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CASE REPORT
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Pseudo-Placentational Endometrial Hyperplasia in Young Beagle Bitch

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ABSTRACT

***Background*:** Pseudo-placentational Endometrial Hyperplasia (PEH) is a well-documented pathological condition in middle-aged to older bitches, yet its occurrence in young, sexually immature canines - particularly those utilized in preclinical toxicity studies - remains profoundly underreported. This gap in literature poses challenges for interpreting uterine lesions in juvenile laboratory Beagles.

***Case*:** A 10-month-old Beagle bitch was euthanized at the study termination age during a repeat-dose toxicity study. No clinically significant abnormalities were observed in clinical observations of this animal. The animal presented with uterine nodules (0.5-0.8 cm diameter) containing serosanguinous fluid during necropsy, initially suggestive of pyometra or pregnancy. Histopathological evaluation revealed absence of inflammation or microbial colonization, contrasting with classical pyometra, and identified complex glandular hyperplasia with placental mimicry, including villous projections lined by cuboidal-to-columnar epithelium. Immunohistochemistry demonstrated epithelial-mesenchymal transition-like features, with strong vimentin expression in mesenchymal-like stromal cells and pan-cytokeratin positivity in epithelial components, while desmin and estrogen receptor alpha remained negative. Histopathological examination and immunohistochemical analysis showed Pseudo-placentational Endometrial Hyperplasia.

***Discussion*:** PEH of Beagle dog is extremely rare in the young Beagle dog. A 100 years ago, PHE has been already described in standard textbooks, and it was not wildly recognized by diagnostic pathology. Previously, it was also known as "deciduoma". The deciduoma of uterine have been found in human, monkeys, dogs, rabbits, and rats. However, it is generally accepted that deciduoma is a neoplastic lesion. As its form of endometrial hyperplasia is similar to the normal histology of the endometrium at placentation sites in normal pregnancy, until 2008, D.H. Schlafer 1st named PEH. The present case describes the morphological and immunohistochemical features of the uterine discovered a 10-month-old Beagle bitch in toxicity and pharmacological study. During the gross examination, nodules with fluid discharge were detected in the uterus, initially prompting suspicion of pyometra or pregnancy. Given that the bitch was housed exclusively with individuals of the same sex, mating was ruled out. Histopathological analysis revealed the absence of discernible inflammation in the uterine tissue; however, structures resembling placental tissue were observed. These findings suggest endometrial hyperplasia mimicking placental morphology due to unknown etiology. Additionally, Vimentin and Pan cytokeratin exhibited strong positive expression in the areas of villous proliferation, suggesting epithelial cells in this region possess the ability to differentiate into mesenchymal tissue. Currently, the etiology of canine PEH remains unclear. It is widely believed that bacteria, foreign bodies, and other factors may contribute to PEH. While PEH may be incidentally discovered and spontaneously regress without intervention, persistent lesions warrant consideration for surgical intervention. In toxicity and pharmacological studies, PEH of the uterus has only been reported in rats. Although Beagle dog is frequently used in toxicity and pharmacological studies, respectively, historical control date for PEH in young Beagle dog has not been reported. So, our case might provide valuable information as a historical for Beagle dog.

Keywords: Beagle dog, Deciduoma, PEH, pseudo-placentational endometrial hyperplasia, uterine lesions.

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INTRODUCTION

Pseudo-placentational Endometrial Hyperplasia (PEH) is a distinct pathological entity characterized by placental-like structural alterations in the endometrium, historically documented in middle-aged to older bitches [9,10]. First formally defined by Schlafer in 2008 [14], PEH diverges from classical deciduomas - previously reported in humans, primates, dogs, rabbits, and rats - by mimicking gestational endometrium without association with pregnancy [2,5]. Existing literature overwhelmingly focuses on mature animals, with no documented cases in sexually immature juvenile Beagles (<12 months old) - a population routinely utilized in regulatory toxicity trials.

This report aims to characterize the fst documented case of PEH in a juvenile Beagle dog within a preclinical toxicity study, establishing foundational histopathological and immunohistochemical criteria to improve diagnostic accuracy and inform future research on endocrine disruptor impacts in susceptible populations.

