Research Article

Cancer Prevention Research

Evidence of a Chemopreventive Effect of Progestin Unrelated to Ovulation on Reproductive Tract Cancers in the Egg-laying Hen

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Abstract

Epidemiologic, laboratory, and animal evidence suggests that progestins and vitamin D may be potent ovarian cancer preventives. Our objectives were to evaluate progestins as reproductive tract cancer chemopreventives in the chicken, determine whether restricted ovulation affected the incidence of reproductive tract tumors, and assess whether vitamin D would confer cancer protection either alone or in addition to progestin. A total of 2,400 two-year-old Single Comb White Leghorns were randomized into six groups (400 each) with hormonal and dietary manipulation for 2 years as follows: (i) no intervention, regular feed/caloric intake, (ii) control, (iii) vitamin D, (iv) the progestin levonorgestrel, (v) vitamin D plus levonorgestrel, and (vi) the progestin Provera (medroxyprogesterone acetate). Groups 2 to 6 were caloric restricted to inhibit ovulation. Our results indicated that caloric restriction decreased egg production by more than 60%, and was associated with a greater than 70% decrease in reproductive tract cancers. Ovulatory events did not differ among the caloric-restricted groups (groups 2–6), except for the group receiving levonorgestrel, which had fewer ovulatory events than controls ($P = 0.046$). After correcting for egg production, birds receiving progestins had significantly fewer reproductive tract cancers [OR, 0.61; confidence interval (CI), 0.39–0.95; $P = 0.03$], with similar proportionate reductions in tumors arising in either the ovary or oviduct. Vitamin D did not significantly affect cancer incidence overall, or add to the cancer preventive effect of progestins. This study suggests a protective effect of progestins against ovarian and oviductal cancers. These data support the concept that progestins provide a chemopreventive effect unrelated to ovulation. *Cancer Prev Res; 6(12); 1283–92. ©2013 AACR.*

Introduction

Epithelial ovarian cancer is a highly lethal malignancy. It is the fourth leading cause of cancer-related deaths among women in the United States and causes more than 100,000 deaths annually in women worldwide (1). Despite intensive research efforts during the past decade directed toward improved detection and treatment of ovarian cancer, the long-term survival of women with ovarian cancer has only improved modestly. Progress in the fight against ovarian cancer has been hampered by a number of factors, including late diagnosis, the molecular heterogeneity of tumors, and the absence of highly curative chemotherapy. Furthermore, the lack of a valid animal model for ovarian cancer has markedly slowed the progress of drug development, not only for new therapies for primary treatment but also notably for agents to prevent the disease, as evaluation of preventive agents often require lengthy clinical trials.

The development of effective chemopreventive agents for ovarian cancer holds great potential for decreasing ovarian cancer mortality. Routine use of the combination estrogen–progestin oral contraceptive pill (OCP) confers a remarkable 30% to 50% reduction in the risk of developing subsequent epithelial ovarian cancer, suggesting that an effective cancer-preventive approach using hormones is possible (2–4). Previously, there has been widespread belief that the protective effect of oral contraceptive use is due to the ability of these agents to inhibit ovulation, thereby decreasing the amount of genetic damage incurred by the ovarian surface epithelium or nearby fallopian tube in OCP users (5). This hypothesis suggests that (i) the benefit from the ovarian cancer protective of OCP use would be confined

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only to young women who are ovulating; (ii) no improvement could be made to OCP formulations to further reduce the risk of ovarian cancer because all OCPs are highly effective at inhibiting ovulation; and (iii) postmenopausal women, who by definition do not ovulate and who represent the group of women at greatest risk of ovarian cancer, could derive no protective effect from postmenopausal administration of the drugs.

Our research findings have led to an alternative hypothesis that the protective effect conferred by OCPs against ovarian cancer may be due to potent and direct biologic effects of OCP progestins on the ovarian epithelium. We have discovered that progestins markedly induce programmed cell death (apoptosis) and differentially regulate expression TGF-β in the ovarian epithelium (6, 7). These two molecular events have been strongly implicated in cancer prevention *in vivo*, and are believed to underlie the protective effects of other well-known chemopreventive agents such as the retinoids and tamoxifen (8). The finding that progestins activate these molecular pathways in the ovarian epithelium provides the rationale for further investigation of progestins as chemopreventive agents for ovarian cancer, and raises the possibility that other agents that similarly activate cancer-preventive pathways in ovarian epithelial cells also may be attractive ovarian cancer preventives. Among these other agents, there is growing evidence in support of vitamin D, including (i) data that sunlight exposure lowers ovarian cancer mortality (9), (ii) case–control evidence that dietary vitamin D lowers ovarian cancer risk (10), and (iii) evidence that vitamin D induces apoptosis in ovarian epithelial cells (11, 12). In this study, we used the chicken ovarian cancer model to prospectively test the preventive effect of two different progestins on reproductive tract cancers and to explore whether cancer-preventive effects might be enhanced by vitamin D.

