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Prolonged Estradiol Exposure and Stromal Fibrosis Influence Treatment Response in Canine Vaginal Hyperplasia

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ABSTRACT

Background: Vaginal hyperplasia is an estrogen-dependent condition commonly observed in bitches during proestrus and estrus. To evaluate how the duration of vaginal hyperplasia in bitches affects its treatment outcome.

Methods: The present clinical study reports 11 cases of vaginal hyperplasia in bitches presented to the Teaching Veterinary Clinical Complex, Bihar Veterinary College, Patna, over a one-year period. Diagnosis was based on clinical examination, vaginal cytology, serum estradiol assay and ultrasonography. Blood parameters (CBC and serum biochemistry) were within normal ranges in all cases. Treatment groups were divided into medical management with human chorionic gonadotropin (hCG, 500 IU intramuscularly at alternate-day intervals for three doses; n = 5) and surgical management by ovariohysterectomy (OVH; n = 6). Clinical outcomes were monitored by the regression of prolapse and changes in serum estradiol concentration.

Result: OVH resulted in a marked reduction of serum estradiol (from 49.5 pg/mL to 12.5 pg/mL), with resolution of hyperplasia in 67% of cases. hCG treatment achieved clinical regression in 60% of cases, consistent with previous reports of variable efficacy. When hyperplasia lasted for 10 days or more, the incidence of fibrosis was 80%, compared to only 20% when it lasted for less than 10 days. Histopathological evaluation revealed stromal fibrosis in non-responsive instances, particularly when hyperplasia persisted for more than 10 days, indicating a strong correlation between prolonged estradiol exposure and the development of fibrotic changes. These findings suggest that while hCG may provide limited therapeutic benefit in field conditions, ovariohysterectomy remains the definitive treatment. Furthermore, the duration of estrogenic stimulation appears to influence stromal remodelling and may predict therapeutic outcomes.

Key words: Bitch, Estradiol, Fibrosis, HCG, Ovariohysterectomy, Vaginal hyperplasia.

INTRODUCTION

Vaginal and vestibular masses in the bitch can originate from various conditions, most commonly vaginal or urethral tumours and vaginal tissue prolapse (Manothaiudom and Johnston, 1991; Bawaskar, 2024). Although vaginal prolapse is an infrequent condition, it has been reported in association with neoplasia or trauma. Hormonal influences are particularly significant around parturition, as declining progesterone and increasing estrogen levels can predispose to prolapse. Vaginal hyperplasia, often mistaken for true prolapse, usually occurs during pro-oestrus and oestrus in younger bitches and resolves once dioestrus begins (Schaefer-Somi and Schaeferes-Okkens, 2005). Epidemiological risk factors like dioestrus stage, parity, breed as well as age (Ergene *et al.,* 2019) play a pivotal role in the disease pathology. Conversely, true vaginal prolapse is rare and happens much less frequently outside the periparturient period.

Reproductive tract disorders in bitches remain a challenge for clinicians, affecting fertility and animal welfare. While conditions such as vaginitis and neoplasia are more common, the actual incidence of vaginal prolapse is relatively low. Its development is closely associated with the effects of oestrogen and relaxin on reproductive tissues, leading to the relaxation and eversion of vaginal folds. A breed predisposition has been identified, with brachycephalic dogs and larger breeds, such as Dobermans and Dalmatians, at higher risk (Antonov *et al.*, 2009). Recognising these factors is crucial for accurate diagnosis and effective management.

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MATERIALS AND METHODS

Clinical case

A case study was performed with 11 cases of vaginal hyperplasia in bitches between December 2023 to March 2025 presented at the Teaching Veterinary Clinical Complex, Bihar Veterinary College, Patna- 14. The prolapsed mass was washed with 0.3% cetrimide solution (Savlon) diluted in normal saline (1:10). Through manual palpation and

differential diagnosis with other vaginal conditions, it was clinically diagnosed as vaginal hyperplasia. It was characterised by a prolapsed vaginal mass (Fig 1), protruding from the vulvar lips. After applying local anaesthesia with 2% lignocaine, the tissue sample was collected using a 6 mm punch biopsy needle (BASCO, India), dipped in 10% formalin and sent for histopathology.

