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ORIGINAL ARTICLE

Spontaneous ovarian adenocarcinoma in the domestic turkey breeder hen (*Meleagris gallopavo*): Effects of photoperiod and melatonin

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Abstract

OBJECTIVES: The effect of photoperiod or melatonin treatments on ovarian adenocarcinoma in turkey breeder hens (*Meleagris gallopavo*) was investigated to evaluate the usefulness of this animal as a model for studying ovarian cancer.
METHODS: In Experiment 1, photoperiod effects were tested by exposing turkeys with ovarian tumors to 8 wks of short days (8:16LD) followed by a 12 wk period of long days (16:8LD). In Experiment 2, exogenous melatonin was administered to turkeys during long day-induced development of ovarian tumors. In both experiments, the stage of tumor growth was scored weekly on a scale of 0 to 4.
RESULTS: It was clear that exposure to short days produced complete regression of tumors, with a mean time to score 0 of 4.4 wks. Following re-exposure to a long photoperiod, all of the same birds showed re-growth of the ovarian tumor with a mean time to first palpable detection of 5.4 wks. When melatonin was administered daily during the long photoperiod (Experiment 2), there was a significant delay in the re-growth of tumors.
CONCLUSION: It was clear from this study that the growth of solid ovarian tumors in the turkey breeder hen was promoted by long photoperiods and ceased, to the point of remission, on short photoperiods. Thus, ovarian adenocarcinoma in turkeys can be completely manipulated by photoperiod. In addition, treatment with melatonin attenuates tumor growth in the turkey hen. The results suggest that the domestic turkey hen is a useful *in vivo* model for studying spontaneous ovarian adenocarcinoma.

Abbreviations:
LD: Light Dark,
wk: week

Introduction

Ovarian cancer continues to be one of the leading causes of death among the female population and the lack of a valid animal model has impeded progress towards prevention and treatment of this deadly disease. Ovarian carcinomas have been identified in avian species. The chicken hen has been suggested as a model for *in vivo* studies of spontaneous ovarian adenocarcinoma. In a detailed report of avian cancers, it was found that laying chicken hens have a high rate of tumors and most of these tumors arise from the ovary. In addition, the incidence of spontaneous ovarian tumors is unusual in chickens less than 2 years of age with adenocarcinoma being the most common type of neoplasia [1]. This detailed report established a foundation for the study of ovarian cancer in the chicken. Therefore, most of the associated research has focused on establishing the chicken as the avian model system in studying the pathophysiology of ovarian cancer. One such report has focused on the expression of antigens frequently expressed in human ovarian cancer. Many of the antibodies for these molecular markers were found to be cross-reactive in the laying hen, strengthening the utility of the hen as a valid animal model for ovarian carcinoma [2]. In addition, a pilot study of cancer chemoprevention using medroxyprogesterone acetate (Depo-Provera) has proven successful in utilizing the chicken for studying the effectiveness of this putative chemopreventive agent [3].

As early as 1909, the histologic and morphologic characteristics of avian-derived tumors have been investigated [4]. Pertinent to the present study, ovarian adenocarcinomas are common tumors in adult chickens that seem to arise from the ovarian surface epithelium [1]. There are few reports of the existence of ovarian adenocarcinoma in turkeys but Walser and Paul [5] characterized these tumors as localized, multilobular masses associated with the ovary and microscopically described them to have solid, acinar, and scirrhous characteristics. The gross and histological features detailed in this early report are consistent with those described in the laying chicken hen. Until now the domestic turkey hen has been neglected as a useful *in vivo* ovarian cancer model and no research has focused on the effects of photoperiod on the progression of ovarian adenocarcinoma in any avian species. It is known that turkey ovarian development and subsequent egg production is regulated, and may be controlled, by daylength. Long daylengths promote and short daylengths diminish ovarian development through neuroendocrine mechanisms involving the hypothalamo-hypophyseal-ovarian axis. Consequently, we hypothesized that changes in photoperiod may be used to manipulate ovarian tumor growth in the turkey breeder hen.

