

Leiomyomas of Oviduct and Its Ventral Ligament of Hens: Immunohistochemical Evaluation and the Plasma Concentration Levels of 17 β -Oestradiol and Progesterone ^[1]

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Summary

Leiomyoma's of the oviduct and its ventral ligament (VL) are common tumours of the domestic fowl which develop after the end of the first laying season. In the present study, macroscopic, microscopic, and immunohistochemical features of the leiomyomas of the reproductive tract and their prevalence were investigated in commercial laying hens. In 60-90 weeks old commercial laying hens, toward the end of the first laying period, the incidence of genital tract leiomyomas were determined as 10.43%. Of these, 95.81% were developed from oviduct VL and 4.19% were oviduct leiomyomas. These tumours were firm, round to oval and microscopically well-circumscribed, consisting of monomorphic spindle cells. In immunohistochemical examination with α -smooth muscle actin, desmin and vimentin, all tumours were found positive with these markers. To investigate the aetiological importance for tumourogenesis, the plasma concentrations of 17 β -oestradiol and progesterone levels were determined. Concentrations of 17 β -oestradiol and progesterone were higher in hens with tumours than that of non-tumour control animals. The results suggested that there was an association between the levels of 17 β -oestradiol and progesterone and the leiomyomas of genital tract in hens.

Keywords: Leiomyomas of hens' oviduct, Incidence, Immunohistochemical evaluation, 17 β -oestradiol and Progesteron levels

Yumurtacı Tavuklarda Ovidukt Ventral Ligamenti Leiomyomları: İmmunohistokimyasal İnceleme ve Plazma 17 β -Östradiol ve Progesteron Düzeyleri

Özet

Ovidukt ve ovidukt ventral ligamenti (VL)nin leiomyomları, ilk yumurtlama periyodunu tamamlayan yumurtacı tavuklarda oldukça sık karşılaşılan tümörlerdir. Çalışmada, ticari olarak yetiştirilen yumurtacı tavukların reproduktif sistemlerinde gelişen leiomyomların sıklığı ve bu tümörlerin makroskopik, mikroskopik ve immunohistokimyasal özellikleri değerlendirildi. İlk yumurtlama periyodunu tamamlamış, 60-90 haftalık yaşta, ticari yumurtacı tavuklarda, reproduktif sistem leiomyomlarının insidensi %10.43 olarak saptandı. Saptanan leiomyomların %95.81'inin oviduktun ventral ligamentinde, %4.19'un oviduktta yerleştiği belirlendi. Makroskopik olarak sert kıvamlı ve yuvarlak-oval şekilli olan bu tümörlerin mikroskopik incelemesinde iyi sınırlanmış mekik şeklindeki hücrelerden oluştuğu belirlendi. İmmunohistokimyasal incelemede, tüm tümörlerin α -smooth muscle actin, desmin ve vimentin primer antikorları ile pozitif reaksiyon verdikleri belirlendi. 17 β -östradiol ve progesteronun tümörogenezisteki etiyolojik öneminin değerlendirilmesi amacıyla plazma konsantrasyonları belirlendi. Tümör tespit edilen yumurtacı tavukların plazma 17 β -östradiol ve progesteron düzeyleri kontrol gruplarındaki yumurtacı tavuklardan anlamlı derecede yüksekti. Elde edilen bu sonuçlar ile, yumurtacı tavukların reproduktif sistemlerinde sıklıkla ortaya çıkan leiomyomların gelişiminin, plazma 17 β -östradiol ve progesteron düzeyleri ile ilişkili olduğu görüldü.