The time from the appearance of prolapse till arrival at VCC was recorded. Rectal temperature was also recorded. Blood samples were collected for a Complete Blood Count, serum biochemistry, including Aspartate Transaminase (AST), Alanine Transaminase (ALT), BUN, creatinine and an estradiol assay. Ultrasonography was performed to assess the presence of an ovarian follicle/cyst.

Treatment

The bitches received two types of treatment and were accordingly classified into two groups, *e.g*. hCG and OVH. The bitches in the hCG group were injected 500 IU of Human Chorionic Gonadotropin (Chorulon®, MSD, India) intramuscularly three times at alternate-day intervals (Knauf *et al.*, 2013), along with a uterine wash of 10 ml of 5% Providone Iodine diluted ten times with Normal Saline for seven days. The bitches in the OVH group underwent ovario-hysterectomy. The recovery in both groups was observed after 7 days. A blood sample was taken at 10 days for the estradiol assay.

Anaesthesia and preparation of surgical site

The bitch was administered with Ringer's Lactate (Rintose, Vetoquinol, India) at the dose of 5 ml/kg/hour. Atropin sulphate (Tropin, NEON Laboratories) was administered subcutaneously at the rate of 0.04 mg/kg as pre-anaesthetic medication. The bitch was anaesthetized by a combination of Tiletamine and Zolazepam (Zoletil 50, Virbac, India) at the dose of 10 mg/kg body weight intravenously. The bitch was placed in dorsal recumbency with endotracheal intubation and ovariotomy was attempted first. The surgical site was sterilised by alternate swabbing with 3% chlorhexidine (Savlon®) and providone iodine (Betadine®).

Ovario-hysterectomy

A mid-ventral skin incision was made two fingers caudal to the umbilicus and then by incising the linea alba and peritoneum, the abdominal cavity was reached. The left ovary was carefully exteriorised, the ovarian pedicle was ligated with Vicryl 1-0 and the ovary was excised at its base. The base was held in place with rat tooth forceps to check for bleeding before returning it to the abdomen. The linea alba, along with the peritoneum, was closed using a continuous lock stitch suture. Subcutaneous continuous sutures were placed to minimise dead space and the skin was closed with an interrupted mattress suture using 1-0 Nylon suture.

Excision of the prolapsed mass

After catheterising the urinary bladder with Romsons' tube no. 8, the vaginal wall was clamped using Rochester-Carmalt forceps and interrupted sutures were placed with Vicryl 1-0 around the circumference (Fig 2B) to ligate all bleeding vessels following modified Schaefer's technique. The hyperplastic mass was excised cranial to the sutures and any bleeding points were ligated with reinforced sutures along the edges.

Post-surgical treatments

The bitch received cefuroxime (C4All 50; Alembic, India) at a dose of 2 mg/kg body weight once daily for 7 days, meloxicam suspension (Melonex, Intas, India) at 0.1 mg/kg body weight for 3 days, pantoprazole (Palapan Vet-10; Oriheal Lifesciences®, India) at 1 mg/kg body weight once daily before breakfast for 10 days and aRBCe as previously described for 14 days. The dressings of the surgical wound were performed every alternate day for 10 days. The bitch had an uneventful recovery and the skin sutures and vaginal sutures were removed on the 10th day.

RESULTS AND DISCUSSION

Of the eleven bitches with vaginal hyperplasia in our study, two were of the Labrador breed and three were Pugs (Table 1 and 2), which corroborates the observation of Antonov *et al.* (2009) that brachycephalic and large breeds had a higher risk of vaginal prolapse. In India, Devarajan *et al.* (2024) and Kumar *et al.* (2025) also reported a case of vaginal hyperplasia in the Labrador breed.

