen size. The dehydration process utilized 80% and 90% acetone

cooled to -5°C . Although a comparative control study with conventionally preserved specimens was not conducted to objectively measure shrinkage, this low-temperature dehydration step was incorporated based on standard plastination principles to help mitigate potential tissue shrinkage and discoloration. Based on subjective evaluation, the specimens remained pliable and odorless, successfully maintaining their structural integrity over a storage observation period of 12 months.

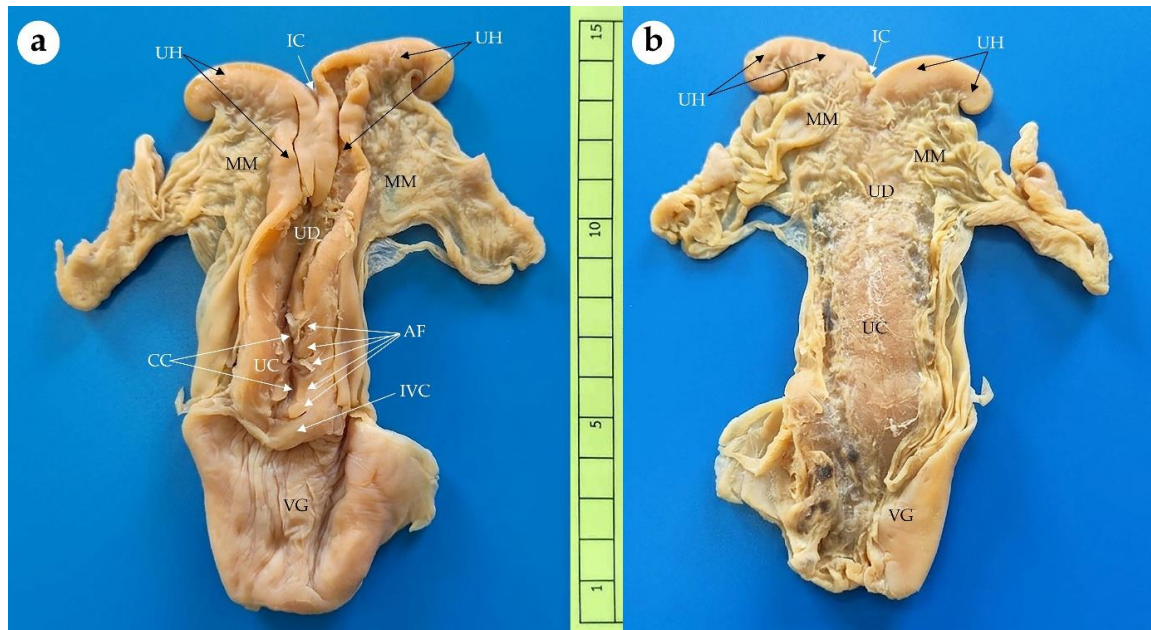


Figure 3 Female reproductive tract of the Thamin Eld's deer (*Rucervus eldii thamin*) preserved using the modified glycerin impregnation technique: (a) dorsal view and (b) ventral view. UH = uterine horn, IC = intercornual ligament, MM = mesometrium, UD = uterine body, UC = uterine cervix, AF = annular fold, CC = cervical canal, IVC = intravaginal part of cervix, VG = vagina.