CASE

In repeated toxicity study, Beagle dogs were sacrificed at the age corresponding to the age of termination study. The dogs were purchased from Fuzhou Zhenhe Experimental Animal Technology Development Co. Ltd. at the age of 6 months. It was housed in group breeding (2-3 animals of the same sex in a cage) and provided with automatic water supply. The temperature in the animal room was maintained at 20.2°C to 24.1°C, with humidity ranging from 48.9% to 70%. Animal care and use conformed with the guidelines of the Institutional Animal Care and Use Committee of Guangzhou General Pharmaceutical Research Institute. During the quarantine period, clinical examination, hematological, electrocardiography and serum biochemical analysis, and urinalysis revealed no abnormalities. Beagle dogs were euthanized by intravenous injection of propofol and exsanguination from the carotid artery before necropsy.

Macroscopically, 3 ovoid distended mass were findings in the right uterine horn, and numerously black fluid were observed in the swollen segmental site of lumen of the uterine. The mucous membrane was covered by numerous villi protruding into the lumen in the segmental portion of uterine section. No abnormal region was observed in the left uterine horn and other organs macroscopically. The uterine was collected and fixed in 10% neutral buffered formalin and embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). For immunohistochemical characterization different antibodies were used. The results for the Pan-cytokeratin[1], Vimentin[1], proliferating cell nuclear antigen (PCNA)[2], Ki-67[2], SMA-α[2] were evaluated as positive and negative immunolabelling.

Histopathologically, the endometrium was disrupted by multilayered masses. The smooth muscle layer of the mass was thin by expansion of the lumen probably. Within the affected uterine, are composed of three distinctive layers including the inner, middle and outer layer (Figure 1A). The inner layer of the mass

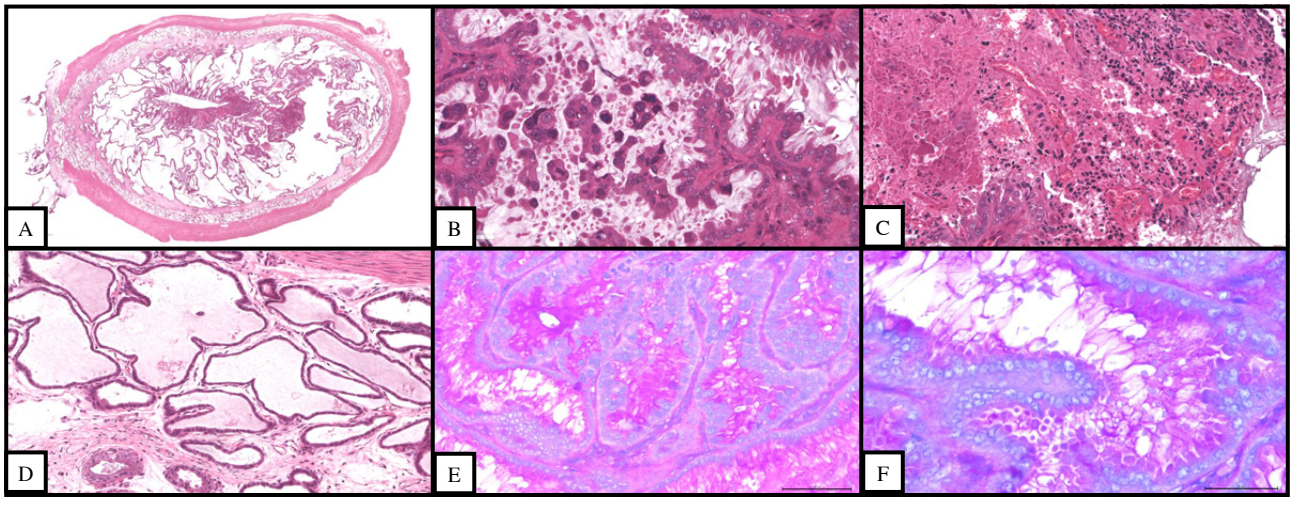

Figure 1. Histopathological findings. A- The hyperplastic endometrium presents a three-layer appearance reminiscent of placenta formation [HE; bar= 3 mm]. B- Numerous syncytium and mucus were seen in the lumens [HE; bar= 60 µm]. C- The superficial aspect of the epithelium is multifocal either eroded or necrotic with accumulation of karyorrhectic debris and fibrin [HE; bar= 90 µm]. D- Abundant of sponge-like cysts with mucus in the out layer [HE; bar= 100 µm]. E & F- Periodic acid-Schiff (PAS) staining was positive in epithelial cells and mucus.