Materials and Methods

Animal housing/husbandry

Single Comb White Leghorn chickens (*Gallus domesticus*) at 770 days of age were selected from the 32nd North Carolina Layer Performance and Management Test located at the Piedmont Research Facility in North Carolina, and comprised nine commercial lines of laying hens (13). A wing band was placed onto each bird for identification. The birds were fed a conventional layer diet (Table 1) and allowed to adapt to their new environment for 2 weeks before the initiation of the study. The birds were confirmed to be free of vertically transmitted diseases (*Mycoplasma gallisepticum*, *Infectious Bursal Disease*) and had a prevalence of cancer of less than 2% as determined by a baseline necropsy of 400 additional birds. The study was performed under Institutional Animal Care and Use Committee (IACUC) oversight of the NC State University IACUC committee.

Diet/treatments

Of note, 2,400 birds were housed in 768 cages. A single feed trough covered four cages so that individual feeding treatments were applied among 192 "replicate" four-cage feed troughs throughout the trideck battery style cage system. The birds were housed at an average density of 994 cm^2 per bird.

Each replicate feed trough of four cages was randomly assigned to one of the following six treatment groups containing 400 hens each: (i) regular feed (conventional layer diet); (ii) feed restricted control (body maintenance diet—caloric restriction to maintain hen weight, but below threshold required for consistent ovulation); (iii) feed restricted with vitamin D_3 (cholecalciferol)–enriched diet; (iv) feed restricted plus the progestin levonorgestrel; (v) feed restricted with vitamin D_3–enriched diet, plus levonorgestrel; and (vi) feed restriction plus the progestin Provera. All commercial strains were equally represented in each treatment group. However, these strains were originally selected for common egg-production parameters and were expected to be similar in their ovarian cancer risks. To assign 400 birds to each treatment group, birds assigned to each treatment group were housed in 112 cages containing three birds and 16 cages containing four birds. Hens in groups 2 to 6 were placed on a body-maintenance diet to reduce ovary and oviduct weights, thereby inducing a state of relative anovulation in accordance with the findings of Dunn and colleagues (14). This dietary manipulation was designed to limit the potential confounding impact of ovulation on ovarian cancer outcome and to allow for a more direct assessment of the impact of the chemopreventive interventions on ovarian cancer risk. A standard diet for birds at this age and stage of egg production contains 13% crude protein and 2,978 kcal/kg of metabolizable energy. The body-maintenance diet for this study provided each hen with adequate amounts of protein, amino acids, and minerals, but only 55% of the calories of a standard diet (356 kcal/bird/d; Table 1). The body-maintenance diet was sufficient to maintain the body weight of the birds, but ovulation was markedly reduced. Feed was provided *ad libitum* via a mechanical trough feeder such that all birds received acceptable nutrient intake. The feed was weighed back every 28 days to determine the feed intake and thereby adjust the drug levels in the feed to ensure the proper daily dose.

Chemopreventives were added into the feed on site using an industrial grade mixer according to mixing protocol parameters published in Feed Manufacturing Technology IV, Kansas State University, Department of Grain Science and Industries. Groups 1, 2, 4, and 6 received the standard daily allowance of 30 IU of vitamin D_3 per day (15), whereas birds in groups 3 and 5 received twice the daily allowance of vitamin D_3 of 60 IU. Progestins were administered in amounts comparable with that in oral contraceptives or hormone replacement therapy, adjusted for the size and metabolic rate of the bird to approximate the human-equivalent dose (chickens metabolize 356 kcal/d, vs. 1,800 kcal for a 70-kg woman). Hens in groups 4 and 5 received Norgestrel at a dose of 0.0125 mg/bird/d (comparable with 0.25 mg/d human dose). Hens in group 6 received Provera at a dose of 0.25 mg/bird/d (comparable

Table 1. Dietary formulations and analysis for control and restricted diets beginning at 770 days of age