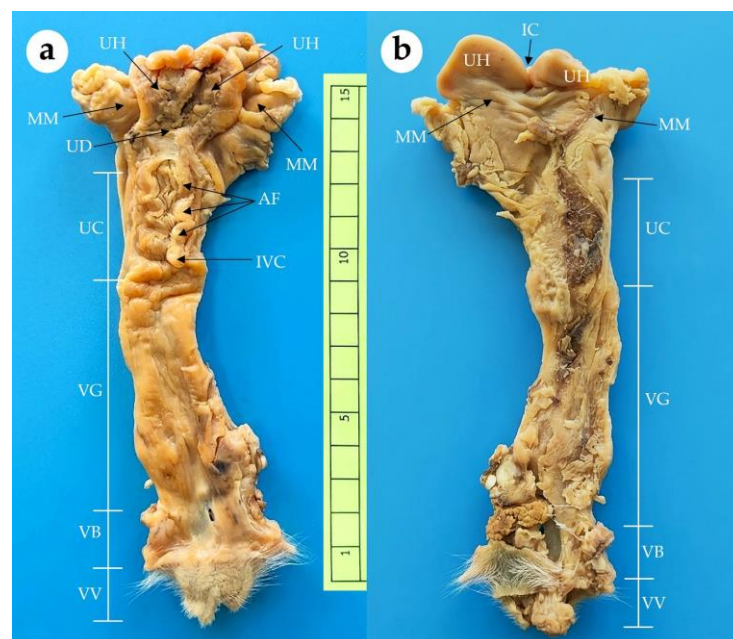


Figure 4 Female reproductive tract of the Fea's muntjac (*Muntiacus feae*) preserved using the modified glycerin impregnation technique: (a) dorsal view and (b) ventral view. UH = uterine horn, IC = intercornual ligament, MM = mesometrium, UD = uterine body, UC = uterine cervix, VG = vagina, VB = vestibule, VV = vulva, AF = annular fold, IVC = intravaginal part of cervix.

Discussion

To our knowledge, this study represents the first description of the female reproductive tracts in the Thamin Eld's deer (*Rucervus eldii thamin*) and the Fea's muntjac (*Muntiacus feae*). Despite the limited number of specimens and the incomplete recovery of one reproductive tract, anatomical examination of the female reproductive tract in these rare species provides important insights into cervid reproductive morphology, with relevance to fundamental anatomical knowledge, reproductive biotechnology, and wildlife conservation. While these two specimens differed in age, body weight, and necropsy conditions, the identified anatomical distinctions still highlight key species-specific adaptations. These findings provide useful comparative insights when placed alongside data from other cervids and small ruminants.

The general anatomical structure of the female reproductive tract in the Fea's muntjac followed the typical small ruminant pattern, consisting of paired ovaries and oviducts, bicornuate uterine horns, a single uterine body, uterine cervix, vagina, vestibule, and vulva. The ovaries in both species were oval in shape and small, consistent with previous reports in other cervids (Mobini *et al.*, 2002; Perez *et al.*, 2013) and in small ruminants (Nickel *et al.*, 1979). In the Thamin's Eld deer, the left ovary contained a dominant follicle, whereas the right ovary exhibited only small follicles without a corpus luteum, indicating a proestrus stage at the time of postmortem examination. In contrast, the Fea's muntjac presented two dominant follicles in the left ovary and one dominant follicle together with a corpus luteum in the right ovary, consistent with the early to mid-luteal phase of the estrous cycle in the ruminants (Hanzen *et al.*, 2000). Interestingly, these observations demonstrate that cyclic ovarian activity remains functional in these species even in older individuals.

The uterus in both species was bicornuate type which was a morphology typical of cervids (Clark, 2011) and small ruminants (Dyce *et al.*, 1996). The uterine horns of the Thamin Eld's deer were longer and gently curved, similarly to the uterine horns in the Pampas deer (Pérez *et al.*, 2013) and in sheep (Constantinescu, 1991). However, the uterine horns of the Fea's muntjac were shorter but exhibited a more distinct curvature, similar to the pattern observed in the red brocket deer (Mayor *et al.*, 2012) and in goats (Nickel *et al.*, 1979). The uterine cervix of both species was thick-walled and had a tortuous cervical canal, characterized by complex transverse annular folds in which the last fold close to vagina projected to the vaginal lumen, forming the intravaginal part of cervix. In small ruminants, the number of annular folds, including the intravaginal portion of the cervix, varies in numbers. Goats typically exhibit four to eight folds (Dayan *et al.*, 2010; Kanthawat *et al.*, 2024), whereas sheep usually possess five to six folds (Kershaw *et al.*, 2005). In the Thamin Eld's deer, six annular folds were identified, whereas the Fea's muntjac exhibited four annular folds. In previous reports, the pampas deer possesses four annular folds (Ungerfeld *et al.*, 2008),

