

Journal of the Hellenic Veterinary Medical Society

Vol 70, No 4 (2019)



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doi: [10.12681/jhvms.22243](https://doi.org/10.12681/jhvms.22243)

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To cite this article:

MARINO, G., QUARTUCCIO, M., SFACTERIA, A., CRISTARELLA, S., & ZANGHÌ, A. (2020). Complete vaginal stenosis and hematocolpus in two bitches with a history of GnRH treatment to postpone puberty. *Journal of the Hellenic Veterinary Medical Society*, 70(4), 1905–1910. <https://doi.org/10.12681/jhvms.22243>

Complete vaginal stenosis and hematocolpus in two bitches with a history of GnRH treatment to postpone puberty

G. Marino, M. Quartuccio, A. Sfacteria, S. Cristarella, A. Zanghì

Department of Veterinary Sciences, University of Messina, Messina, Italy

ABSTRACT. Two mixed-breed bitches (18 and 19 months), that had been treated, one year before, with deslorelin acetate implant to postpone puberty, were hospitalized and monitored during their first heat. The heat was presumed by the owners, that observed vulvar swelling in both cases but no vulvar bloody discharge. The following diagnostic procedures were employed: physical genital tract examination, vaginoscopy, vaginal cytology, endocrine assay, ultrasound and X-ray using vaginal infusion of iodum and pneumobladder as positive and negative contrasts. In bitch 1, vaginal cytology and progesterone levels confirmed the presence of an ovulatory “dry” oestrus, without cytological presence of red blood cells, progressing to dioestrus. Ultrasound showed preovulatory follicles and, in the following days, transition to corpora lutea. The caudal abdomen presented a large ovoid cystic structure filled with echoic fluid, next to the bladder. Radiographic scans demonstrated a normal bladder profile, while the contrast medium failed to enter into the cranial vagina. On the basis of these findings, the bitch 1 was submitted to laparotomy 10 days after the end of oestrus. A vaginal dilatation (10x5 cm), from which brown fluid was aspirated, was found and resected together with uterus and ovaries. Bitch 2 had the same diagnostic route and findings, but she was laparotomized 3 months after the heat. During this period no spontaneous regression of the lesion was observed. At laparotomy, the vaginal dilatation (8x4 cm) was only aspirated and the bitch regularly neutered. In both cases, cytology of the fluid taken from the vaginal sac revealed superficial epithelial cells and abundant degenerate red blood cells. Histology (bitch 1) confirmed the vaginal origin of the dilatation and revealed an additional Gärtnner duct cyst. The abnormality (hematocolpus) probably originated by an inadequate drainage of proestrous bloody discharge because of a severe vaginal stenosis. A congenital origin of the lesion was unlikely; it was strongly suspected that the treatment of the prepubertal bitches interfered, by an irreversible way, with the normal development of the vagina.

Keywords: Dog, hematocolpus, deslorelin, ultrasound, pathology

Corresponding Author:

Marino Gabriele, Department of Veterinary Sciences - University of Messina
Via Palatucci, Polo Universitario Annunziata, 98168 Messina, Italy
Email address: marinog@unime.it

*Date of initial submission: 26-03-2019
Date of revised submission: 05-08-2019
Date of acceptance: 11-08-2019*

CASE HISTORY

Two mixed-breed female dogs (bitch 1 and 2), not related to each other, treated at 6 months of age with 4.7 mg deslorelin acetate implant (Suprelorin®, Virbac), inserted into the interscapular region, were hospitalized and monitored during their first heat, observed at the age of 18 and 19 months, respectively.

All procedures were approved for ethical implications by the Center for Animal Reproduction and Assisted Insemination of the University of Messina. The extra-label use of the drug, not designed for prepubertal female dogs, was approved by owners in agreement with literature (Trigg et al., 2006; Sirivaidyapong et al., 2012; Marino et al., 2014; Kaya et al., 2015). Even the diagnostic trials had the owners' consent.