Experimental and clinical reports in mammals suggest that there is a link between cancer development and pineal function [6, 7, 8, 9]. Most of these reports suggest a clear inhibition of cellular proliferation by pineal melatonin and therefore a reduction in the progression of many neoplasms. In addition, melatonin is known to reduce DNA damage by acting as a potent free radical scavenger and antioxidant [10]. These reports and many others suggest that a decrease in melatonin might enhance tumor development. In fact, pinealectomy enhanced the incidence of mammary adenocarcinoma in the rat induced by the chemical carcinogen 9,10-benz-anthracene (DMBA) [11]. Furthermore, melatonin supplementation abolished the effect of pinealectomy on the growth of induced melanoma in the hamster [12]. Also, melatonin synergized with IL-2 in controlling tumor growth in mice [13]. In addition, 90 percent of mice housed in long days and treated with the chemical carcinogen DMBA developed squamous cell carcinoma as compared to the absence of tumors in DMBA-treated mice housed in short days. The implantation of melatonin capsules significantly counteracted the effects of long days in these mice [14]. Therefore, from the existing literature in mammals, it is apparent that melatonin can exert a functionally significant effect on the growth of some neoplasms. No similar reports of the anticarcinogenic effects of melatonin exist for avian species. The current study focused on the evaluation of photoperiod and melatonin supplementations as effectors of tumor growth in domestic turkey hens with ovarian cancer.

Materials and Methods

Husbandry

All turkey breeder hens were maintained in floor pens in the same light-controlled research facility. All management and husbandry practices were identical between treatments. All birds were at least 2 years of age at the start of the study. The building was not temperature controlled but was insulated and the rooms mechanically ventilated. Feed and fresh water were provided *ad libitum* throughout the study. A pelleted breeder feed was provided throughout the study that was calculated to contain 16% crude protein, 3.5% calcium, and 2,970 kcal ME/kg of feed. All photoperiods were provided using incandescent light with a mean intensity level of 54 lx at turkey head height.

Experiment 1

This experiment was conducted to determine the effect of photoperiod on the maintenance and development of ovarian tumors in turkey breeder hens. A flock of 2 year old turkey breeder hens maintained in long photoperiods (16:8LD) were rectally palpated for the presence of ovarian tumors and 15 birds had palpable tumors. Each of these 15 hens was given a tumor score, with score 0 representing nonpalpable tumor and score 4 being the greatest tumor size. The tumor score was obtained by rectal insertion of a digit and rating (0–4) both the distance of insertion until tumor was palpable and the size of the tumor. A score 1 was a barely detectable, hard, irregular object with easily discernable

margins and required deep penetration. A score 4 was a massive, hard, irregular mass with no clear margins and required very little penetration. Although rather subjective, this technique proved to be a rapid, non-invasive means of accurately identifying the presence and magnitude of ovarian tumors. All 15 birds with palpable tumors were placed in one floor pen and given an 8 wk period of short photoperiod (8:16LD). Immediately following this 8 wk period, all 15 birds were re-exposed to a long photoperiod (16:8LD) for 12 wks. In a typical turkey hen this photoperiod protocol would result in a complete cessation of lay on 8:16LD within 2–3 wks and full ovarian development and egg production within 3–4 wks of exposure to the 16:8LD photoperiods. Tumor scores were determined weekly by palpation throughout the study. The mean time to regression of tumor to a score 0 was determined during the short day exposure and the mean time to regeneration of the tumor to a score 1 was determined during the long day exposure. Any deceased birds were immediately necropsied and tissue obtained for histological analysis. Tissues were fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin, and examined microscopically.