Anahtar sözcükler: Yumurtacı tavuk, Ovidukt, Leiomyom, İnsidens, İmmunohistokimyasal inceleme, 17 β -östradiol ve progesteron düzeyleri



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INTRODUCTION

The frequently observed spontaneous tumours of the unknown aetiology in domestic fowls are leiomyoma of the ventral ligament (VL) of the oviduct [1,2]. These tumours are capsulated, round, solid masses and grow as solitary within VL of the oviduct [3,4]. They are benign and consist of smooth muscle fibers located in a fibrous stroma [1]. Leiomyomas of the oviduct VL commonly arise after the end of a laying period [5]. They can reach considerable dimensions without impairing production in layer hens [6]. It has been suggested that there is an association between the number of eggs produced in a laying period and the incidence of leiomyomas [4]. The prevalence of these tumours varied from 0% to 60% [1]. Although most genital organ leiomyomas in laying hens develop from the oviduct VL, occasional oviduct leiomyomas can also be seen [2]. In humans, uterine leiomyomas are the most common type of reproductive tract tumours in women and leiomyomas of the oviduct in sexually mature domestic hens share important features with human uterine leiomyomas [7-9]. The high prevalence and ability to induce leiomyomas of the oviduct VL in hens means that this species could be of interest as a model for the study of similar tumours in mammals as well as women because the remarkable similarities between the ovulatory cycles of women and hens [3,7-11].

Oestrogen and progesterone hormones were suggested to have etiological importance on the tumourogenesis of the oviduct magnum region adenomas, oviduct adenocarcinomas and leiomyomas of the oviduct VL [1,5]. However, no such tumours could experimentally be induced in chicks by administration of only oestrogen or progesterone [1]. On the other hand, concurrent i.m. administration of oestrogen and progesterone to three weeks old chicks repeated for five weeks was shown to cause leiomyomas of the oviduct VL [1]. Fredrickson et al. [8] reported that there were no changes in the plasma concentrations of oestrogen and progesterone in oviduct gland tumours. In addition, such studies on the plasma concentrations of oestrogen and progesterone in leiomyomas of the oviduct VL are limited [5]. However, there are no studies investigating the association between these hormones and the leiomyomas of oviduct.

This study was undertaken to investigate the immunohistochemical profile of the leiomyomas of the oviduct and its VL and to better characterize the relationship between etiological importance of 17 β -oestradiol and progesterone on the tumourogenesis of these tumours.

MATERIAL and METHODS

Animals and Tissue Processing

The study materials were 60-90 weeks old hens which

were at the end of a laying period. A total of 1600 commercial laying hens slaughtered in a privately owned company in Ankara were investigated. Each hen was given with a wing number. Then, blood samples were collected; sera samples were obtained within 4 hours and stored at -18°C until analysis. Following the slaughter, internal organs, were examined for tumours lesions. When a tumour was observed it was grossly evaluated and fixed in 10% buffered formalin solution for microscopical investigation. Then, using routine techniques, 5-6 micron sections were cut from paraffin blocks and stained with haematoxylin and eosin for histopathological examination. To demonstrate the tumour components, Masson's Trichrome and Van Gieson were used.

Biochemical Analysis

Plasma concentration levels of 17 β -oestradiol and progesterone were determined according to the standards laid out by the USA Center for Disease Control/National Institute of Health Manual and Biosafety in Microbiological Laboratories, 1984. For the determination of 17 β -oestradiol concentration, an immunoassay kit (Boehringer-Manheim 1776002) was used [12,13]. In this assay, 50 microliters of serum sample from each of the 167 cases of leiomyomas and for the control 60 cases with no tumours were incubated with peroxide labelled 17 β -oestradiol and progesterone. Rabbit anti-17 β -oestradiol or rabbit anti-progesterone antibodies were then added, depending on which hormone was tested, and unbound labelled 17 β -oestradiol or progesterone was removed by washing. Hormone found in the serum and bound to the antibody of interest was determined by a colorimetric mean using hydrogen peroxide/TMB. Staining density showing the hormone concentration level was read in a spectrophotometry at 450 nm. Students't test was used to compare plasma concentrations of 17 β -oestradiol and progesterone between the groups of hens with and without tumour.