	Diets		
Ingredients (%)	Conventional (regular feed)	Body maintenance (feed restricted)	Body maintenance with D_3 (feed restricted)
Corn	69.45	31.55	31.50
Wheat midds	10.05	7.00	7.00
Corn gluten meal	3.85		
Soybean hulls		57.00	57.00
Soybean meal (48%)	4.65		
Fat	2.10	0.50	0.50
Ground limestone	7.45	1.50	1.50
Bicarbonate of soda	0.60		
Salt	0.25	0.30	0.30
Trace mineral mix	0.05	0.05	0.05
Vitamin premix	0.05	0.05	0.05
T-premix	0.05		
Vitamin D_3 (2,970 IU/lb)			0.05
Choline Cl	0.22	0.05	0.05
Phosphate mono/D	0.85	1.85	1.85
Lysine (78.8%)	0.21		
DL Methionine		0.10	0.10
Selenium premix (0.06%)	0.05	0.05	0.01
Propionic acid (50%)	0.10	0.05	0.05
Calculated analysis			
Crude protein, %	12.9	10.3	10.3
Me, kcal/kg	2,978	1,626	1,624
Calcium, %	3.00	1.27	1.27
Total phosphorus, %	0.45	0.58	0.58
Lysine, %	0.80	0.44	0.44
TSAA, %	0.40	0.30	0.30
Analyzed values			
Crude protein, %	13.0	10.5	10.4
Calcium	3.18	1.32	1.45
Total phosphorus	0.46	0.60	0.65

with 5 mg/d human dose). Treatments were administered continuously through the feed for 2 years with the Norgestrel and Provera levels adjusted in the feed based upon the average feed consumption of the birds in each treatment replicate (i.e., consumption of the four cages) to maintain a consistent dosing of drug over time.

Outcome measures/statistics

The trial ended after 2 years of treatment when the chickens were 4 years of age. Surviving chickens were euthanized by cervical dislocation, necropsied, and the ovary and oviduct of each hen examined for evidence of reproductive tract cancers under the direct supervision of a board-certified veterinary pathologist (J. Barnes) with experience in avian pathology. A standard protocol was followed to determine if cancer was present or absent, the distribution and degree of cancerous lesions if present, and presence of other lesions. After gross examination of the reproductive tract, samples of ovary and oviduct from all hens, along with possible metastatic lesions in other tissues of cancerous hens, were removed and placed into 10% buffered neutral formalin. After fixation in formalin for 72 hours, tissues were transferred to 70% ethyl alcohol, and subsequently trimmed, processed by paraffin embedding, and stained with hematoxylin and eosin for histopathologic examination. Characterization of reproductive tract cancers as to type, stage, and grade was performed as previously described (16).

The primary objective of the study was to evaluate progestins as reproductive tract cancer preventives. A secondary objective was to evaluate whether a modest dietary enrichment with vitamin D_3 would provide ovarian cancer protection, or confer additional ovarian cancer protection to that provided by progestin. Fredrickson published data (17) showing a cumulative incidence of ovarian adenocarcinoma of 14% in chickens followed from ages 2 to 4. For purposes of power calculation, we assumed a 9% ovarian cancer incidence in untreated birds that were feed restricted

and thus had decreased ovulation. We calculated that 378 hens would be required in each experimental group to demonstrate a 50% reduction in the incidence of ovarian cancer (from 9% to 4.5%) as compared with controls at a power of 0.80 and significance of 0.05 with a one-sided test.

Treatment effects were analyzed using multiple logistic regression with cancer occurrence as the dependent variable. Confounders included the vitamin D treatment effect, and the strain identity (as a categorical variable). Results were nearly unaffected by inclusion or exclusion of these confounders in the model. In addition, all models included total egg production for the replicate (cage region; up to 15 hens), to control for effects of ovulation on cancer risk. We saw no evidence of replicate effects whether related to outcome or egg production or on-study mortality. Therefore, the unit of observation was the individual hen. All *P* values shown are two-tailed.

Results

A total of 1,234 birds remained at trial termination. With removal of additional birds for which treatment assignment was unclear, the analyzed data set had 1,209 birds. The majority of birds that expired during the study died of natural causes other than ovarian cancer. Mortality was variable across replicates, but unrelated to treatment group. The variability was consistent with random premature death, i.e., the number of birds at the end of the study was not related to replicate. Moreover, the fact that almost identical numbers of birds remained in each group at trial termination is consistent with there being no major differences between treatment groups in cancer-related mortality before the end of the trial. For example if the incidence of deaths related to non–cancer-related causes (which comprised the overwhelming majority of deaths) was similar across the whole flock throughout the study, then differences in cancer-related mortality would be evident in marked differences in the numbers of birds remaining in each group at the end of the trial. In fact, the numbers of birds remaining in each group at the end of the study were similar across treatment groups. Among the restricted-feed hens, premature mortality was not associated with treatment ($P = 0.80$ by ANOVA; Fig. 1A). The analysis presented later excluded these hens that had died before trial termination from the analyses, to avoid assuming that the hens that died early were all cancer free. Analyses were then repeated inclusive of all the hens, assuming that all hens expiring early were cancer free. This decreased all the cancer rate estimates of course, but the ORs, confidence intervals (CI), and *P* values were all very similar or identical.