At presentation, bitch 1 (Europe) did not show any sign of disorders. The vulva was well developed, increased in volume, dry with wrinkled skin. Digital stimulation of the perivulvar skin evoked a lateral displacement of the tail, accompanied by a stiffening of the posterior limbs. No bloody discharge was observed. Abdominal palpation allowed appreciating an ovoid and firm structure consistent with an enlarged bladder for position and size. The cervix and the uterus were not detectable by palpation. Digital vaginal exploration showed a normally shaped vestibulo-vaginal channel, although slightly stenotic at the cingulus level. Vaginal cytology that was performed at two-day interval during proestrus-oestrus and weekly during dioestrus confirmed a normal transition from proestrus to dioestrus. Cornification during oestrus reached percentage of 80 % lasting approximately 9 days. Neutrophils were detected only at the dioestrus onset, in concomitance to the presence of intermediate and parabasal cells. Red blood cells were not detected in any phase. Vaginoscopy was performed with a rigid endoscope (TCI-Endoscope, length 43 cm, Karl Storz) without sedation, at two-day interval during proestrus-oestrus until dioestrus onset. The introduction of the instrument was facilitated by a shunt system (Minitube), which also provided fixation to the vaginal wall allowing pumping air to facilitate the visualization of the vagina. Typical mucosal folds changed in appearance during the period of study, passing from oedematous, pink, and round (late proestrus) to pale and crenulated (oestrus) up to flattened and flaccid (dioestrus). No septa were observed at every level. The lumen was slightly stenotic and the bottom of the vagina and the cervix were not visualized, giving the appearance of a blinded vagina.

Blood sampling was performed every two days until dioestrus onset. The obtained serum was processed for the determination of progesterone, using an Enzyme Linked Fluorescent Assay (BioMeuriex, Minivid). Circulating progesterone progressively increased from values of 0.9 ng/ml at presentation to 24 ng/ml at dioestrus onset. Ultrasound examination of the abdomen (Esaote, MyLab 40 Vet, 8 MHz convex probe, 12 MHz linear probe) was performed every two days until dioestrus onset. At presentation, multiple cavitary structures in the ovaries, with an anechoic content, 0.6-0.8 cm in diameter, compatible with preovulatory follicles were seen. At ovulation, an increase in echogenicity of such structures was evident together with the finding of a small amount of liquid withheld in the ovarian bursa. The uterus showed normal appearance, a diameter of about 1.5 cm and the absence of intrauterine fluid. For the whole period of monitoring, a large cystic oval formation, at least 8-10 cm long and 4-5 cm wide, thick-walled, and with the presence of numerous and dense echoes within the lumen, was found (Fig. 1).

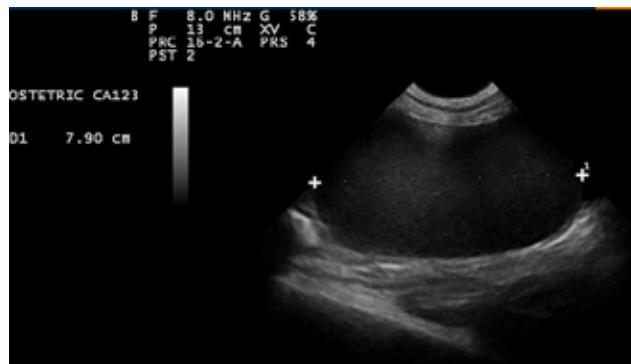


Figure 1. Bitch 1 (Europe). Caudal abdomen. Ultrasound appearance of the large oval cystic structure (hematocolpus) with anechoic content.

This undefined cystic formation was found next to a second cystic oval anechoic structure, more attributable to the bladder. The ultrasound-guided catheterisation of the bladder confirmed the nature of the second structure. By contrast, the introduction of ultrasound-guided catheter into the vagina, presented difficulties in the progression, stopping cranially, for the presence of a thin hyperechoic septum (Fig. 2). The abdominal X-ray examination (Univet, 300HS) was carried out on dorsoventral and lateral views. In order to enhance the contrast of the vaginal area, iopamidol (Iopamiro 300, Bracco Imaging) was inoculated in vagina throughout a Foley catheter that was inserted and fixed in the caudal vagina.

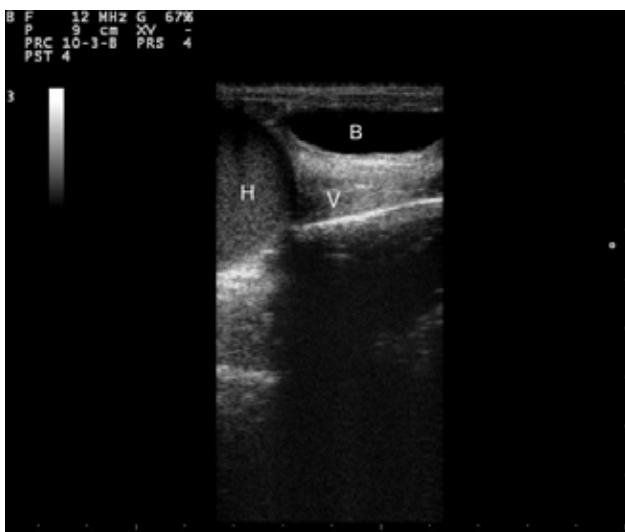


Figure 2. Bitch 2 (Moon). Appearance of continuity between the hematocolpus (H) and the vagina (V). Visible even a partial scan of the bladder (B).