Experiment 2

This experiment was conducted to determine the effect of melatonin on the long day induced regeneration of palpable tumors in turkey breeder hens. All hens selected for this study were different from those utilized in Experiment 1. Twenty-two hens with palpable abdominal tumors were scored and exposed to 8 wks of short photoperiods. The weekly tumor score was determined during this time to ensure that all hens were at score 0 prior to the start of treatments. Following the 8 wk short photoperiod, the hens were selected for two treatment groups (N = 11 birds/treatment) and placed in one of two floor pens maintained under identical husbandry practices. Birds were distributed to each treatment group based on the previous maximum tumor growth scores in a manner that would ensure an even distribution of high responders (score 4) and low responders (score 1). All birds were exposed to 15 wks of long photoperiod (16:8LD, lights on: 0100). At the start of exposure to long photoperiod (week 1), a daily 1 ml intramuscular injection of 50μg/ml melatonin or diluent was administered to each hen (1500hrs) and continued throughout the remainder of the study. Doses of melatonin were prepared weekly by dissolving the appropriate amount in 0.5 ml of 95% ethanol and diluting to 500 ml with distilled water. All control birds received diluent (water\ethanol) injections instead of melatonin. The melatonin dose (50.0 μg/ml) used in the present experiment has been reported to produce significant immunoenhancement in Japanese quail [15]. Weekly tumor scores (0–4) were obtained for each hen in each treatment group throughout the study. Data for the average weekly tumor score for each treatment group were plotted.

Statistical Analysis

Data for Experiment 2 was analyzed by regression analysis using the General Linear Model (GLM) procedure of the SAS Institute (17). The linear model $(Y=\exists_1 \curvearrowright time+\exists_2 \curvearrowright time \curvearrowright X_{treatment})$ was constrained to go through the origin and involves only 2 parameters, time and treatment. Thus, the linear regression analysis compares two slopes corresponding to the two treatments and a zero intercept, so that the mean score at week 0 is modeled as zero. The coefficient of determination (R^2) was 0.98 so that the linear model explains almost all of the variation in the 11-bird averages (though explained variation may be less for individual birds).

Results

Ovarian Adenocarcinoma: Gross and Microscopic Morphology

The microanatomy of ovarian adenocarcinoma in the turkey breeder hen is shown in Figure 1. This tissue was taken from a deceased turkey in Experiment 1 with a tumor growth score of 4 and is typical of necropsied, score 4 tumors. The tumor is composed of a single layer of cuboidal cells lining a slitlike space (bottom). These acinar structures produce a cribriform pattern within the tissue that is consistant with existing literature. The upper left corner of the plate shows a small follicle with nearby neoplasia (top). The cellular source of ovarian cancer is generally regarded to be the ovarian surface epithelium in mammals and birds. Whether this involves one or both of the follicular epithelium or cortical epithelium in birds is not known. The follicular epithelium seems a particularly likely candidate because of the extremely rapid growth rate of avian ovarian follicles during development and the high rate of ovulation. In domestic birds ovulation can occur nearly daily and follicular development and ovulation are hormone dependent. From the present study we cannot determine the exact cellular source of ovarian cancer in turkeys. However, in Figure 1 it appears that the follicular surface is involved.

Figure 2 (right side) illustrates a typical gross morphology of the maximally developed tumor (score 4) obtained from the same bird as in Figure 1 as compared to a normal mature ovary obtained for comparison. The tumor was solid and had a cauliflower-like appearance with irregular masses bulging from the central tumor, which had obliterated most if not all of the normal ovarian surface structures. In fact, score 4 tumors occupy the majority of space in the abdominal cavity. Dark cystic structures are occasionally seen on this tumor and they contain clear amber colored fluid. There were no apparent, macroscopic metastases of the tumor in the thoracic or abdominal areas. The tumor was attached to, and over-grown from, the ovary. This bird had significant ascites with a large volume of amber colored fluid throughout the abdominal cavity.