Immunohistochemistry

For each sample 3 μm sections were cut and immunohistochemically stained for vimentin (V9, DAKO, Carpinteria, CA, USA, M0725, 1:25), desmin (DE-R-11, DAKO, Carpinteria, CA, USA, M724, 1:50) and α -smooth muscle actin (1A4, DAKO, Carpinteria, CA, USA, M0635, 1:50) (Inter-Species Code N° 10 145). Antigen retrieval was performed by microwave treatment in citrate buffer (pH 6.0). After blocking the endogenous peroxidase activity with 0.3% H_2O_2 , non-specific antibody binding was blocked by non-immune goat serum. Further, the sections were incubated with primary antibodies for 30 min at room temperature. Then, each section was stained by a modified labelled streptavidin-biotin (LSAB) method using a standard reagent kit (LSAB2 code K0675, Dako Corp., Carpinteria, CA). Antigen-antibody binding was visualised by 3,3'-diaminobenzidine. The sections were counterstained with Mayer's haematoxylin. For each of the three methods, the internal

positive control was represented by normal cells or structures, found close to the tumors. In the above-mentioned conditions, we considered that an external positive control was not necessary. Negative controls were achieved by replacing the primary antibody with normal mouse serum.

RESULTS

In 167 of 1600 hens (~10.43%) which were toward the end of the first laying period, genital organ leiomyomas were determined. These tumours were only localized in the oviduct and its VL. Of these leiomyomas, 160 (95.81%) had localized in the oviduct VL and 7 (4.19%) in the oviduct (Fig. 1). All of the leiomyomas of the oviduct VL was located in the centre of the ligament (Fig. 1A and B). The size of these tumours varied from 0.5 x 0.6 x 0.5 cm to 7 x 6.8 x 6.4 cm. These tumours were solid, round to oval, firm and in cut surface, they were white yellowish and lobulated. The outer surface of the tumours contained many prominent blood vessels. Some of these leiomyomas showed whitish whorled cut surfaces (Fig. 1D). In three cases, the cut surfaces were red or pinkish with necrosis. Leiomyomas developed from oviduct had similar appearances of the leiomyomas developed from the VL. The number of these tumours in any given case varied from one to seven. These leiomyomas were round, firm, white-yellowish and 0.5 to 1.5 cm in diameter (Fig. 1C). These tumours were sharply

circumscribed, with a line of cleavage in the surrounding myometrium that results in easy shelling out of these lesions. In five cases, with multiple leiomyomas, some were attached to the oviduct serosa with a stalk.

Microscopically, well-circumscribed leiomyomas of the VL and oviduct were observed to consist of monomorphic spindle cells that were arranged in interweaving fascicles surrounded by a thin layer of fibrous tissue (Fig. 2A, B and C). The muscle fibers were stretched in various directions and crossing each other. These tumours were highly hyperaemic and had fibrous tissue and hyalinised collagen fibers that divided the tumour into nodules in their structure. Typical smooth muscle cells were uniform, elongated, with abundant eosinophilic cytoplasm and had cigar-shaped nuclei (Fig. 2A). The muscle fibers were located parallel to each other. The nuclei of smooth-muscle cells were bland ended in longitudinal sections, sometimes the nuclei palisade in a pattern. The nuclei appear round in transverse sections. These leiomyomas showed no cytological atypia and had the mitotic index of < 8 at high-power fields. Only in three cases, coagulative tumour cell necrosis was observed in the centre of the tumours. Invasion was not observed in any case. In 125 cases (74.8%), inflammatory reaction was observed in leiomyomas especially around the vessels (Fig. 2B). This inflammatory reaction was moderate to severe infiltration of small lymphocytes and a few activated large lymphoid cells accompanied by some plasma cells and histiocytes. The inflammatory reaction