All bitches under this study had their CBC and biochemistry within normal range and did not have pyrexia. Ultrasonography revealed the presence of ovarian follicles (Fig 1A), which corroborated the blood estradiol level above 15 pg/ml (Table 2 and Fig 3) and cornified cells in vaginal cytology (Fig 1B). Recovery of bitch with vaginal hyperplasia following surgical excision with simple interrupted peri-vaginal sutures. Vaginal Hyperplasia in bitch (the urethral opening at the base) (Fig 2A). Complete Recovery after 21 days post-surgery (Fig 2C). Blood estradiol concentration greater than 25 pg/ml has been reported in bitches in late proestrus, estrus and cases of delayed ovulation or anovulation (Concannon *et al*., 2009). Estradiol receptor alpha (ERα) is expressed in superficial and deep epithelium and in stromal connective tissue (Ithurralde *et al*., 2013). In fact, stromal ERα is required for estrogen-induced epithelial proliferation (Cunha *et al*., 2004), which supports the notion

Table 1: Resolution of Vaginal Hyperplasia in bitches with Ovariohy-sterectomy (OVH).

Breed	Prolapse-OVH Interval (days)	Resolution of Vaginal Hyperplasia
Pug	3	Resolved completely
Pomeranian	5	Resolved completely
Spitz	4	Resolved completely
Pug	10	Resolved completely
Labrador	18	Not resolved
German shephard	16	Not resolved

that long-lasting estradiol exposure results in epithelial keratinisation, as observed in vaginal cytology.

Following ovariohysterectomy in six bitches with vaginal hyperplasia, the mean estradiol level dropped from 49.5 ng/ml to 12.5 ng/ml (Fig 3), consistent with the anestrous concentration as described by Concannon *et al*. (2009). Of the five bitches, that were treated with hCG, three responded with a return of estradiol level at or below 15 pg/ml (Table 2). Knauf *et al.* (2013) reported that the three consecutive hCG treatments were only effective in 67% of canine ovarian cyst cases, a result similar to ours, where we found it to be effective in only 60% of cases.

In field conditions where diagnostic facilities are limited, the only treatment available for vaginal hyperplasia is hCG. Only a few cases respond to hCG and it remains unclear whether the failure was due to the inability of hCG to induce ovulation or regress the ovarian cyst, leading to decreased blood estradiol levels, or if vaginal hyperplasia failed to regress despite lowered estradiol levels. Therefore, ovariohysterectomy was performed to remove the source of estrogen and study the resolution of vaginal hyperplasia. The results showed that 4 out of 6 bitches experienced complete resolution of vaginal hyperplasia (Table 1). Histopathological studies indicated that the two bitches that did not respond to ovariohysterectomy had stromal fibrosis (Fig 4A, 4B).

Previous studies have shown that estradiol increases both the total and cross-linked collagen in the vaginal wall

Table 2: Resolution of Vaginal Hyperplasia in bitches treated with hCG.

Breed	Prolapse-hCG interval (days)	Blood estradiol level (pg/ml)		Ovarian condition	Resolution of vaginal hyperplasia
		Before hCG	After hCG		
Pomeranian	8	39	12	Ovulation	Resolved completely
Pug	6	45	38	Cyst	Not resolved
Labrador	9	67	52	Cyst	Not resolved
Golden retriever	15	48	8	Ovulation	Not resolved
Pomeranian	12	85	15	Ovulation	Resolved completely



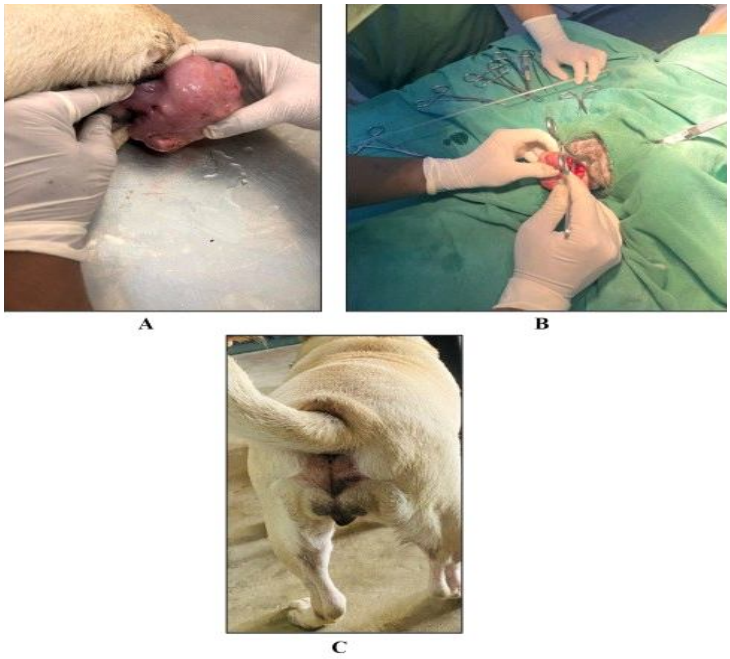
A) Ovary with follicles at the caudal border of the left kidney, corresponding with an estradiol concentration of 38 pg/ml. B) Vaginal cytology showing the presence of cornified epithelial cells (x1000), implying a high blood estradiol level.