Figure 3 illustrates a typical gross morphology of undeveloped ovaries from cancerous hens after forced regression of the mature ovaries to the juvenile state

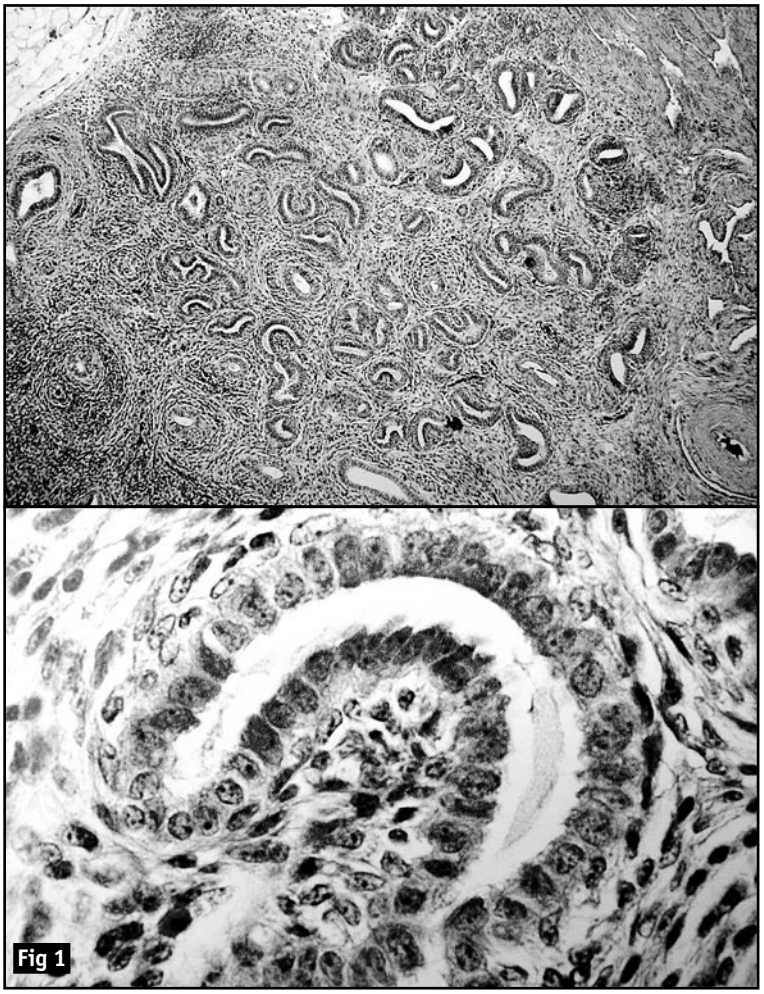

Figure 1. Microscopic anatomy of ovarian adenocarcinoma in a Large White turkey breeder hen. This hen had palpable signs of maximally progressed ovarian cancer with a score 4. Following necropsy, ovarian tissue fixed in 10% formalin was sectioned and stained with hematoxylin and eosin. Typical of avian ovarian adenocarcinoma, the tumor is composed of single layers of cuboidal cells lining slitlike spaces. Magnification: x 200 (top) and x 500 (bottom).