whereas the red brocket deer exhibits five annular folds (Mayor *et al.*, 2012). These findings further illustrate the variability in the number of annular folds among cervid species, emphasizing interspecific differences in reproductive morphology. Interestingly, both species exhibited an intravaginal portion of the cervix together with a fornix vaginae, although these structures were indistinct in the Fea's muntjac. This observation supports the similarity of cervical features in these cervids to those described in small ruminants (Kershaw *et al.*, 2005; Kanthawat *et al.*, 2024). Such structures are functionally important for the application of transcervical AI in cervids (Morrow *et al.*, 2009). The vagina in both species was muscular, with a broad lumen and longitudinal mucosal folds, resembling the morphology described in other cervids (Ungerfeld *et al.*, 2008; Mayor *et al.*, 2012) and small ruminants (Nickel *et al.*, 1979; Constantinescu, 1991). The vulva in the Fea's muntjac was formed by relatively small, hair-covered labia, a feature consistent with small ruminants (Nickel *et al.*, 1979). Taken together, these findings demonstrate that while the overall reproductive anatomy of the Thamin Eld's deer and Fea's muntjac conforms to the general cervid and small ruminant pattern, distinct species-specific modifications are evident in ovarian morphology, uterine horn curvature, annular fold number, and characteristics of the intravaginal part of cervix. Such variations highlight the value of comparative anatomical investigations in advancing our understanding of cervid reproduction, with direct relevance to wildlife management, conservation strategies, and veterinary education.

As mentioned above, the basic reproductive anatomy of cervids is important for advancing reproductive biotechnology in wildlife species. A previous report highlighted that the adaptation of artificial reproductive techniques developed for small ruminants to endangered cervids has been limited by at least two principal limitations: (i) insufficient knowledge of the genetics and reproductive biology of these species, and (ii) the difficulty of safely manipulating animals for artificial insemination (AI) without inducing excessive stress or injury (Morrow *et al.*, 2009). Up to date, AI in small ruminants is commonly performed using either laparoscopic intrauterine insemination or nonsurgical transcervical intrauterine procedures (Faigl *et al.*, 2012). The laparoscopic AI techniques have been adapted and are now routinely applied in farm-raised cervids, including red deer (Fennessy *et al.*, 1990; Haigh and Bowen, 1991) and Fallow deer (Asher *et al.*, 1990). Although the laparoscopic approach generally yields higher and more consistent pregnancy rates, its widespread application remains limited by technical complexity and the high cost of specialized equipment (Kang *et al.*, 2025). Alternatively, nonsurgical approaches to AI employing a speculum-guided French catheter have also proven effective in farmed deer and can be completed within a relatively short time per animal (Willard *et al.*, 2002). Transcervical insemination without the aid of transrectal palpation can be achieved through digital manipulation of the

catheter and cervix via the vaginal route, with visualization of the intravaginal portion of the cervix facilitated by a vaginal speculum and light source (Duarte *et al.*, 2023). Furthermore, Morrow *et al.* (2009) suggested that in smaller cervid species, successful cervical catheterization requires the use of a fine pipette, such as an appropriately sized intravenous or urinary catheter fitted with a sterilized, round-ended stainless steel stylet to facilitate catheter introduction. Consequently, detailed knowledge of the fundamental anatomy of the uterine body, circular rings, intravaginal part of cervix, and vagina in Thamin's Eld deer and Fea's muntjac in this study provides essential information for the design of flexible cervical catheters of appropriate size and shape for successful use in transcervical AI technique. Such anatomical insights are critical for refining nonsurgical AI methods and improving artificial reproductive technologies aimed at conserving endangered cervid species.