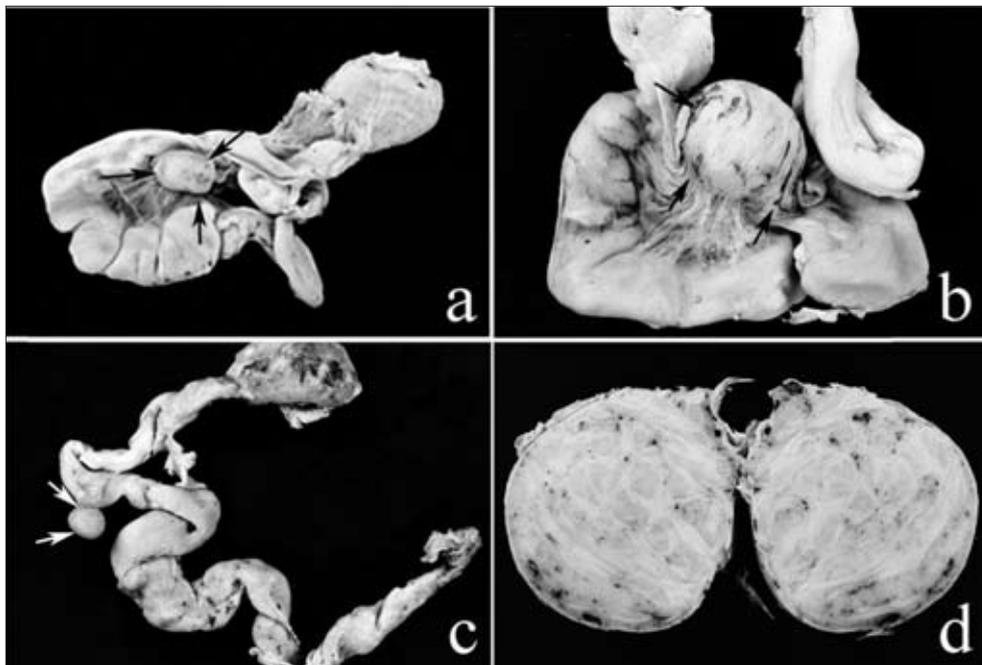


Fig 1. Macroscopic features of leiomyomas in hens. A- Solid round leiomyoma (arrows) within the centre of the VL, B- Large leiomyoma with prominent vascularization (arrows), C- Leiomyoma (arrows) developed from oviduct serosa, D- Cut surfaces of the leiomyoma shows a well-demarcated whitish and whorled mass

Şekil 1. Yumurtacı tavuklarda leiomyomların makroskopik özellikleri. A- Ventral ligamentin merkezinde sert kıvamlı ve yuvarlak şekilli leiomyom (oklar), B- Vaskülarizasyonun (oklar) belirgin olduğu leiomyom, C- Ovidukt serozasından gelişen (oklar) leiomyom, D- Çevresinden iyi sınırlanmış, kesit yüzü beyazımtırak renkte belirgin girdap-vari yapıların seçildiği leiomyom

