

Immunohistochemical Expression of Estrogen and Progesterone Receptors in Oviduct Adenocarcinomas in Laying Hens

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Abstract: The aim of this study was to demonstrate the presence of estrogen and progesterone receptors (ERs and PRs, respectively) in oviduct adenocarcinomas in laying hens. In all, 32 oviduct adenocarcinomas (18 primary tumors and 14 intestinal metastases) from Babcock B-380 laying hens 16-18 months old were stained with commercially available monoclonal antibodies against mouse-anti-human ERs and mouse-anti-human PRs. In the microscopic examination, 13 tumors (7 oviduct adenocarcinomas and 6 intestinal metastases) stained positively for ERs and 12 tumors (4 oviduct adenocarcinomas and 8 intestinal metastases) gave a positive reaction to PR antibody. The staining pattern of the tumor cells with both antibodies was nuclear and cytoplasmic. In conclusion, we proved the existence of ERs and PRs in chicken oviduct adenocarcinomas, which may have roles in the formation of these tumors.

Key Words: Chicken, estrogen receptor, progesterone receptor, immunohistochemistry, oviduct adenocarcinomas

Yumurtacı Tavukların Ovidukt Adenokarsinomlarında Östrojen ve Progesteron Reseptörlerinin İmmunohistokimyasal Olarak Gösterilmesi

Özet: Bu çalışma, yumurtacı tavuklarda görülen ovidukt adenokarsinomlarında östrojen (ER) ve progesteron (PR) reseptörlerinin varlığını tespit etmek ve bu tümörün oluşumunda steroid cinsiyet hormonlarının olası rollerini belirlemek amacıyla yapıldı. Çalışmada, 16-18 aylık B-380 genotipi ticari yumurtacı tavuklarda tespit edilen 32 ovidukt adenokarsinomu olgusu kullanıldı. İmmunohistokimyasal incelemeler için 18 olguda oviduktan, 14 olguda tümörün bağırsak metastazlarından doku örnekleri alındı. Doku örnekleri ticari olarak sağlanan mouse-anti-human östrojen reseptörü ve mouse anti-human progesteron reseptörlerine karşı ticari olarak hazırlanan monoklonal antikor ile boyandı. Mikroskopik incelemelerde, 7'si ovidukt, 6'sı bağırsak metastazları olmak üzere 13 olguda neoplastik hücrelerin çekirdek ve sitoplazmalarında ER ile pozitif boyanma tespit edildi. PR antikoru ile boyanan kesitlerde ise 4'ü ovidukt, 8'i bağırsak metastazları olmak üzere 12 olguda tümör hücrelerinin çekirdek ve sitoplazmalarında pozitif immün boyanma vardı. Sonuç olarak, immunohistokimyasal bulgular tavukların ovidukt adenokarsinomlarında östrojen ve progesteron reseptörlerinin varlığını ortaya koydu ve bu tümörün oluşumunda steroid cinsiyet hormonlarının rolü olabileceği kanısına varıldı.

Anahtar Sözcükler: Tavuk, östrojen reseptörü, progesteron reseptörü, immunohistokimya, ovidukt adenokarsinomu

Introduction

Tumors in laying hens are common and various, and are most often found in genital organs (1,2). Genital tract tumors can be observed in laying hens older than 1 year (3-5). Leiomyomas and oviduct adenocarcinomas are the most common primary tumors of the oviduct in laying hens (6-10). Oviduct adenocarcinomas usually arise from the magnum of the oviduct (1,9,11,12), and occasionally originate from the infundibulum and uterus (11,13).

Magnum adenocarcinomas of the oviduct originate as a result of gland epithelium secreting albumin and settle in the propria mucosa (1,11,14). Oviduct adenocarcinomas are quite malignant. Tumorous cells can pass the lamina muscularis of the oviduct and metastasize in the abdominal cavity (11); therefore, the tumor can metastasize via implantation, forming multiple metastatic nodules on serosal surfaces of the abdominal organs (11,13).