For long-term preserved samples, the modified glycerin impregnation technique is effective for sustaining female reproductive organs of cervid species, yielding specimens with qualities highly suitable for both educational and museum purposes. The preserved genital tracts of Thamin Eld's deer and Fea's muntjac retained their natural anatomical configuration, including delicate structures. This preservation of fine detail enhances the instructive value of the specimens, allowing veterinary students and professionals to appreciate both gross and subtle anatomical features. Compared with traditional formalin fixation, which has long been used in anatomy teaching but poses health hazards due to its toxicity (Leung *et al.*, 2006), glycerin impregnation offers a safer alternative (Bernal *et al.*, 2022). Furthermore, while plastination techniques have been recognized as a preferred option due to their odorless nature and ease of handling, they are often costly and technically demanding (Fruhstorfer *et al.*, 2011). Importantly, the modified glycerin impregnation method provides a more accessible and cost-effective approach, while still maintaining tissue softness, flexibility, and durability, which are qualities essential for repeated handling in classroom and exhibition settings (Bernal *et al.*, 2022; Tienthai *et al.*, 2025). Additionally, the enhanced visibility of annular folds and the cervical canal in specimens preserved by glycerin impregnation, compared with fresh necropsy material, suggests that this technique may improve both anatomical teaching and research by facilitating recognition of structures that are otherwise difficult to distinguish. In this experiment, the female genital tracts were dehydrated using 80% and 90% acetone at -5°C, rather than at 4°C throughout the step, with the duration adjusted according to the availability of specimens from small ruminants as previously performed by Tienthai *et al.* (2025). As a result, only minimal shrinkage and discoloration were observed, further confirming the effectiveness of the method in preserving tissue integrity. This supports its applicability for long-term specimen preservation of at least 12 months, as demonstrated by the glycerin impregnation of female livestock reproductive organs

reported by Tienthai *et al.* (2025). In terms of preservation properties, glycerin is broadly acknowledged as a safe, non-toxic substance for human use, underscoring its suitability for biological specimen preservation (Nashed *et al.*, 2003). Besides, it possesses plasticizing effects, a hygroscopic nature, and strong penetrating ability. These properties make glycerin an effective agent for maintaining tissue integrity and flexibility in isolated visceral organs of various animals (Ahmed *et al.*, 2024; Daneil and Kukor, 2024; Harris *et al.*, 2025).

The primary limitation of the present study is the small sample size, restricted to a single female reproductive tract from each species. Because Thamin Eld's deer and Fea's muntjac are endangered and strictly protected, specimens could only be obtained opportunistically following natural mortality. Accordingly, the anatomical descriptions presented herein are primarily descriptive and baseline in nature, and they may not fully account for individual, physiological, or age-related variations within these species. Nonetheless, given the extreme scarcity of anatomical data for these rare cervids, these findings provide essential baseline morphological information that can support future non-invasive clinical diagnostics and conservation management.

In conclusion, this study presents the first anatomical data of the female reproductive tract in Thamin Eld's deer and Fea's muntjac. The findings enhance comparative anatomy and provide essential insights for future nonsurgical AI in cervids. The modified glycerin impregnation technique proved effective, producing durable, flexible, and odorless specimens. Taken together, these results advance cervid reproductive knowledge while offering safe, practical tools for conservation, veterinary practice, education and museum exhibition.