The positive contrast medium showed a radiological stop in the vagina, emphasized by the presence of a substantial amount of iodum between the septum and the balloon of the Foley catheter. Then, pneumocystography was obtained by blowing filtered air into the bladder throughout a Foley catheter. The bladder, of normal appearance and content, had no connection with the undefined cystic formation. In light of these findings and the diagnostic suspicion of an abnormal collection in the vagina/uterus, the bitch was submitted to exploratory laparotomy and eventual ovariohysterectomy 10 days after the end of the oestrus. After premedication with intramuscular injections of tramadol hydrochloride (2 mg/kg b.w.; Contramal, Grunenthal Italia) and acepromazine maleate (0.05 mg/kg b.w.; Prequilan, Fatto), the patient was induced with diazepam (0.5 mg/kg b.w.; Diazepam, Intervet) and propofol (3 mg/kg b.w.; Propovet, Zoetis) administered intravenously. After intubation, anaesthesia was maintained with isoflurane in 100% oxygen. Laparotomy was performed on the linea alba. In proximity to the bladder, but in continuity with the uterus cranially and with the vagina caudally, an impressive fluid-filled dilatation was found (Fig. 3), including the cervix. About 200 ml of brown fluid was aspirated from the dilatation (Fig. 4). Furthermore, a second cyst inside the main cystic structure was found, from which 10 ml of yellowish mucoid fluid was aspirated (Fig. 5). The fluids were cytologically evaluated during laparotomy. The first fluid showed only epithelial vaginal cells, many of which were cornified and abundant degenerate red blood cells (Fig. 6).



Figure 3. Europe. Externalization of the formation caudal to the body of the uterus.

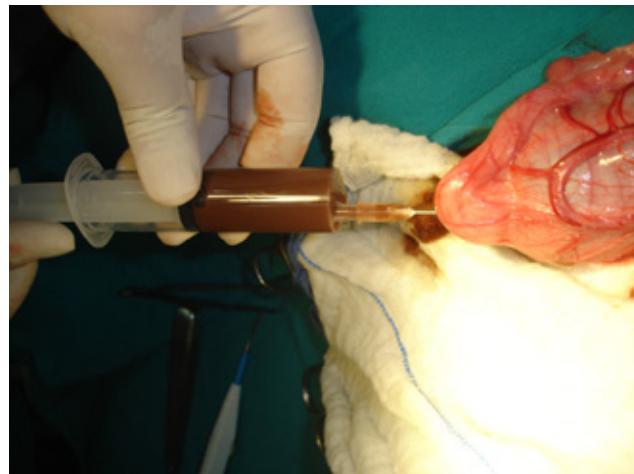


Figure 4. Moon. Intraoperative aspiration of the brown fluid contained in the hematocolpus.



Figure 5. Moon. Different aspect of the content of the second cavity (Gärtner duct cyst).

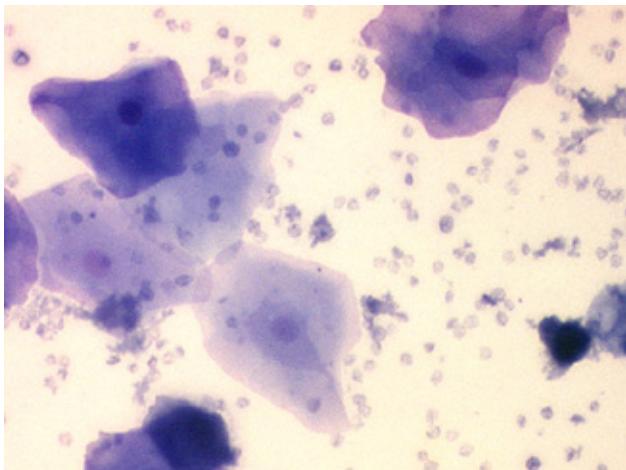


Figure 6. Moon. Superficial epithelial cells and degenerate red blood cells in the fluid aspirated from the hematocolpus. May - Grünwald Giemsa. Obv 20x.

The second fluid presented parabasal-like epithelial cells and mucus. In both fluids no bacteria and inflammatory cells were detected. A diagnosis of hematocolpus was done. After ligating the vaginal arteries and branches of the internal pudendal arteries, the dilated vagina was amputated at 2/3 level and removed together with uterus and ovaries. The caudal wall of the dilated vagina was inspected to confirm the absence of communication with the caudal portion of the vagina (Fig. 7).