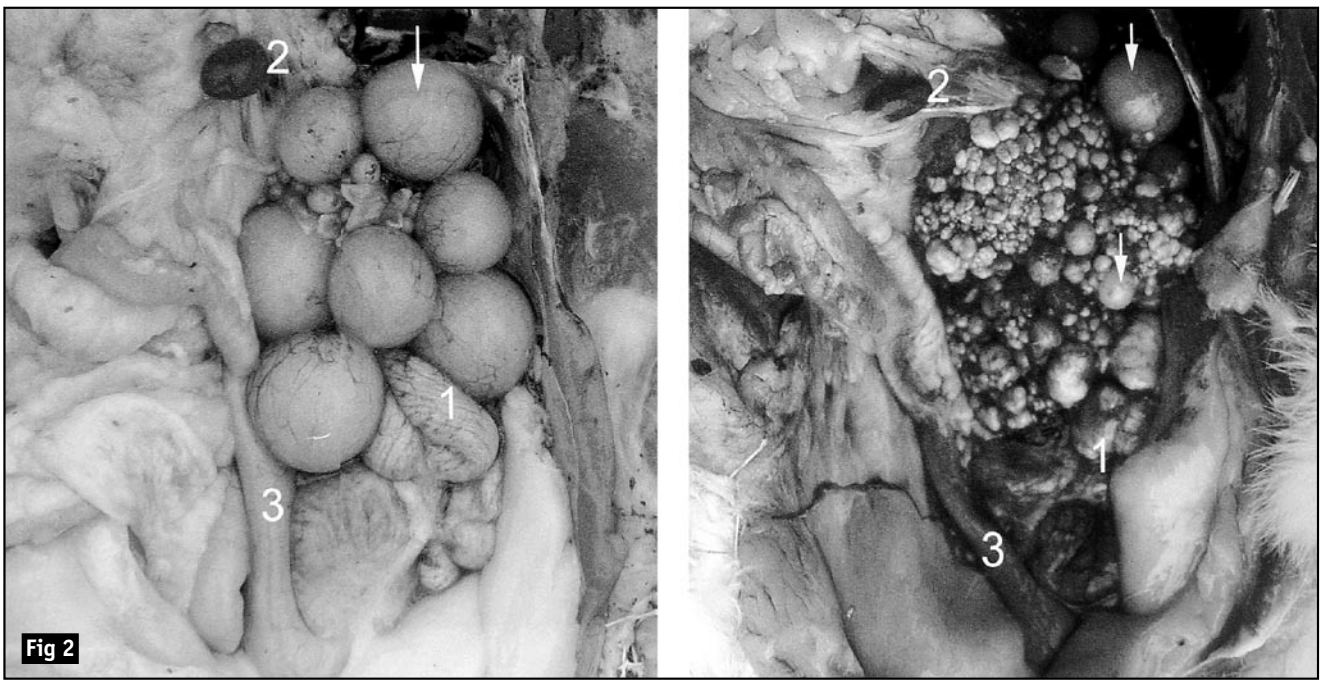

Figure 2. Macroscopic anatomy of a mature ovary showing characteristics of ovarian adenocarcinoma (right) as compared to a normal mature ovary (left). Both birds had the same exposure to long days (16:8LD), which allows for normal maturation of ovarian tissue. Note the large solid tumors and displacement of normal follicles in the diseased ovary as compared to the normal tissue. Arrows indicate ova. 1 = oviduct; 2 = spleen; 3 = large intestine.