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The etiology of genital tract tumors is not known, but a relationship between high egg production and the prevalence of oviduct tumors has been shown (15,16). Furthermore, the etiology of leiomyomas and oviduct adenocarcinomas has been suggested to be related to long-term exposure to the steroid sex hormones estrogen and progesterone (10). The existence of progesterone and estrogen receptors (PRs and ERs, respectively) has been shown immunohistochemically in normal oviducts (17,18). ERs and PRs in oviduct tumors and metastases have been quantified (9), but the expression of these receptors has not been shown immunohistochemically. The aim of this study was to demonstrate the presence of ERs and PRs in oviduct adenocarcinomas and metastases in laying hens so as to gain a better understanding of the possible etiological role of steroid sex hormones in these tumors.

Materials and Methods

We obtained 32 oviduct adenocarcinomas from Babcock B 380 laying hens 16-18 months old during the slaughter of 10,475 chickens at a private chicken slaughterhouse in Afyon, Turkey. Detailed descriptions of the tumors were previously reported (5). Tumor samples were fixed in 10% neutral buffered formalin, processed to wax, and cut at 5 μ m. Upon gross and light microscopic examination, 18 of the 32 tumors (58%) were diagnosed as primary oviduct adenocarcinomas and 14 (42%) as intestinal metastases. Although metastases were seen in the pancreas, spleen, mesenterium, gizzard, ovarium, and intestine, including the duodenum, jejunum, ileum, colon, and cecum, only duodenal metastases were used for immunohistochemical examination. The tissue sections from the tumorous growths in the oviducts and duodenum were stained using a streptavidin-biotin complex (Strep-ABC) technique (19), with commercially available mouse-anti-human ER (Clone 6F11, Biocare Medical, CA, USA) and mouse-anti-human PR (1A6, Biocare Medical) monoclonal antibodies. In short, formalin-fixed and paraffin-embedded tissue sections were dewaxed using xylene for 15 min, rehydrated in ascending series of alcohol, and subjected to antigen retrieval in citrate buffer (pH 6.0) for 20 min in a pressure cooker. After blocking the endogenous peroxidase activity with 3% H₂O₂ (Peroxidazed 1, PX968G, Biocare Medical) for 10 min and rinsing in PBS, protein blocking solution (Background Eraser, BE965G,

Biocare Medical) was applied for 10 min. Primary antibodies (1:100 diluted in PBS) were then applied for 1 h at room temperature. The slides were then rinsed in PBS and biotinylated secondary goat-anti-mouse antibody (Biocare Medical) was applied for 10 min. After 10 min of incubation with streptavidin-peroxidase (Biocare Medical), amino-ethyl carbazole (AEC) was applied for 15 min for color development. The slides were finally counterstained with Mayer's hematoxylin and covered with glycerin gel. Chicken oviduct and canine mammary tumors were used as positive controls. Negative controls were achieved by replacing the primary antibody with PBS. Stained cells and intensity of the staining were evaluated by scoring from 0 to 3+ in a semi-quantitative system, as previously described (19). In brief, the scoring was as follows: 0: unstained; +: faint staining; ++: moderate staining; +++: strong staining. All slides were evaluated in a blind manner by the authors, independently; in cases of disagreement the slides were re-evaluated by consensus. Pearson's chi-square test was used to test the difference between the 2 groups and the level of $P < 0.05$ was considered significant (SPSS 11.5, SPSS Inc., Chicago, IL, USA).

Results

In microscopic examination, ERs and PRs were observed to be positively stained, both in normal and tumorous oviducts. The staining pattern of the tumor cells was nuclear and cytoplasmic, both for ER and PR antibodies (Figures 1 and 2). While lamina epithelialis was the unique stained component in normal oviducts, in addition to this a positive reaction was observed in the tumorous cells in the submucosa of the oviducts and in the metastatic epithelium of the intestines. In some cases normal oviduct epithelium was stained positively for both ERs and PRs, even if the tumor adjacent to the normal oviduct was stained negative. In all, 13 tumors (40%) (7 from oviduct adenocarcinomas and 6 from intestinal metastases) stained positively for ER, while 12 tumors (37%) (4 from oviduct adenocarcinomas and 8 from intestinal metastases) gave a positive reaction to PR antibody (Table 1). In ER staining there were 3 cytoplasmic and 4 nuclear stained tumors in the oviducts (Figures 3 and 4). In ER staining of intestinal metastases, 4 cytoplasmic and nuclear stained tumors, and 2 cytoplasmic stained tumors were observed. In oviduct PR staining 3 cytoplasmic and nuclear stained tumors and 1