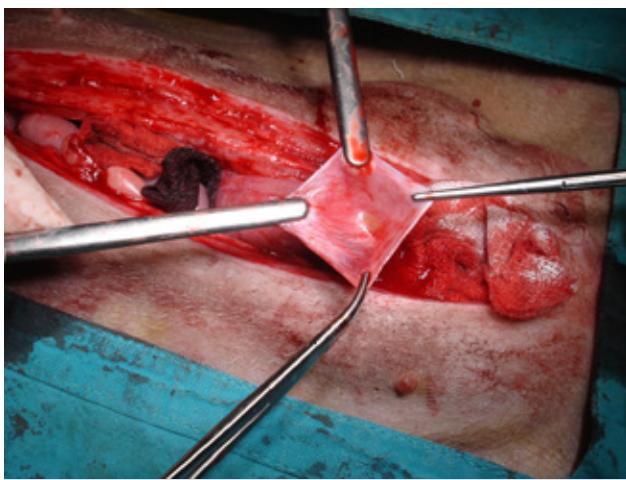


Figure 7. Europe. Visualization of the blinned caudal wall of the hematocolpus.

The removed uterine horns were 10 cm length and 1.5 cm in diameter, with a spiral trend according to the phase of the cycle (dioestrus). The ovaries, respectively of 1.5 x 1.0 x 0.5 cm on the right and 2.5 x 1.0 x 0.6 cm on the left, presented both newly formed corpora lutea. The cervix was found inside the dilated area, projecting in it, surrounded by large haemorrhagic areas (Fig. 8).



Figure 8. Europe. Gross appearance of the mucosa of the hematocolpus with haemorrhagic areas. Following the dorsal median fold the cervix is visualized.

Specimens were processed for histopathological examination. The vaginal mucosa was thinned and partially degenerate, sometimes covered by a multi-layered epithelium. In the cranial part, the mucosal epithelium appeared monolayered with strong polarization, similar to endocervical epithelium. Inflammatory cells were not detectable in the mucosa and the underlying layers. In some areas the submucosa was characterized by haemorrhagic extravasation (Fig. 9). A large cavitary structure lined by a single layered epithelium was found resembling a Gärtnner duct epithelium (Fig. 10).

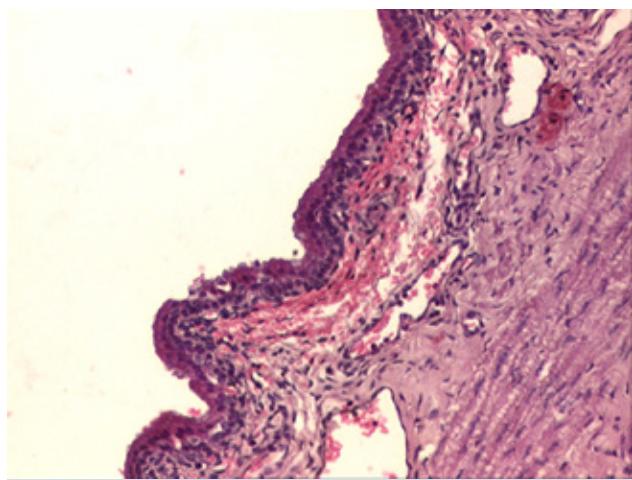


Figure 9. Europe. Hematocolpus. Vaginal mucosa close to the cervix covered with a columnar epithelium. Note the absence of inflammatory infiltrates and the hemorrhagic extravasation. Haematoxylin and eosin. Magnification 20x.

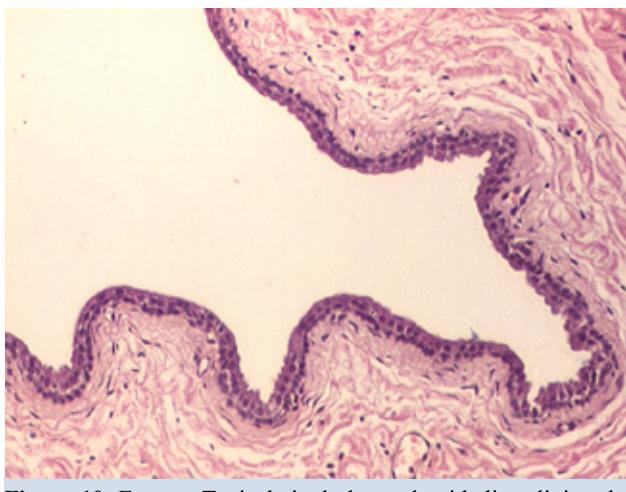


Figure 10. Europe. Typical single layered epithelium lining the Gartner's cyst. Haematoxylin and eosin. Magnification 20x.