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References

- Adamovic D, Cepic Z, Adamovic S, Stosic M, Obrovski B, Moraca S and Miloradov MV 2021. Occupational exposure to formaldehyde and cancer risk assessment in an anatomy laboratory. *Int J Environ Res Public Health*. 18(21): 11198.
- Ahmed O, Gaballa MMS, Abumandour MMA, Al-Otaibi AM, Choudhary P and El-Shafey AA 2024. Morphometric and histopathological evaluation of modified Elnady's plastinated tissue compared to non-plastinated tissue: Highlighting its relevance for teaching and research. *Anat Histol Embryol*. 53(3): e13046.
- Asher GW, Kraemer DC, Magyar SJ, Brunner M, Moerbe R and Giaquinto M 1990. Intrauterine insemination of farmed fallow deer (*Dama dama*) with frozen-thawed semen via laparoscopy. *Theriogenology* 34: 569-577.
- Bernal V, Aburto P, Pérez B, Gómez M and Gutierrez JC 2022. A technical note of improvement of the Elnady technique for tissue preservation in veterinary anatomy. *Animals* 12(9): 1111.
- Clark SG 2011. Reproductive anatomy and physiology of whitetail deer and other select cervids, sheep and goats. *Clin Theriogenol*. 3(4): 517-521.
- Constantinescu GM 1991. *Clinical Dissection Guide for Large Animals: Horse, Ox, Sheep, Goat, Pig*. St. Louis, MO: Mosby Year Book. 164 pp.
- Daniel JA and Kukor I 2024. Evaluation of Elnady preserved tissues as a teaching aid for undergraduate animal science courses. *Transl Anim Sci*. 8: txae077.
- Dayan M, Besoluk K, Eken E and Ozkadif S 2010. Anatomy of the cervical canal in the angora goat (*Capra hircus*). *Kafkas Univ Vet Fak Derg*. 16: 847-850.
- Duarte GS, Galindo DJ, Baldini MHM, da Fonseca JF, Duarte JMB and Oliveira MEF 2022. Transcervical artificial insemination in the brown brocket deer (*Subulo gouazoubira*): A promising method for assisted reproduction in deer. *Sci Rep*. 13: 17369.
- Dyce KM, Sack WO and Wensing CJG 1996. *Textbook of Veterinary Anatomy*. Philadelphia, PA: W.B. Saunders Company. 848 pp.
- Elnady FA 2016. The Elnady technique: An innovative, new method for tissue preservation. *ALTEX* 33(3): 237-242.
- Faigl V, Vass N, Javor A, Kulcsár M, Solti L, Amiridis G and Cseh S 2012. Artificial insemination of small ruminants - a review. *Acta Vet Hung*. 60(1): 115-129.
- Fennessy PF, Mackintosh CG and Shackell GH 1990. Artificial insemination of farmed red deer (*Cervus elaphus*). *Anim Prod*. 51: 613-621.
- Fruhstorfer BH, Palmer J, Brydges S and Abrahams PH 2011. The use of plastinated prosections for teaching anatomy--the view of medical students on the value of this learning resource. *Clin Anat*. 24(2): 246-252.
- Gray TNE, Brook SM, McShea WJ, Mahood S, Ranjitsingh MK, Miyunt A, Hussain SA and Timmins R 2015. *Rucervus eldii*. The IUCN Red List of Threatened Species 2015. e.T4265A22166803.
- Haigh JC and Bowen G 1991. Artificial insemination of red deer (*Cervus elaphus*) with frozen-thawed wapiti semen. *J Reprod Fertil*. 93: 119-123.
- Hanzen CH, Pieterse M, Scenczi O and Drost M 2000. Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation per rectum. *Vet J*. 159: 161-170.
- Harris GG, Jones JM and McNary TG 2025. Analysis of the Elnady technique on cadaveric tissues: A low barrier-to-entry to increase tissue handling comfort, longevity, and safety. *Cureus* 17(12): e99483.
- Kang T-C, Lai I-L and Shen P-C 2025. Laparoscopic artificial insemination in small ruminants: Technological integration, economic evaluation, and future perspectives. *Front Vet Sci*. 12: 1667887.
- Kanthawat S, Srisuwatanasagul K, Thatsanabunjong F, Chaivoravitsakul N, Panyaboriban S and Srisuwatanasagul S 2024. Morphophysiological assessment of the cervix during the reproductive cycle and early pregnancy in does using computed tomography and oxytocin receptor immunohistochemistry. *Animals* 14(19): 2793.
- Kershaw CM, Khalid M, McGowan MR, Ingram K, Leethongdee S, Wax G and Scaramuzzi RJ 2005. The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. *Theriogenology* 64(5): 1225-1235.
- Korzekwa AJ and Kotlarczyk AM 2021. Artificial reproductive technology (ART) applied to female cervids. *Animals* 11(10): 2933.
- Leung KK, Lu KS, Huang TS and Hsieh BS 2006. Anatomy instruction in medical schools: Connecting the past and the future. *Adv Health Sci Educ Theory Pract*. 1(2): 209-215.
- Likhithaswamy HR, Madhushankari GS, Selvamani M, Kumar KPM, Kokila G and Mahalakshmi S 2022. Assessing the quality of long-term stored tissues in formalin and in paraffin-embedded blocks for histopathological analysis. *J Microsc Ultrastruct*. 10(1): 23-29.
- Mayor P, Lopez-Plana C and Lopez-Bejar M 2012. Anatomicohistological characteristics of the tubular genital organs of the female red brocket deer (*Mazama americana*) in the Peruvian Amazon. *Anat Histol Embryol*. 41(6): 436-444.
- Mobini S, Heath AM and Pugh DG 2002. *Theriogenology of sheep and goats*. In: *Sheep and Goat Medicine*. DG Pugh (ed). Philadelphia, PA: Saunders. 129-186.
- Monfort SL, Brown JL, Bush M, Wood TC, Wemmer C, Vargus A, Williamson LR, Montali RJ and Wildt DE 1993. Circannual inter-relationships among reproductive hormones, gross morphometry, behaviour, ejaculate characteristics and testicular