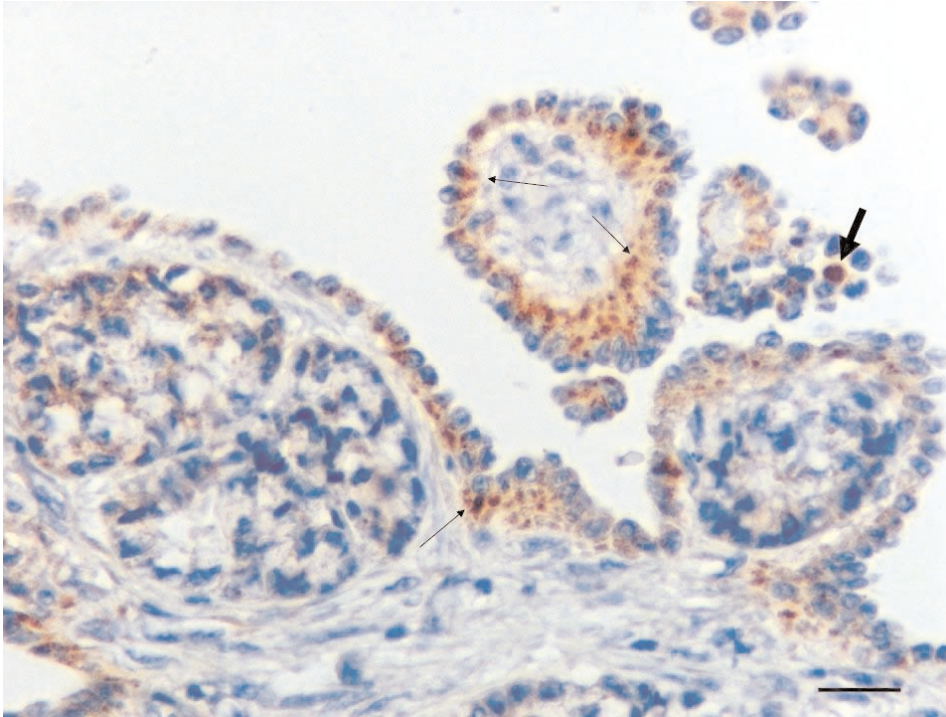


Figure 1. Both intracytoplasmic (thin arrows) and intranuclear (thick arrow) positive reaction in tumorous glands, ER, oviduct. Bar = 30 μ m.