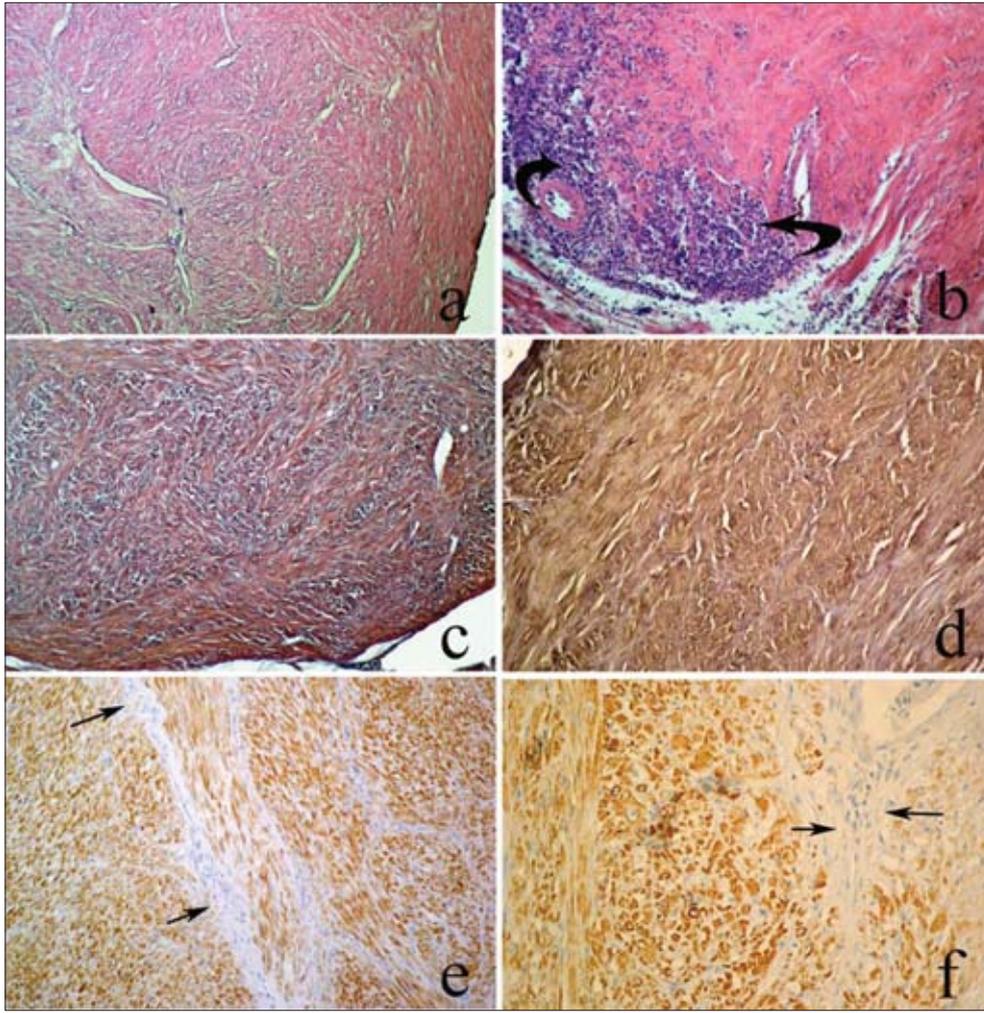


Fig 2. Microscopic (A, B and C) and immunohistochemical (D, E and F) feature of leiomyomas. **A-** The well developed leiomyoma of the oviduct ventral ligament, fascicles of smooth muscle fibers without necrosis HxE, x 80, **B-** The histological section show interlacing spindle-shaped smooth muscle cells, which infiltrated lymphoid cells (*arrows*) HxE, x 80, **C-** Typical smooth muscle cell are uniform, elongated, with abundant eosinophilic cytoplasm and had cigar-shaped nuclei. Spindle tumour cells located in various direction. Masson's Trichrome Stain, x 160, **D-** Diffuse and intense immunopositivity for vimentin antibody avidin biotin peroxidase complex (ABC) x 80, **E-** Immunohistochemical staining for desmin antibody showing strong positivity while the stromal tissue negative immunoreactivity (*arrows*) for desmin antibody, ABC x 80, **F-** Tumour cells showed strong cytoplasmic immunopositivity and stromal tissue negative immunoreactivity (*arrows*) for α -SMA antibody, ABC x 160

Şekil 2. Leiomyomların mikroskopik (A, B ve C) ve immunohistokimyasal (D, E ve F) özellikleri. **A-** Ovidukt ventral ligamentinden gelişen düz kas demetlerinden oluşan leiomyom, HxE, x80, **B-** Birbirinin içine geçen mekik şekilli düz kas hücreleri arasına infiltre lenfoid hücreler (*oklar*) HxE, x80, **C-** Yoğun eozinofilik sitoplazmalı ve çekirdekleri sigara şeklinde, uzun, uniform düz kas hücreleri. Mekik şekilli tümör hücreleri farklı yönlerde uzanmakta. Masson'un Trichrome Boyası, x160, **D-** Vimentin primer antikoruna ile diffuz ve yoğun boyanma. Avidin biotin peroksidaz kompleksi (ABC) x80, **E-** Tümör hücreleri desmin primer antikoruna ile pozitif olarak boyanırken çevredeki bağ doku hücrelerinin reaksiyon vermediği (*oklar*) görülmekte, ABC x80, **F-** Tümör hücreleri α -SMA antikoruna ile immunpozitif olarak boyanırken stromal dokuda negatif immunreaksiyon (*oklar*), ABC x160