Egg-production data were available at the level of the replicate (Fig. 1B). As reported previously, caloric restriction significantly lowered egg production in the flock by more than 60% (18). In comparison with feed-restricted untreated controls, levonorgestrel lowered mean egg production by 18% ($P = 0.046$ by Wilcoxon test; Fig. 1C). Total egg production in the other treatment groups was not significantly different from controls. Egg production was highly variable between replicates within treatment groups, so subsequent logistic regression analyses were adjusted for these differences in egg production by inclusion in the model as a predictor. Controlling for ovulation could not be done for individual birds because they were not caged individually. Egg counts were available only for feeding units. Thus, we controlled for total cumulative egg production, and also for other egg-production summaries: average number of eggs per bird, and cumulative production at several time points. The results were nearly unaffected by these various approaches to concerns about confounding due to ovulation differences, suggesting that there was no important confounding. Among the restricted-feed hens, premature mortality was not associated with the egg-production totals ($P = 0.62$ by linear model; $P = 0.10$ by ANOVA; Fig. 1D). The replicates were constructed to maximize balance in the distribution of nine strains across treatment and within replicate. This was achieved very well; all Pearson residuals for the independence model were at most 1.6 in absolute value.

Adenocarcinomas were lobulated, firm, pale tan or gray. They occurred with similar frequency in the ovary and oviduct, often in both organs. Ovulation frequently continued even when large tumors replaced most of the ovary (Fig. 2A). The reproductive tract cancers demonstrated a spread pattern similar to that of human ovarian cancers. Ascites and carcinomatosis were common in advanced cases (Fig. 2B). Microscopically, tumors were typical albuminous adenocarcinomas that were highly variable even among lobules within the same tumor (Fig. 3A–C). Characteristic cytoplasmic ovalbumin granules were most numerous in tumors from hens that were still ovulating. Tumor emboli were present within ovarian lymphatics in advanced cases (Fig. 3D).

Overall, reproductive cancers occurred in 33.3% of group 1 full-fed birds and in 10.3% of group 2 caloric-restricted birds. On the basis of histopathology, 26.3% birds in the full-fed group had ovarian adenocarcinoma compared with 6.3% of birds in the calorie-restricted control group. Caloric restriction alone thus resulted in a near 75% reduction in ovarian cancer. The remainder of the cancer-positive birds in these groups had oviductal adenocarcinomas. The histologic appearance and subtypes of the avian cancers did not vary relative to treatment.

We combined the evidence for the two progestin treatments in a model using all the restricted-feed groups and controlling for vitamin D treatment as well as egg totals. Progestin treatment was associated with a significant reduction overall in reproductive tract tumors. For ovarian and oviductal cancers combined, the OR was 0.611 (CI, 0.392–0.953; $P = 0.03$; Table 2). The estimated protective effect was similar individually for ovarian and for oviductal cancers, but the *P* values exceeded 0.05. For the ovary, the OR was 0.648 (CI, 0.387–1.09; $P = 0.10$); for the oviduct, the OR was 0.825 (CI, 0.479–1.42; $P = 0.34$; Table 2). As expected due to the effect of ovulation suppression, in comparison with the full-fed control group, all of the restricted-feed groups had significantly fewer reproductive