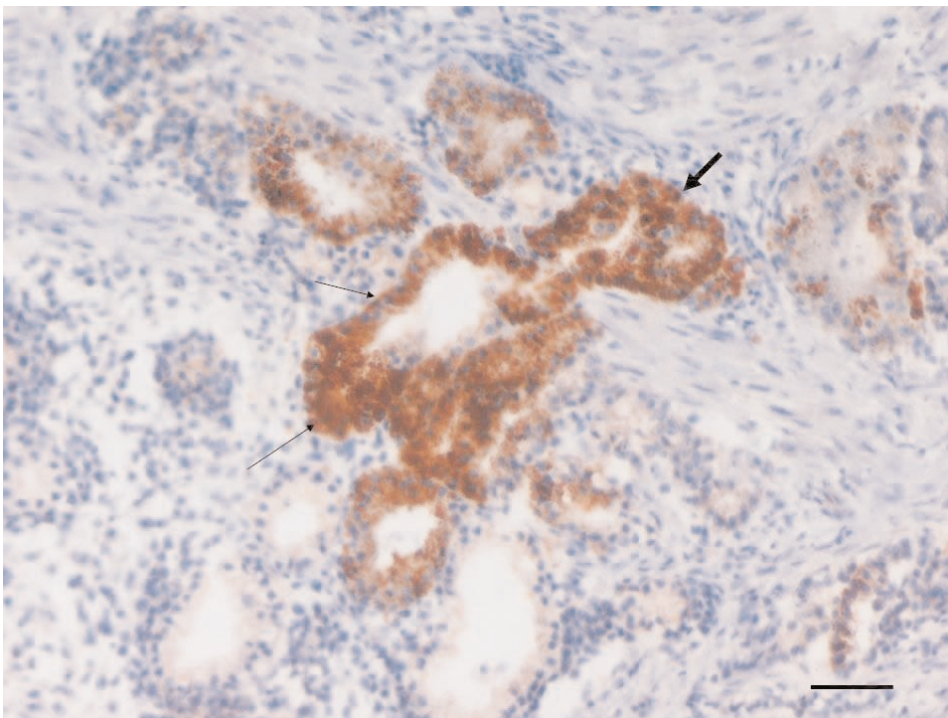


Figure 2. Both intracytoplasmic (thin arrows) and intranuclear (thick arrow) positive reaction in tumorous glands, PR, oviduct. Bar = 60 μ m.

Table 1. Positive and negative oviduct adenocarcinomas and intestinal metastases.

Groups	Number of positive-stained cases			Number of negative-stained cases			Total tumors
	Oviduct adenocarcinoma	Intestinal metastases	P value	Oviduct adenocarcinoma	Intestinal metastases	P value	
ER	7	6	NS	11	8	NS	32
PR	4	8	NS	14	6	NS	32
Total	11	14		25	14		64

NS: Not significant ($P > 0.05$).

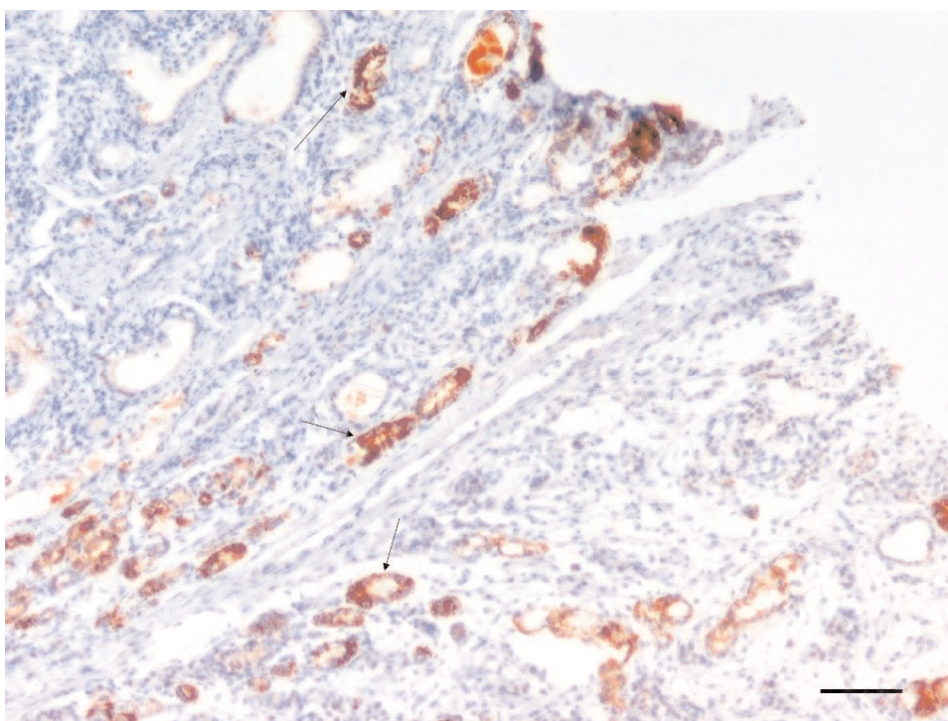


Figure 3. Positive reaction with ER in tumorous glands (arrows), oviduct. Bar = 120 μ m.

cytoplasmic stained tumor were seen (Figure 5). In PR staining of the intestinal metastases, 4 cytoplasmic and 4 nuclear stained tumors were observed (Figure 6) (Table 2).