was confined to the leiomyoma and did not extend in the surrounding non-neoplastic tissue. Within the tumour mass, the blood vessels were extremely enlarged and in some cases the tumourous tissue pressured through the vessel lumen in a finger-like appearance that gave the impression of papillary hyperplasia to the blood vessel.

Distinction of the muscle cells and the fibrous tissue

elements were made with special stains. Masson's Trichrome and Van Gieson stain revealed collagen bundles, which gave a lobular appearance to the tumourous tissue (*Fig. 2C*). Immunohistochemically, all samples had diffuse and intense expression of vimentin (*Fig. 2D*). The positive reactivity was also detected in the cytoplasm of the spindle cells with desmin and α -SMA (*Fig. 2E* and *F*). In general, reactivity for the muscle markers desmin and α -SMA was

Table 1. Plasma concentration of 17 β -oestradiol and progesterone levels in laying hens with and without leiomyomas**Tablo 1.** Leiomyomlu ve kontrol grubundaki yumurtacı tavuklarda plazma 17 β -östradiol ve progesteron seviyeleri

Parameter	Groups	N	X \pm SE	t
Progesterone	Control	60	0.588 \pm 0.11	2.208*
	Leiomyomas	167	1.054 \pm 0.11	
17 β -oestradiol	Control	60	132.78 \pm 26.39	2.423*
	Leiomyomas	167	234.00 \pm 32.39	

* $P < 0.05$

strong and diffuse. Because the stromas of the leiomyomas were not stained with desmin and α -SMA, the positive reactions were multi lobular in appearance.

In biochemical analysis, plasma concentrations of 17 β -oestradiol and progesterone in hens with leiomyomas were significantly ($P < 0.05$) higher than that of control animals. Plasma concentrations of 17 β -oestradiol and progesterone of 60 controls and 167 leiomyoma observed hens were summarized in *Table 1*. Plasma concentrations of 17 β -oestradiol and progesterone in hens with leiomyoma were significantly higher than that of controls.

DISCUSSION

The most common tumours of genital tract in poultry are adenomas and adenocarcinomas [4]. The other common tumours of the genital tract are leiomyomas of the oviduct and its VL [2]. Studies on the prevalence of these tumours are limited. Although it is previously speculated that the incidence of these tumours varied from 0 to 60%, in post-mortem examination of hens. Reece [14] reported the prevalence of oviduct VL leiomyomas as 0.11%. In other studies, the prevalence was reported to vary from 1.36% to 7.28% [2,15]. Only in one report, the incidence of oviduct leiomyomas was reported as 0.02% [2]. In the present investigation, the prevalence of oviduct and its VL were found as 4.19% and 10.43%, respectively. Therefore, our results regarding to the incidences of these tumours were relatively higher compared to those of the previous reports. It has been suggested that a difference in the prevalence of the leiomyomas can occur depending on the breeds and lines [1]. Therefore, hereditary influence might the reason for higher findings in this study.

Leiomyomas consist of complex interlacing fascicles of smooth muscle fibers, with little or no mitotic activity [1,4]. These tumours are rarely accompanied by a focal to diffuse lymphocytic infiltration in humans [16]. Some unknown factors and pathogenesis were suggested for underlying causes of lymphoid infiltration. However, only in one report, in the majority of laying hens, moderate lymphocytic infiltration was reported [2]. Similarly, in 74.8% of leiomyomas of the present study, moderate to severe lymphocytic infiltration was found. Although the cause is not clear, this inflammatory infiltration is suggested to be

an immunological reaction against the tumorous tissue as indicates a direct cytotoxic effect by an autoimmune mechanism.