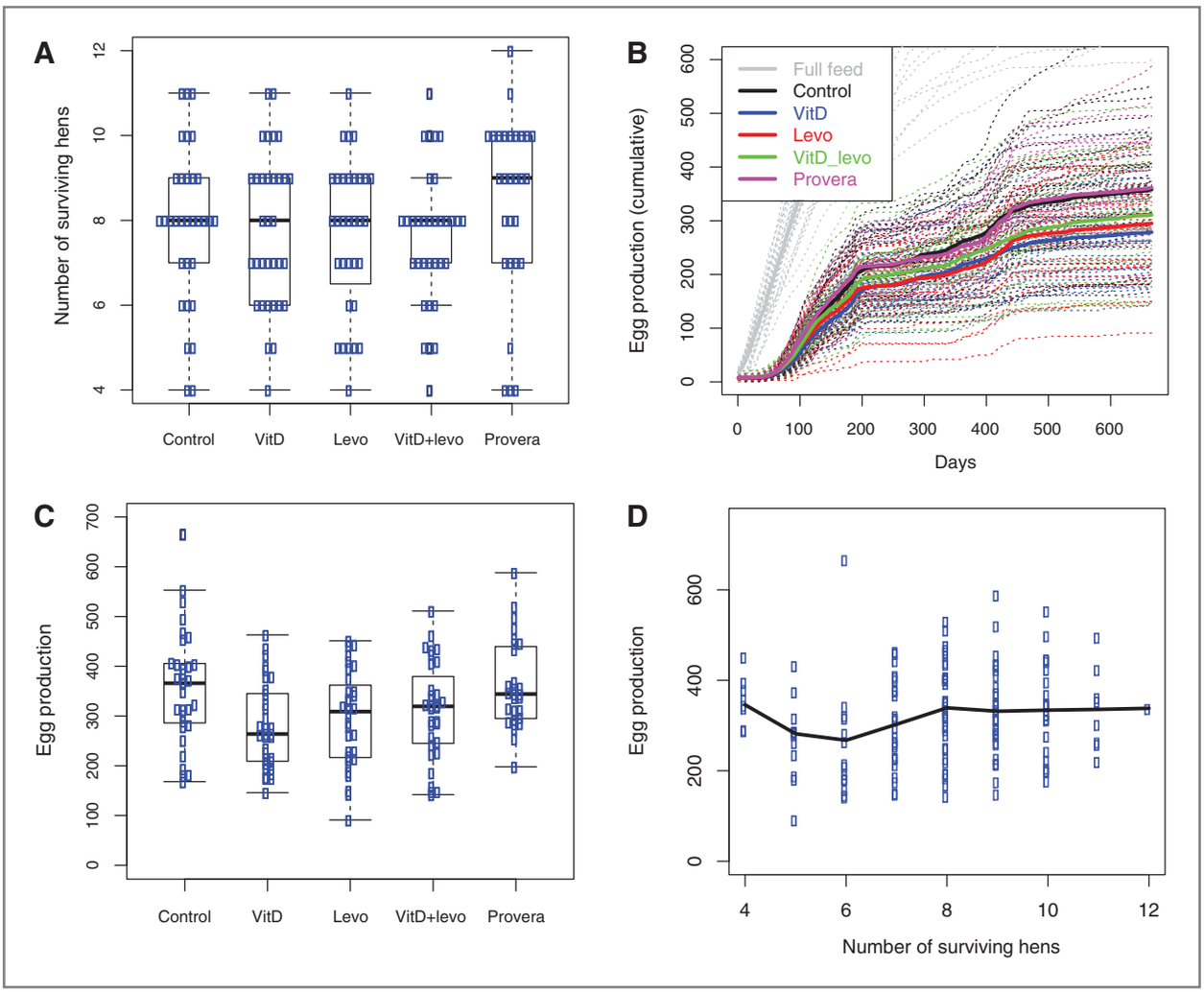

Figure 1. Boxplots in A and D show means, quartiles (box extents), whiskers (box extents × 1.5), and outliers. A, number of hens surviving to necropsy per cage, by treatment group. B, cumulative production of eggs per cage, by treatment group. Thick lines are the means for each treatment group. C, total egg production, per cage, by treatment group. D, total egg production, per cage, by number of surviving hens. VitD, vitamin D; Levo, levonorgestrel.

tract cancers, including both oviductal and ovarian cancers. The effects of treatment on the incidence of reproductive tract tumors in each of the hormone-treated groups are seen in Table 3. Individually, none of these single-treatment group comparisons are statistically significant, but the treatment effects are suppressive, ranging from 13% to 48% in all but one of the eight comparisons after correcting for egg production. Vitamin D supplementation did not significantly impact the occurrence of reproductive tract tumors, or enhance the cancer-protective effects of progestins.

Discussion

The results of this study demonstrate a chemopreventive protective effect of progestins against reproductive tract cancers in the chicken ovarian cancer animal model. As compared with untreated controls, chickens receiving the progestins Provera or levonorgestrel had significantly fewer ovarian and oviductal cancers. Our results are consistent with the findings reported in two prior studies demonstrating that progestins lower ovarian cancers in chickens (19, 20). In both of these studies, however, the experimental design did not control for the potential confounding influence of ovulation on ovarian cancer outcome. Although progestin-containing hormonal interventions markedly lowered ovarian cancer rates, there was also a concomitant marked drop in the number of ovulatory events associated with progestin exposure. Thus, the authors concluded that progestin-containing regimens lowered ovarian cancer incidence, but could not conclude that the protective effect was due to a true chemopreventive effect of the progestin unrelated to ovulation. In contrast, because the confounding influence of ovulation on ovarian cancer risk was controlled for in our study, our findings suggest a chemopreventive biologic effect of progestins that is independent of ovulation. In fact, the protective effect of progestins demonstrated