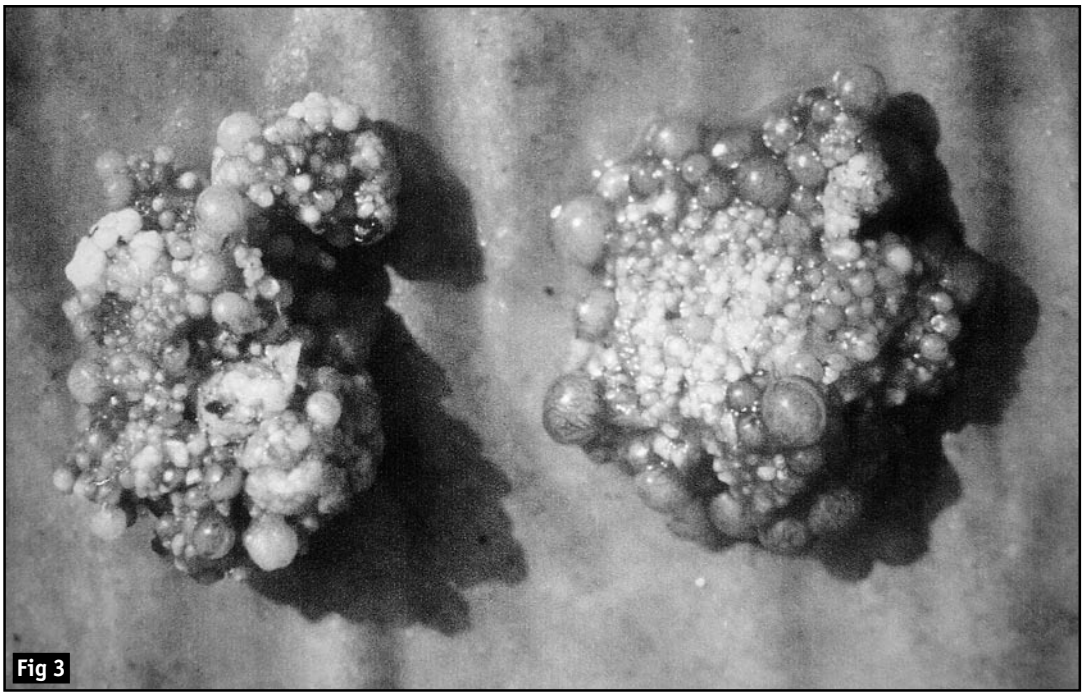

Figure 3. Undeveloped ovaries from turkeys with previously palpable ovarian tumors. Both hens were forced to regress their ovaries (and tumor) to the juvenile state by exposure to 8:16LD photoperiods for 8 wks. Note the white nodules that appear to be remnants of the regressed tumor.

Table 1. Regression of palpable ovarian cancer to score 0 during exposure to 8 weeks of short photoperiod (8:16LD) and regeneration of ovarian cancer to first palpable detection (score 1) during exposure to 12 wk of long photoperiod (16:8LD). *=deceased hen

Bird	*Starting Score*	*Weeks to Regress*	*Weeks to Regenerate*	*End Score (12wk)*
A	3	5	*	*
B	4	6	7	4
C	2	3	4	2
D	4	5	9	3
E	3	3	8	4
F	2	3	5	3
G	3	8	*	*
H	3	3	2	3
I	4	3	7	4
J	2	2	*	*
K	3	5	4	3
L	4	7	5	4
M	3	6	2	3
N	2	4	6	2
O	3	3	*	*
		$\bar{x}$ ± SEM 4.4±1.8	5.4±2.3	

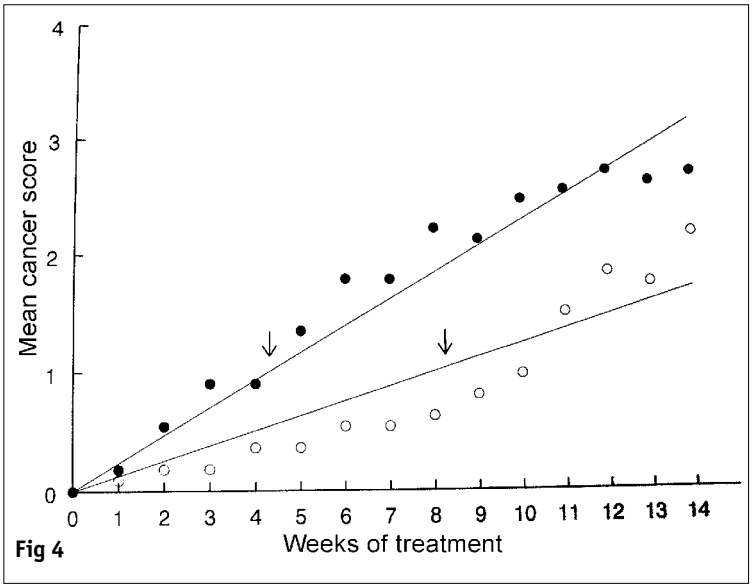

Figure 4. The effect of melatonin on the long day-induced regeneration of turkey ovarian adenocarcinoma. A daily 1 ml intramuscular injection of 50 μg/ml melatonin or diluent was given to each bird. Each data point represents the mean weekly tumor score (N = 11 birds/treatment): melatonin (open circles), diluent (solid circles). Lines are best-fit linear regressions of the mean values indicated by the symbols. The photoperiod was 16:8LD throughout the 15-week experiment. Arrows represent the mean time to first palpable tumor. The regression coefficient for the controls (r=0.296) was significantly different (P > T=0.0001) from that of the melatonin treated group (r= 0.127).

by exposure to short photoperiods for 8 wks. Neither ovary contained mature follicles, typical of a hen forced out of production by short daylengths. However, both still had what appears to be visible tumor presence though clearly in remission. This was seen as nodular, very firm, white growths that resemble atretic follicles. However, these follicles are less symmetric than atretic follicles and may be partially buried within the ovarian stroma and present on the surface of follicles.