Tumor cells from both oviduct adenocarcinomas and intestinal metastases stained more intensely than normal oviduct epithelium, and staining of the intestinal metastases was more intense than that of the primary oviduct tumors (Table 3). There was no staining in poorly differentiated and sclerotic tumors with anti-ER and -PR antibodies.

Discussion

It is well known that steroid sex hormones are involved in the differentiation and protein synthesis of epithelium in the glands and lumen in normal oviducts, and these processes are mediated by PRs and ERs (17,18). Ovaries of laying hens ovulate and produce estrogen and progesterone continuously, and the oviduct is a target organ for these hormones. Therefore, there is a significant relationship between a high plasma estrogen concentration, and the estrogen:progesterone hormone ratio and oviduct tumors (8). Better laying hens have long

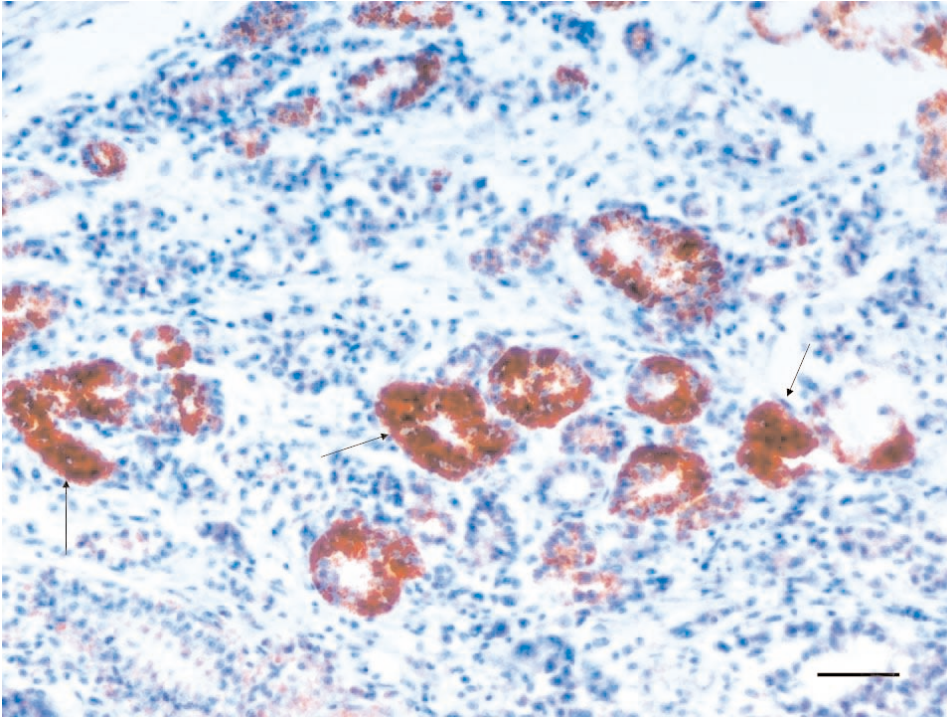


Figure 4. Nuclear staining in tumorous cells (arrows), oviduct, ER. Bar = 60 µm.