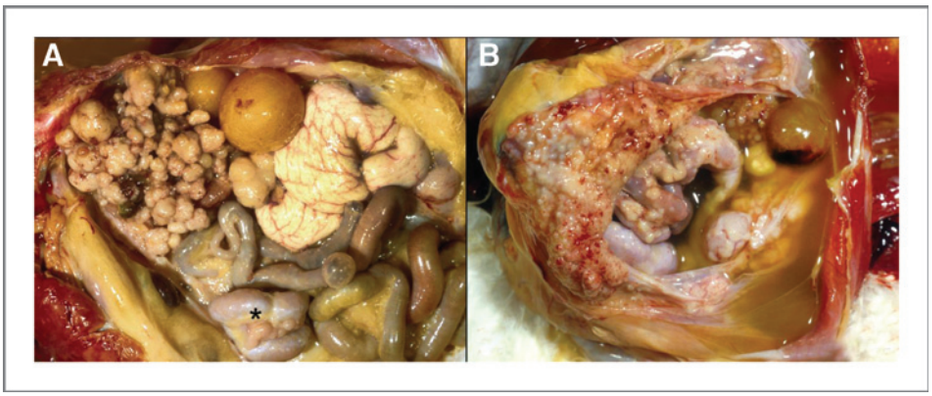

Figure 2. Ovarian adenocarcinomas in 4-year-old hens. A, a large adenocarcinoma replaces most of the ovary, but the hen is continuing to ovulate and produce eggs from an unaffected area. Lobulated appearance of the tumor results from neoplasia developing within vascular spaces in the walls of the follicles. Tumor has spread to the duodenum and pancreas (*), but generalized involvement of serosal membranes has not occurred. B, mesentery and peritoneum are thickened because of confluent metastatic adenocarcinoma. Ascites develops primarily from occlusion of lymphatics.

in this study is quite understated, and even more remarkable given the dramatic reduction in cancer incidence that had already occurred in the flock as a consequence of caloric restriction to inhibit ovulation. The 40% reduction in reproductive tract cancers in birds on progestins occurred in the background of an already reduced cancer incidence of more than 70% associated with caloric restriction. We did not observe a corresponding cancer-preventive effect of