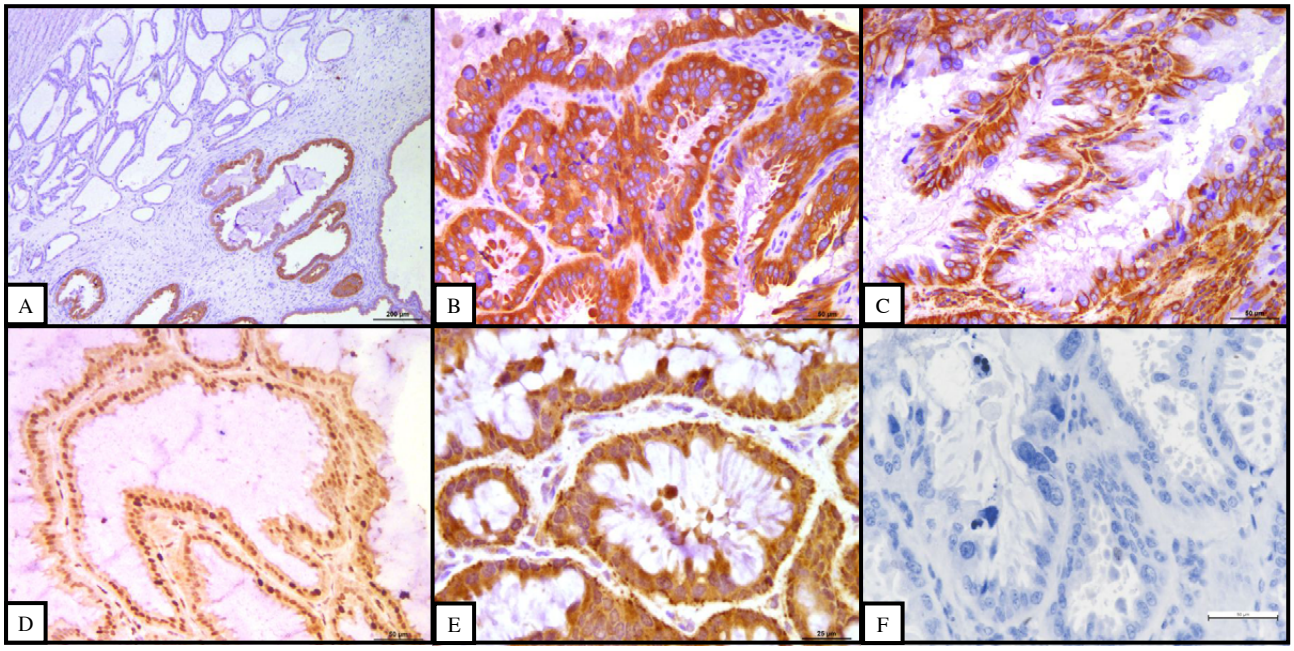

Figure 2. Immunohistochemical characteristics. Pancytokeratin is strongly positive in most cell membranes and cytoplasm of the inner layer, but weakly positive (A & B) in the glandular hyperplasia layer. Vimentin is strongly expressed in smooth muscle cells, endothelial cells and inner layer glandular epithelial cells (C). PCNA (D) and ER-α (E) were strongly expressed in the nucleus of glandular epithelial cells. Ki-67 was negative in the uterine (F).

is composed of long villous fold lined by columnar to pseudostratified epithelium with vacuolated cytoplasm and large vesiculate nuclei, often forming papillary projections and cysts filled with mucus and protein. Periodic acid-Schiff (PAS) staining was positive in epithelial cells and mucus (Figure 1E & F). Many multinucleated syncytia were found within the luminal epithelium, suggesting the possibility of syncytial trophoblast formation and true placenta formation (Figure 1B). In addition, Necrosis, vascular proliferation, and syncytium were common findings in this area (Figure 1C). The middle layer of the mass consisted of a bundle of loose fibrous connective tissue that is clearly expanded by edema. The outermost layer was made up of notably dilated gland lined by attenuated ciliated cuboidal cells and amount of globular to homogeneous, proteinaceous material (Figure 1D). These properties are very similar to the endometrium observed in the maternal placenta during normal pregnancy.

The cytoplasm of endometrial epithelial cells and necrotic epithelial cells were strongly positive immunolabelling to the Pan cytokeratin. Interestingly, weakly positive of luminal epithelial cells in the outermost layer (Figure 2A & B). The smooth muscle cells, the endothelial cells showed a diffuse, intense positive to vimentin. Curiously, some of the glandular epithelial cells in the inner layer are strongly positive (Figure 2C). The nuclei of most endometrial epithelial cells were strongly positive for PCNA (Figure 2D), indicating highly proliferative activity of the endometrium. ER-α was expressed strongly in the nuclei of endometrial epithelial cells (Figure 2E) and starry-formed cells in the transitional and mesometrial regions. No proliferative cells were position for Ki-67 in the uterine (Figure 2F).