Fig 1: Ultrasonography and cytology of bitches presented with vaginal hyperplasia.

of guinea pigs (Balgobin *et al*., 2013). Additionally, Montoya *et al.* (2015) reported that a low dose of oestrogen applied in the vaginas of rats resulted in an overgrowth of collagen fibres. In a study with bitches, Linharattanaruksa *et al.* (2019) reported that the proportion of collagen to smooth muscle in the bitches' cervix at estrus was higher compared to other stages of the cycle. In fact, as has been discussed earlier, stromal fibroblasts having higher concentration of ERs (Cunha *et al.*, 2004) release growth factors like Epidermal Growth Factor and Insulin-Like Growth Factors (Zhou *et al.*, 2018) that stimulate the mitosis of the basal epithelium, leading to upward movement and keratinisation in later stages (Fig 1A). A study reported higher occurrence of pyometra among the older dogs *i.e.*,

Table 3: Duration of hyperplasia/estradiol exposure affects the success rate of vaginal hyperplasia following ovario-hysterectomy/hCG treatment.

Duration of Prolapse	No. of bitches with fibrosis	Recovery with ovario-hysterectomy/hCG
Below 10 days	1/5 (20%)	4/6 (67%)
After 10 days	4/5 (80%)	1/5 (20%)



A. Vaginal Hyperplasia in bitch (the urethral opening at the base), B. Peri-vaginal sutures after clamping the prolapsed part with Rochester-Carmalt forceps, C. Complete Recovery after 21 days post-surgery.

Fig 2: Recovery of bitch with vaginal hyperplasia following surgical excision with simple interrupted peri-vaginal sutures.

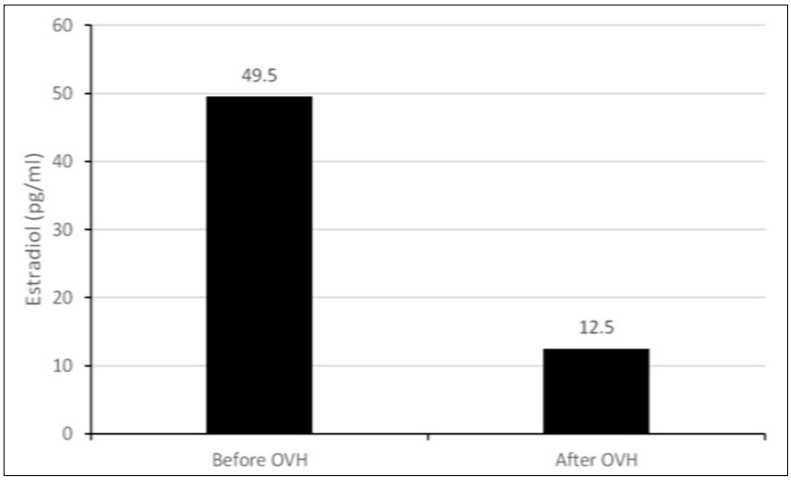


Fig 3: The mean blood estradiol level of bitches (n=6) before and after ovariohysterectomy with vaginal hyperplasia.