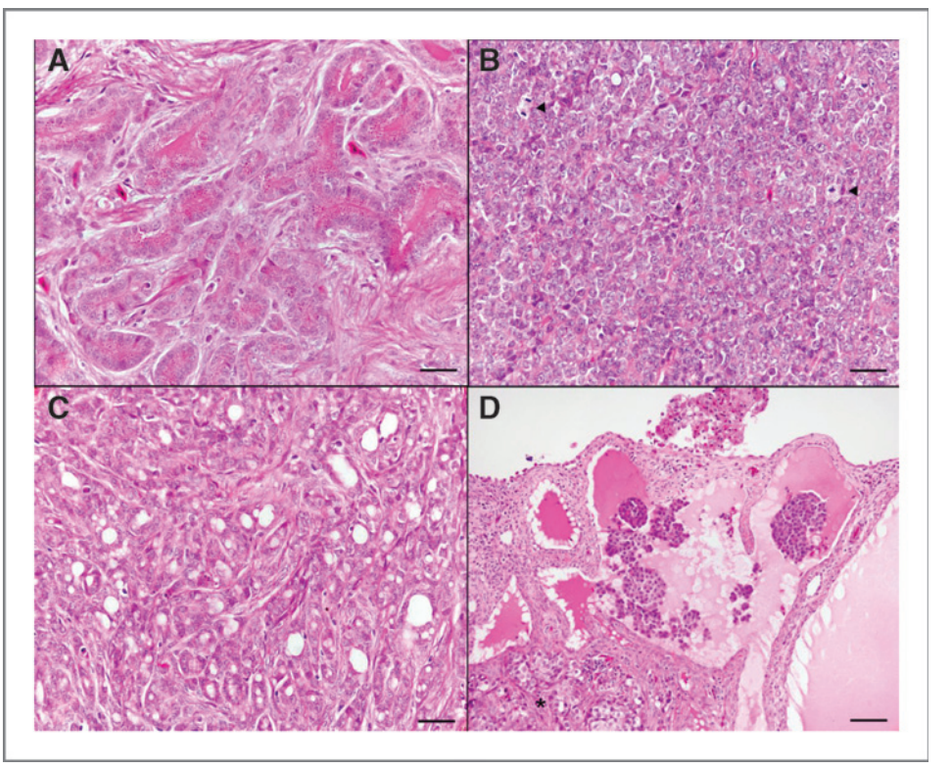

Figure 3. Ovarian adenocarcinomas in 4-year-old hens. A, grade 1 cancer in a hen that is still ovulating. Tumor is composed of tubules and acini lined by well differentiated, relatively uniform epithelial cells separated by varying amounts of interstitial connective tissue. Mitotic figures are not present. Albumin granules, the secretory product of avian ovarian adenocarcinomas, are in the apical cytoplasm of most cells and indicate that the hen was in production. B, grade 2 cancer in a hen that is no longer ovulating. Cells are in sheets that are poorly organized and do not form distinct tubules or acini. Groups of cells are separated by fine connective tissue and occasional mitotic figures are present (arrowheads). Cells have modest eosinophilic cytoplasm but lack albumin granules, which is typical for a hen that is not ovulating. C, grade 3 cancer. Cells are pleomorphic, typically stellate, or spindle-shaped. Mitotic figures are usually numerous but are not present in this field. Tubules are infrequent and poorly formed. Lack of albumin granules is typical for grade 3 adenocarcinomas regardless of reproductive status. D, multiple tumor emboli are located within a follicular lymphatic in the cortical area of the ovary. Emboli that have implanted and begun to differentiate can be seen in bottom left (*). Bar, 25 μm (A–C), 50 μm (D).

Table 2. Impact of progestin treatment on reproductive tract cancers

			Logistic regression, controlling for eggs	
Cancer endpoint	Controls[a] Incidence *N*/total (%)	Progestin treated[b] Incidence *N*/total (%)	OR (95% CI)	*P* Wald *P* Lik
Reproductive (ovary+oviduct)	48/503 (9.54%)	44/707 (6.22%)	0.611 (0.392–0.953)	0.03 0.03
Ovary	33/503 (6.56%)	32/707 (4.53%)	0.648 (0.387–1.09)	0.10 0.10
Oviduct	29/503 (5.77%)	32/707 (4.53%)	0.825 (0.479–1.42)	0.34 0.34

NOTE: All birds were feed restricted.
[a]Controls: no-treatment group plus vitamin D-only group.
[b]Progestin-treated: levonorgestrel or Provera, either with or without vitamin D.

vitamin D in this study. However, we did not anticipate that caloric restriction would have as profound an inhibitory impact on cancer outcomes as was observed. Thus, our study was not sufficiently powered to demonstrate an effect of vitamin D. In addition, the dose of vitamin D that we administered (60 IU or twice the daily allowance) may not have been sufficient to maximize the cancer-protective benefits of the vitamin as suggested by Vieth (21).

Taken together with our prior finding in primates that progestins activate known chemopreventive surrogate endpoints in the genital tract (6, 7), our findings in chickens provide further support for the hypothesis that progestins are potent chemopreventive agents in the reproductive tract. Recently published human data suggest that a biologic effect related to progestins may be a major mechanism underlying the cancer-preventive effect for both the OCP as well as pregnancy, which confers potent protection against subsequent ovarian cancer and is associated with high serum progesterone levels. (i) Analysis of the data from the Cancer and Steroid Hormone Study (CASH) has

Table 3. Cancer outcomes, individual treatment groups versus the control (no-treatment; feed restriction-only) group

				Logistic regression, simple		Logistic regression, controlling for egg totals	
Cancer endpoint	Cancers in controls	Treatment	Cancers in treated	OR (95% CI)	*P* Wald *P* Lik	OR (95% CI)	*P* Wald *P* Lik
Ovary	16/253 (6.32%)	Vitamin D	17/250 (6.8%)	1.08 (0.533–2.19)	0.829 0.829	1.07 (0.502–2.29)	0.855 0.855
Ovary	16/253 (6.32%)	Levonorgestrel	12/236 (5.08%)	0.794 (0.367–1.71)	0.556 0.555	0.871 (0.388–1.96)	0.738 0.738
Ovary	16/253 (6.32%)	Vitamin D and levonorgestrel	12/235 (5.11%)	0.797 (0.369–1.72)	0.564 0.562	0.733 (0.332–1.61)	0.44 0.438
Ovary	16/253 (6.32%)	Provera	8/236 (3.39%)	0.52 (0.218–1.24)	0.139 0.129	0.521 (0.219–1.24)	0.141 0.131
Oviduct	17/253 (6.72%)	Vitamin D	12/250 (4.8%)	0.7 (0.327–1.5)	0.358 0.355	0.868 (0.38–1.98)	0.736 0.736
Oviduct	17/253 (6.72%)	Levonorgestrel	9/236 (3.81%)	0.55 (0.24–1.26)	0.158 0.149	0.723 (0.301–1.74)	0.47 0.466
Oviduct	17/253 (6.72%)	Vitamin D and levonorgestrel	11/235 (4.68%)	0.682 (0.312–1.49)	0.336 0.331	0.705 (0.316–1.57)	0.393 0.389
Oviduct	17/253 (6.72%)	Provera	12/236 (5.08%)	0.744 (0.347–1.59)	0.446 0.443	0.746 (0.347–1.6)	0.453 0.451