- histology in eld's deer stags (*Cervus eldi thamin*). J Reprod Fertil. 98(2): 471-480.
- Morrow CJ, Penfold LM and Wolfe BA 2009. Artificial insemination in deer and non-domestic bovids. Theriogenology 71(1): 149-165.
- Nashed G, Rutgers RPG and Sopade PA 2003. The plasticisation effect of glycerol and water on the gelatinisation of wheat starch. Starch-Starke 55: 131-137.
- Nickel R, Schummer A and Seiferle E 1979. Urogenital system. In: The Viscera of the Domestic Mammals. 2nd ed. A Schummer and R Nickel (eds). Berlin and Hamburg: Verlag Paul Parey. 282-392.
- ONEP 2020. "The Office of Natural Resources and Environmental Policy and Planning: Biodiversity Information Facility." [Online]. Available: <https://thbif.onep.go.th/>. Accessed May 18, 2026.
- Perez W, Vazquez N and Ungerfeld R 2013. Gross anatomy of the female genital organs of the pampas deer (*Ozotoceros bezoarticus*, Linnaeus 1758). Anat Histol Embryol. 42(3): 168-174.
- Pichardo-Molinero MR, Jardon-Xicotencatl S, Oliver-González MR and García-Tovar CG 2024. Implementation of a new solution for the preservation of anatomical specimens made of non-toxic substances. Open J Vet Med. 14: 56-67.
- Phraluk O, Punkong C, Thongphakdee A, Siriaronrat R, Wajjwalku W and Thongtip N 2010. Monitoring ovarian cycles by fecal progesterone analysis in Thamin Eld's deer hinds (*Rucervus eldii thamin*). Thai J Vet Med. 44(3): 317-323.
- Supannachart P, Tangphatsornruang S, Pootakham W, Sonthirod C, Tongsimma S, Wangkumhang P, Wilantho A, Thongphakdee A, Sanannu S, Kongjak P, Vechmanus T, Panyalert S and Suriyaphol G 2026. Genome-wide SNP data reveal genetic relatedness and structure in an ex-situ population of threatened Fea's muntjac (*Muntiacus feae*) (Artiodactyla, Cervidae). ZooKeys 1283: 223-239.
- Tienthai P, Intarapanya J and Chuesiri P 2025. Innovative glycerin impregnation of female livestock genital organs and satisfaction assessment for veterinary anatomy education in Thailand. Thai J Vet Med. 55(3): 12.
- Timmins R, Steinmetz R and Chutipong W 2016. *Muntiacus feae*. The IUCN Red List of Threatened Species 2016. e.T13927A22160266.
- Ungerfeld R, González-Pensado S, Bielli A, Villagrán M, Olazabal D and Pérez W 2008. Reproductive biology of the pampas deer (*Ozotoceros bezoarticus*): A review. Acta Vet Scand. 50(1): 16.
- von Horst C, von Hagens R, Sora CM and Henry RW 2019. History and development of plastination techniques. Anat Histol Embryol. 48(6): 512-517.
- World Association of Veterinary Anatomists 2017. Nomina Anatomica Veterinaria. 6th ed. Editorial Committee, Hannover.
- Willard ST, Neuendorff DA, Lewis AW and Randel RD 2002. A comparison of transvaginal artificial insemination procedures for use in commercially farmed deer. Small Rumin Res. 44: 135-140.
- ZPOT 2026. "The Zoological Park Organization of Thailand under the Royal Patronage of H.M. the King." [Online]. Available: <https://encyclopedia.zoothailand.org/>. Accessed May 19, 2026.