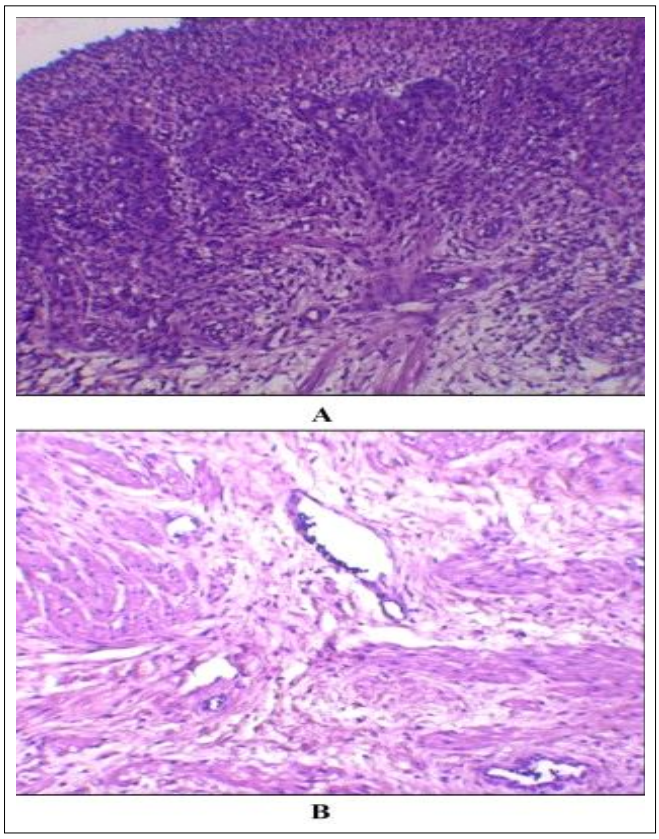


Fig 4: A) Histological section (H and E,10x) of vaginal mass of a bitch shows marked epithelial hyperplasia with stratified squamous thickening and early keratinisation consistent with estrogen-induced changes during estrus. No evidence of dysplasia, inflammation or neoplasia is present; B) Histological section (H and E, 10x) of the vaginal mass shows dense fibrous connective tissue with interlacing collagen bundles and scattered spindle-shaped fibroblasts. Small to medium blood vessels exhibit mild perivascular fibrosis. These features indicate stromal changes secondary to chronic vaginal hyperplasia associated with hormonal stimulation in intact bitches.

>7-year age (78.26%) followed by 4-7 years age group (17.39%) and least occurrence in 1-3 years age group (4.34%) (Achary *et al*., 2024). Higher incidences in adult bitches of more than 7 years of age might be attributed to other concurrent co-morbid conditions thus amplifying the severity of clinical conditions as well as incidences of the disease (Robaj *et al.,* 2018). A study showed incidence of pyometra, fetal maceration and pseudopregnancy was found to be the highest in Labrador (35.92%, 26.92% and 23.33%, respectively), uterine infection in Labrador and German Spitz (15.38% each) and TVT in local breeds (51.51%) of dogs (Bhuyan *et al.,* 2024).

Another interesting fact from this study is the correlation between the duration of vaginal hyperplasia/estradiol exposure and the occurrence of fibrosis as detected in histopathology. When hyperplasia lasted for 10 days or more, the incidence of fibrosis was 80%, compared to only 20% when it lasted for less than 10 days (Table 3). This aligns with the findings of Montoya *et al*. (2015) in rats, where a low dose of estrogen applied to the vagina for a prolonged period resulted in fibrosis and thickening. Additionally, an inverse relationship was observed in our study, where the occurrence of fibrosis negatively impacted the natural resolution of vaginal hyperplasia following hCG treatment or ovario-hysterectomy. Thus, it can be inferred that as the exposure to estrogen is prolonged, the collagen fibres are laid down for an extended period of time, which does not get digested once the source of estrogen is withdrawn.

CONCLUSION

Vaginal hyperplasia in bitches is an estrogen-driven condition. Ovariohysterectomy proved more effective than hCG therapy, offering consistent resolution by eliminating the source of estrogen. Prolonged estradiol exposure was associated with stromal fibrosis, reducing treatment success. Early intervention is therefore key to better outcomes.

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expenditure for the diagnosis and treatment of the bitches was borne by the pet owners.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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