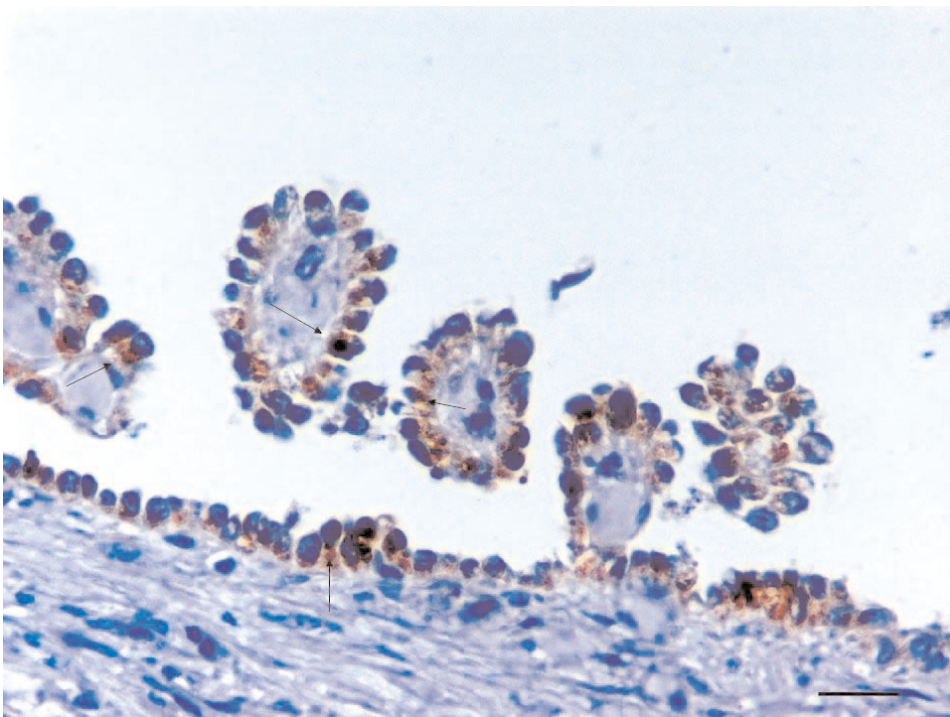


Figure 5. Cytoplasmic staining in tumorous cells (arrows), oviduct, PR. Bar = 30 µm.

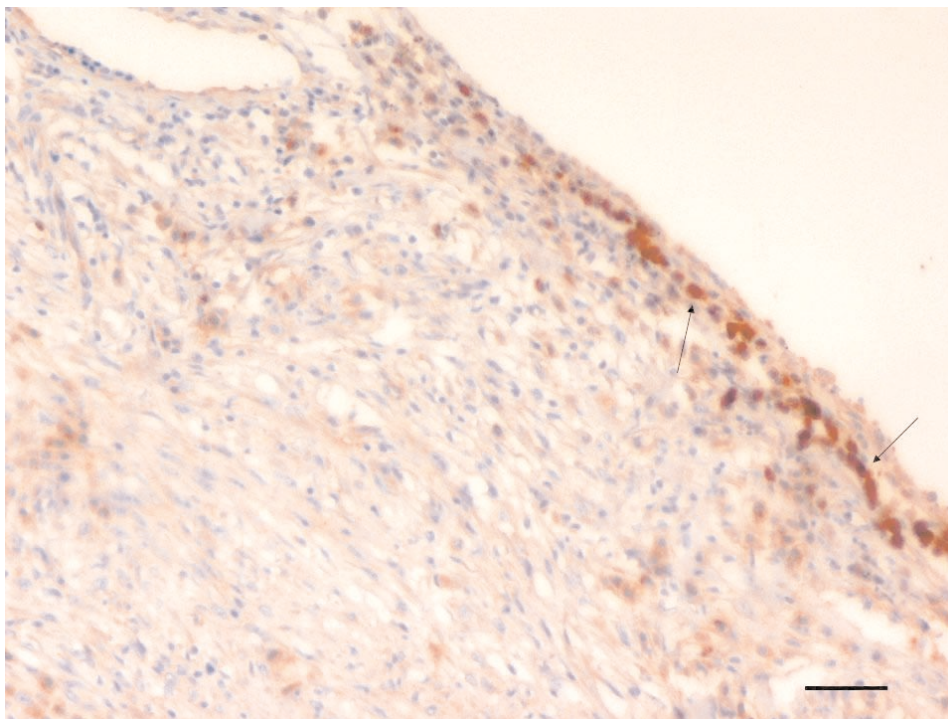


Figure 6. Nuclear staining in intestinal metastases (arrows), PR. Bar = 60 μm.