Experiment 1

Table 1 shows the weeks required for regression of palpable ovarian cancer in each hen to a score 0 during exposure to 8 wks of short photoperiod (8:16LD). The starting score represents the tumor score of each hen while on long photoperiods and just prior to exposure to short photoperiod. The weeks to score 0 represent the time required for the starting tumor score to become nonpalpable. In every hen, the tumor score dropped dramatically from the starting score with a mean time (± S.E.M.) of regression to score 0 for all hens of 4.4 ± 1.8 wks (range 2 to 8 wks). From these data, it is clear that complete regression of palpable tumors was observed in all birds by the end of exposure to short photoperiod. Table 1 also shows data for the time to regeneration of first palpable ovarian cancer (score 1) in these same hens, during re-exposure to long photoperiod (16:8LD). All birds were at a starting tumor score of 0 with no palpable tumors. The regeneration of palpable tumor was seen in all birds with a mean time of regeneration of 5.4 ± 2.3 wks (range 2 to 9 wks). Each asterisk represents a deceased bird and it should be noted that all deceased birds died immediately after the transition from short to long photoperiod. Also in Table 1, palpable tumors in all hens continued to regenerate throughout the 12 wk period as indicated by the scores at the end of 12 wks of 16:8LD photoperiods. Note that 8/11 hens had regained a tumor score identical to that at the start of the test. The 16:8LD photoperiods induced egg production in these hens, however all birds ceased lay by the end of the 12 wk period.

Experiment 2

Figure 4 illustrates the effect of melatonin on the long day-induced regeneration of ovarian adenocarcinoma in turkey hens. It was clear that melatonin treatment significantly slowed the regeneration of tumors in hens placed in a long photoperiod. However, melatonin treatment did not prevent the eventual reoccurrence of tumors and all hens eventually had tumors by the 15 wk time point. The mean time to regeneration of first detectable (palpable) tumors was 4.5 and 8.5 wks for the controls and melatonin treated hens, respectively.

Discussion

The current study confirms the existence of ovarian adenocarcinoma in the turkey, which is similar to chicken ovarian cancer at least in terms of gross and microscopic morphology [1]. Interestingly, this tumor could be regressed and placed in complete remission by exposure of the hen to short daylengths. Re-exposure to long daylengths caused regrowth of the tumor to full size. Thus, ovarian adenocarcinoma in turkeys can be completely manipulated by photoperiod. In addition, the regeneration of these tumors was slowed by treatment with the pineal hormone melatonin. This is not to say that these tumors are photoperiod dependent, but rather that they can be regulated by photoperiod. This most likely occurs via neuroendocrine mechanisms that ultimately affect pituitary gonadotrophins and ovarian steroids and would be consistent with these tumors being hormone (estrogen?) dependent.

The effect of long photoperiod on tumor growth in the present study is consistent with reports in mammals. Mice housed in a short photoperiod (6:18LD) as compared to a longer photoperiod (12:12LD) develop smaller tumors when injected with a colon adenocarcinoma cell line [18]. It was also found that in the adult female deer mouse (*Peromyscus maniculatis*), 90 percent of mice housed in long days and treated with the chemical carcinogen DMBA developed squamous cell carcinoma as compared to the absence of tumors in DMBA-treated mice housed in short days. Since melatonin treatments could reverse the effects of DMBA in the long day mice and the fact that this photoperiod-induced carcinogenesis was found to be independent of gonadal steroids, it was suggested that the effects of photoperiod on tumor growth were mediated by melatonin alterations of immune responses [14]. Melatonin effects on immune responses have been well documented in mammals [19].