Leiomyomas of oviduct and its VL in poultry have been described only by histomorphology. However a final diagnostic decision cannot be made depending on one criteria only. Therefore, specific markers might be necessary to show that the tumour is originated from smooth muscle tissue. Leiomyomas have been commonly described to be immunohistochemically positive for vimentin and α -Smooth Muscle Actin (α -SMA). These tumours could also be stained with desmin [7]. These markers are used in human and veterinary pathology as indicators of smooth muscle tumours [3,7,17,18]. Vimentin is an intermediate filament and expressed in most mesenchymal cells. Vimentin is considered a nonspecific marker commonly expressed in less differentiated tumours and its usually associated with expression of other markers [19]. Desmin is a cytoskeletal intermediate filament that is expressed in skeletal, cardiac, and smooth muscle [10]. α -SMA is a cytosolic intermediate filament that is involved in the mechanism of contraction and that is specific to smooth muscle cells [10,19]. α -SMA and desmin are conventional smooth muscle markers and the use of both antibodies is recommended in immunohistochemical studies as poorly differentiated smooth muscle tumours may react with antibodies to either desmin or α -SMA [3,19]. The tumours in this series showed immunohistochemical staining for vimentin, desmin and α -SMA. The marked and diffuse positivity to these antibodies confirm that the tumours of oviduct and its VL were leiomyomas.

Development and function of genital canal is regulated by steroidal sex hormones [17]. Therefore, such hormones were thought to play important roles in the development of genital organ tumours [5,20,21]. The dorsal oviductal ligament suspends the oviduct and continues ventrally as the fanlike oviduct VL the free edge of which is reinforced by smooth muscle. It is known that oviduct VL is involved in oviduct peristaltics and egg drop [22]. The administration of diethylstilbesterol (DES) via skin implant was shown to increase the VL size and diameter. Hyperplasia of smooth muscles at the free border of the oviduct VL was reported in DES administered 22 weeks old laying hens [1]. The relationship between the genital tract tumours and the plasma concentrations of oestrogen and progesterone

were investigated previously. Fredrickson et al.^[11] has found no correlation between the plasma concentration of these hormones and the magnum glandular tumours. On the other hand, Anjum and Payne^[5] reported that plasma concentration of oestrogen but not progesterone was higher in hens with oviduct tumours than in non-tumourous hens. In the present study, plasma concentration levels of both 17 β -oestradiol and progesterone were found higher in laying hens with leiomyomas of oviduct and its VL. Their levels were found approximately twice the levels of non-tumourous control animals. Therefore, our results partially correlate with the previous study regarding the high levels of 17 β -oestradiol in tumourous hens, and differ from it by high level of progesterone. While administration of oestrogen or progesterone alone was reported not to cause tumours, concurrent administration of these hormones were shown to induce leiomyoma of the VL^[11]. Although many organs bear smooth muscles, higher incidences of leiomyomas in the oviduct might be explained by the presence of receptors for oestrogen and progesterone on the smooth muscle cells of this organ. These cells are known to be under the effect of these hormones during a laying season^[20,23]. Therefore, longer hormone effects on oviduct might be thought to increase the incidence of tumour. In this respect, steroid sex hormones were thought to be effective on leiomyomas of VL in poultry animals.

Leiomyomas of oviduct and its VL in hens share several histologic and biological features with human uterine leiomyomas^[8-10]. Our histochemical and immunohistochemical findings confirm that tumours found on the oviduct and its VL of hens are derived from smooth muscle cells and there are association between tumorigenesis and ovarian sex steroid hormones.

In conclusion, leiomyomas of the oviduct and its VL in hens were investigated both by histological and immunohistochemical means. In addition, a probable association between the plasma concentration levels of 17 β -oestradiol and progesterone was discussed and high levels of these hormones were thought to play a role in the tumourogenesis of these tumours. Therefore, if the aetiology of these tumours in hens were better understood, that would help in explaining similar tumours in other animals and humans. In this respect, more research is still needed to explain why these tumours arise in such high incidences.

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