DISCUSSION

This report describes a rare case of pseudo--placentational endometrial hyperplasia in a young Beagle bitch from a preclinical toxicity study. The morphological presentation, including glandular hyperplasia and placental mimicry without significant inflammatory infiltration, represents a novel finding in this species and age group. The absence of notable inflammatory infiltrates contrasts with classical descriptions of pyometra, where neutrophilic inflammation is characteristic [11,12]. This observation, coupled with the lack of bacterial colonies on histological examination, supports an alternative pathogenesis for this case. The presence of placental-like structures in the absence of pregnancy or known trophoblastic disease raises questions about aberrant differentiation pathways in the canine endometrium.

The placentlike area of the single columnar epithelium and mucin PAS staining were strongly positive, indicating abnormal accumulation of glycogen or neutral glycosaminoglycans at this site. This

phenomenon may be associated with the generation of advanced glycation end products (AGEs) and activation of their receptor [6]. This activation induces oxidative stress and pro-inflammatory cytokine release, thereby promoting fibrosis and tissue remodeling [3,16]. Notably, immunohistochemical analysis revealed vimentin positivity in mesenchymal cells and pan-cytokeratin positivity in epithelial components, suggesting the potential involvement of epithelial-mesenchymal transition (EMT). The aberrant activation of the TGF-β/Smad signaling pathway, particularly the phosphorylation status of Smad2/3, may serve as the central mechanism driving this biological process [8]. Unlike classical endometrial hyperplasia, this case exhibited strong ERα positivity in stromal cells, suggesting that ERα signaling may drive endometrial hyperplasia via ligand-dependent pathways [1]. Furthermore, dysregulated ERα activation could establish a positive feedback loop by upregulating TGF-β1 expression, exacerbating fibrosis [17]. These findings challenge the conventional association of proliferative endometrial hyperplasia with pregnancy or chronic inflammation, while providing novel insights for preclinical toxicology research: PAS staining may serve as an adjunctive indicator for assessing endometrial glycometabolic abnormalities, and in-depth analysis of the ERα-TGF-β/Smad axis and EMT-related molecules could elucidate the molecular basis of fibrosis and structural disruption.

PEH remains a rare clinical entity in veterinary medicine, with no established epidemiological data in the general pet dog population. However, it demonstrates a markedly higher reported incidence of 0.3%-0.5% in preclinical toxicological studies involving laboratory Beagle dogs compared to domestic pet canines [9,13]. The lesion predominantly affects middle-aged intact bitches and is histopathologically characterized by: 1) placental-like structures (vascular-rich stromal cores lined by a single layer of columnar epithelium), 2) PAS-positive material deposition indicative of abnormal glycogen or neutral mucopolysaccharide accumulation, and 3) intense estrogen receptor alpha (ERα) immunoreactivity in stromal cells, suggesting aberrant ERα signaling activation as a pivotal pathogenic mechanism [4]. Contributing factors include endocrine imbalances (elevated estrogen levels), environmental endocrine disruptors (e.g., bisphenol A), foreign bodies, and genetic predisposition with apparent breed predilections. Notably, chronic low-dose toxin exposure in laboratory settings may exacerbate lesion development [10,15].

Current treatment protocols for endometrial hyperplasia lack standardization. For mild cases, anti-estrogen medications (e.g., tamoxifen) or progestogens (e.g., medroxyprogesterone acetate) can be used to inhibit the ERα signaling pathway, though thrombotic side effects must be monitored. Severe or atypical cases with malignant potential require surgical intervention, such as hysterectomy with bilateral salpingo-oophorectomy [7]. The current challenges in the diagnosis and treatment of PEH stem from non-uniform diagnostic criteria and an incomplete understanding of molecular mechanisms. Future research should focus on elucidating the interplay between the ERα-TGF-β/Smad axis and dysregulated glycometabolism (e.g., AGEs-RAGE pathway) to develop targeted therapeutic strategies, thereby improving clinical management outcomes.

MANUFACTURERS

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Authorship contributions. The authors Bo Liu and Minhua Hu contributed to equally to this work.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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