In birds, the effect of long photoperiod on tumor growth has not been investigated. The current report is the first report of long day stimulation of ovarian tumorigenesis in a bird. However, there are reports of photoperiod effects on immune function in birds. In the Japanese quail, it was observed that constant light caused significant immunosuppression as compared to birds exposed to LD cycles and melatonin treatments abolished this constant-light immunosuppression [15]. In addition, photostimulation of turkeys with a long photoperiod to regain ovary function significantly inhibits cellular and humoral immune responses as compared to turkeys kept on short photoperiods [20]. Therefore, as in the mammal, a decrease in melatonin and subsequent immunosuppression could contribute to the observed regeneration of ovarian tumors in long photoperiod. However, many neoplasms are dependent on steroids and it would certainly be reasonable to assume that gonadal steroids are also involved in the photoperiod effects on tumor growth observed in the present study. However, it has been reported that there is no relationship between gonadal steroids

and ovarian adenocarcinoma in chickens [1]. Further experiments are needed to determine the exact mechanisms of regression and regeneration of ovarian cancer in the turkey model. It is notable that the duration of light per se (8 hr extended to 16 hr) is not responsible for development of the ovarian adenocarcinoma. An intermittant photoperiod of 2L:12D:2L:8D given daily (only 4hr light per day total) will also induce ovarian adenocarcinomas (Siopes, unpublished). This intermittant photoperiod induces full ovarian development and egg laying in control hens.

The apparent lack of macroscopic metastases of the ovarian tumor of turkey hens was somewhat unexpected as this occurs in chickens [3]. We have also observed a lack of macroscopic metastases in other turkey hens necropsied after mortality from spontaneous ovarian tumors (unpublished). So any absence of apparent metastasis is not likely due to experimental procedures such as acute, induced tumors or lack of chronic exposure to the tumor. The ovarian adenocarcinoma in the turkey appears to be a localized tumor.

The photoperiodic regulation of reproduction in turkeys provides for certain advantages that would make the turkey an appropriate *in vivo* model. The fact that complete regression and regeneration of the ovarian tumor can be produced by alteration of photoperiod allows for repeated measures analysis within individual hens. This greatly reduces the total number of birds needed to evaluate the chemotherapeutic potential of novel agents. It also greatly improves the evaluation of preventive and therapeutic treatments to the tumor by control of their application to regulated stages of the tumor ranging from remission to full development. The simple, fast, and noninvasive diagnostic technique for this tumor is another plus for the turkey model, and supplemental diagnostic techniques would only improve the model.

It was not surprising that melatonin treatment could slow the growth of ovarian adenocarcinoma by a mean time of 4 wks as compared to control hens (Figure 4). Melatonin is widely recognized to exert an inhibitory action both on carcinogenesis and tumor growth in a variety of experimental situations [21]. Also, the result in the present study is consistent with several *in vitro* reports of the oncostatic properties of melatonin. Melatonin has been reported to reduce cell proliferation in many cell types [22, 23, 24]. Most appropriately, melatonin has been shown to reduce cell proliferation of a human ovarian adenocarcinoma cell line [25]. These *in vitro* results certainly provide evidence for a direct oncostatic effect of melatonin on tumor cells. However, there is also evidence of an indirect effect on tumor growth by stimulation of immune responses. In recent years, a close link between melatonin and immune function has been established in mammals and birds. In birds, melatonin has been shown to counteract the immunosuppressive effects of constant light or pinealectomy in the Japanese quail [15, 26]. Furthermore, melatonin administration to turkey poults significantly accelerated the development of cellular and humoral immune responses [27]. Therefore, melatonin given to turkeys during the long photoperiod may act to stimulate immune functions prior to tumor regeneration, thereby slowing the progression of tumor development in these birds. Although the current study does not determine the exact mechanism of action by melatonin, it certainly lends support to the therapeutic potential of melatonin in cancer prevention and treatment. In fact, it has been shown that concomitant treatment of melatonin and IL-2 induces tumor regression and increases overall survivability in patients with advanced solid tumors [13].

From the results in the present study, it was clear that the regression and regeneration of ovarian adenocarcinoma in the turkey breeder hen could be controlled by photoperiod. In addition, melatonin treatments significantly slowed the regeneration of tumors in long photoperiods. Taken together, the results from the present study support the turkey breeder hen as a useful animal model for studying ovarian adenocarcinoma and are the first account of photoperiod and melatonin regulation of tumor growth in an avian system.

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