Table 2. Number of cytoplasmic only, nuclear only, and both cytoplasmic and nuclear staining slides in oviduct adenocarcinomas and intestinal metastases.

Groups	Tumor	Number of cytoplasmic only staining	Number of nuclear only staining	Number of both cytoplasmic and nuclear staining	Total
ER	Oviduct adenocarcinoma	3	4	-	7
	Intestinal metastases	2	-	4	6
PR	Oviduct adenocarcinoma	1	-	3	4
	Intestinal metastases	4	4	-	8

Table 3. Distribution and intensity of receptors in oviduct adenocarcinomas and intestinal metastases.

Groups	Tumor	Normal luminal epithelium	Neoplastic glandular epithelium	Neoplastic glandular epithelium	Normal glandular epithelium
ER	Oviduct adenocarcinoma	+	++		
	Intestinal metastases			+++	+++
PR	Oviduct adenocarcinoma	+	++		
	Intestinal metastases			+++	+++

laying periods and have a high estrogen concentration in plasma. Therefore, it is possible that selection for high egg production will predispose hens to these tumors. Moreover, total ERs and PRs were higher in malignant tumors than in normal magnum (9,12). Selection for high egg production may predispose hens to this tumor and estrogen is considered a weak initiator of carcinogenesis or a growth stimulator for hormonally dependent tumors (8). Total ER and PR receptor content increases from normal magnum tissue to primary malignant tumors and metastatic growths (9). In the present study, ERs and PRs in the metastatic growths tended to stain stronger than those in the primary tumors and normal oviducts. This finding is in agreement with the literature (8,9), which suggests that there is a relationship between the intensity of staining and receptor contents in normal tissue, primary tumors, and metastatic growths.

Chicken PRs are expressed in 2 isoforms, PR-A and -B, and the cellular responsiveness to progesterone depends on the ratio of PR-A to -B expression. In laying hens there is approximately equimolar expression of PR-A and -B in the oviduct; however, in estrogen-withdrawn chicks and aged non-laying hens, PR-A is the dominant isoform (18). Hen oviducts used in this study were from animals with problems related to egg production; therefore, PR 1A6 antibody was used to recognize both PR-A and -B isoforms. A significant proportion of the tumors did not stain with anti-ER or anti-PR antibodies. It was noteworthy that these tumors were usually poorly differentiated and sclerotic.

In tumorous and normal cells, most ERs are located in the cell nucleus, whereas most PRs are located in the cytosol (9). In the present study, both ER and PR staining were cytoplasmic and nuclear in primary tumors and intestinal metastases (Table 2). Only nuclear staining with ERs was observed in the primary tumors, but not in

intestinal metastases. While there was no concurrent cytoplasmic and nuclear staining with ERs in oviduct adenocarcinomas, there was both cytoplasmic and nuclear positive staining with ERs in intestinal metastases. Cytoplasmic staining with PRs was more common in primary tumors than in intestinal metastases; however, the nuclear stained cells were present only in intestinal metastases. No correlation was observed between the location of the staining and the grade of the tumor. We did not observe any staining pattern for ERs and PRs limited to the cytoplasm or the nucleus, whereas Anjum et al. (9) showed that most ERs were located in the nucleus of tumorous cells and most PRs were located in the cytosol. Both ERs and PRs showed nuclear, cytoplasmic, and nuclear-cytoplasmic staining in this study. Only epithelial cells were immunoreactive, while mesenchymal cells did not stain positively for ERs or PRs. Positive staining for ERs and PRs in normal oviduct epithelium adjacent to negatively stained tumors could be due to the expression of the receptors in normal epithelium, but not in the tumor cells. Quantitative analyses revealed that ER and PR content in primary malignant tumors and metastatic growths is higher than in normal oviducts (9). In the present study, ER and PR staining in tumorous epithelium was more intense than that in normal oviduct epithelium. These findings are compatible with the literature (9). In conclusion, the presence of ERs and PRs in oviduct adenocarcinomas and intestinal metastases is consistent with hormone-dependent tumors, and these receptors may have plausible roles in the formation and metastases of these tumors.

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