Bitch 2 (Moon) had perfectly comparable findings. She was monitored until 3 months after the dry heat. During dioestrus, ultrasound monitoring of the vaginal dilatation was performed weekly, without evident changes in size and appearance. At laparotomy, the main cyst (hematocolpus) and the secondary smaller cyst (Gartner duct cyst) were only aspirated and left in place; ligature was done at the uterine body level to remove uterus and ovaries. Moon had rectilinear uterine horns of 10.5 cm in length and 0.8 cm in diameter according to the phase of the cycle (anoestrus). The ovaries, sized respectively 1.8 x 0.9 x 0.7 cm on the right and 1.8 x 0.9 x 0.8 cm on the left, presented regressing corpora lutea. The uterine mucosa showed an initial picture of segmental hyperplasia. During follow-up, the stump was monitored by ultrasound and no changes were detected for a period of 1 year after the surgery.

DISCUSSION

Disorders of the development of the vagina and vestibulum are occasionally reported in the bitch and include vaginal septa, vaginovestibular stenosis, vestibulovulvar stenosis, segmental aplasia of the vagina (Gee et al., 1977; Wadsworth et al., 1978; Holt and Sayle, 1981; Wykes and Soderberg, 1983; Hawe and Loeb, 1984; Root et al., 1995; Archbald and Wolfsdorf, 1996; Kyles et al., 1996). No breed and genetic predisposition for these abnormalities have been reported. Clinical signs reported in dogs with vaginal abnormalities include chronic vaginitis, mating difficulty, urinary incontinence, chronic urinary tract infections, dystocia and ambiguous external genitalia (Holt and Sayle, 1981; Wykes and Soderberg, 1983; Root et al., 1995; Archbald and Wolfsdorf, 1996;

Kyles et al., 1996), despite some bitches with vaginal septa or circumferential stenosis are asymptomatic (Wykes and Soderberg, 1983; Root et al., 1995; Kyles et al., 1996). The terms hydrocolpus, mucocolpus, hematocolpus, pyocolpus refer to the abnormal distension of the vagina for the accumulation of fluid, mucus, blood and pus, respectively. These conditions may be related to a difficult drainage of the vagina and the few reported cases in the bitch were generally secondary to developmental disorder of the vestibulo-vaginal tract (Gee et al., 1977; Wadsworth et al., 1978; Hawe and Loeb, 1984; Tsumagari et al., 2001; Viehoff and Sjollema, 2003; McIntyre et al., 2010; Marinho et al., 2013; Alonge et al., 2015).

GnRH agonists are used as anticonceptuals in the canine species. Deslorelin acetate is a GnRH agonist developed as an implant, whose main indication is transitory contraception in adult male dogs. Recent studies have proved the efficacy of the treatment in prepubertal dogs to postpone puberty (Trigg et al., 2006; Sirivaidyapong et al., 2012; Marino et al., 2014; Kaya et al., 2015). There are few data about the side effects of GnRH agonists in veterinary medicine, and they are generally considered safe and totally reversible. Persistent oestrus related or not to ovarian cysts, uterine disorders, urinary incontinence and hair abnormalities have occasionally been described (Arlt et al., 2011; Fontaine and Fontbonne, 2011; Palm and Reichler, 2012). In previous studies on prepubertal bitches, delayed epiphyseal closure, hip dysplasia, transient juvenile vaginitis and marked atrophy of the internal genital tract have been reported with minimal or no clinical impact (Marino et al., 2014; Kaya et al., 2015).

In this paper, two bitches with an abnormal distension of the vagina (hematocolpus) were studied. It was remarkable that both bitches received an implant of deslorelin acetate in a prepubertal time. The presence of an obstruction in the genital tract caused an obstacle to normal drainage and an accumulation of fluid cranially to the stenotic point. When cycling female dogs have such obstructions the collection of fluid mixes with the typical bloody discharge of proestrus; these animals show the so-called dry heats (without bloody discharge from vulva) (Viehoff e Sjollema, 2003). Specific genes involved and heritability of developmental disorders of the genital tract have not yet been fully defined in bitches (McIntyre et al., 2010). Developmental disorder of the gonad and kidney may be associated finding, as consequence of

failure of gene expression or lack of bloody supply in the genital segment (McIntyre et al., 2010). Some causes may even be acquired after birth, since the development of the internal and external genitalia is completed by puberty. There was a reasonable suspicion that the treatment received by prepubertal bitches contributed to the determinism of the lesion. The treatment applied at prepubertal time is able to arrest

the development of the genital tract throughout the period of treatment (Marino et al., 2014). In some female dogs, these changes are probably not completely reversible at vaginal level.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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