demonstrated that progestin-potent OCPs confer greater protection against ovarian cancer than OCPs containing weak progestin formulations (22). (ii) Further support for progestins as ovarian cancer preventives has come from an analysis of data from the WHO by Risch, demonstrating a 60% reduction in the risk of nonmucinous ovarian cancer in women who have ever used Depo-medroxyprogesterone acetate, a progestin-only contraceptive (23). Progestin-only OCPs do not reliably inhibit ovulation, but are nevertheless contraceptively effective, presumably due to direct biologic effects on the reproductive tract. These effects include alteration of cervical mucous and the endometrium that adversely impact sperm migration and embryo implantation respectively (24). Up to 40% of women using the progestin-only OCPs can ovulate (23–26). Thus, the 60% reduction in ovarian cancer from a progestin-only OCP is further evidence that progestins have a direct chemopreventive effect on the ovary. (iii) Epidemiologic evidence has shown that twin pregnancy is more protective against subsequent ovarian cancer than singleton pregnancy. Previously, it was presumed that women who have twins would be at greater risk of ovarian cancer, presumably due to an increased likelihood of more lifetime ovulatory events as compared with women who do not have twins, and the notion that increased ovulation would confer greater risk of ovarian epithelial damage. Because women with twin pregnancy have higher progesterone levels than women with singleton pregnancy, it has been proposed that the epidemiologic data regarding twin pregnancy are supportive of the notion of a biologic effect of progesterone as conferring ovarian cancer protection, and that the effect is dose dependent (27). (iv) Finally, pregnancy at a later age is more protective than pregnancy early in life. In fact, a pregnancy after the age of 35 is twice as protective against ovarian cancer as a pregnancy prior to the age of 25. It has been proposed that this would suggest a protective effect of pregnancy that is unrelated to effects on ovulation, and supporting the hypothesis that pregnancy may clear premalignant or damaged cells from the ovary (27, 28).

The pathogenesis of ovarian cancer is not completely understood, but it is likely that ovarian cancer risk is influenced by a number of hormonal and environmental factors that either thwart or promote carcinogenesis in the reproductive tract. These can include direct biologic effects of steroid hormones or gonadotropins in the reproductive tract epithelium, and even inflammatory mediators that can directly or indirectly cause neoplastic transformation (29). Progestins can potentially confer a cancer-protective role via a number of possible mechanisms unrelated to ovulation. Activation of apoptosis or of TGF-β signaling, both shown previously to be induced by progestins in the ovary in a primate model, would be potent repressors of carcinogenesis through clearance of genetically damaged cells or via induction of cellular differentiation, rendering cells more resistant to neoplastic transformation (7). In addition, progestins may inhibit gonadotropins as well as lessen endometriosis or other inflammatory mediators that can promote ovarian cancer risk (29).

It is interesting that we observed a similar reduction in cancer incidence in the chicken ovary and oviduct in response to both caloric restriction and progestins. This would support the premise that the pathogenesis of carcinogenesis may be similar in the two organs. Indeed, it has recently been proposed that human epithelial ovarian cancers may actually arise from cells that originated in the fallopian tube (30–33). This hypothesis is speculative, but supported by the finding that most ovarian cancers have a serous histology similar to that of the fallopian tube. In addition, fallopian tube cancer risk is markedly elevated in women with BRCA-related hereditary risk of ovarian cancer, and an unusually high incidence of histologic and molecular signatures associated with dysplasia have been identified in the fimbriated end of the fallopian tube in prophylactic oophorectomy specimens from women at high risk (33, 34). Furthermore, careful examination of the fallopian tube in women with serous pelvic carcinoma has demonstrated a high incidence of endosalpinx involvement, or of coexistent tubal carcinomas, with similar alterations in p53 noted in the pelvic and fallopian tube lesions, suggesting that the lesions might be genetically related (35, 36). It is possible that the fimbriated end of the fallopian tube may be susceptible to neoplasia when exposed to dysplastic cells shed from the ovarian surface epithelium or even in response to ovarian stromal factors released during ovulation. Given that oviductal and ovarian carcinomas are both common in the chicken and appear to respond similarly to dietary and hormonal interventions, it may be possible to use this animal model further to better characterize the relative importance of the ovary or fallopian tube as the site of origin for these cancers.

In conclusion, the results of the current study suggest that progestins have a chemopreventive effect independent of ovulation against reproductive tract cancers. Our data provide further support to our prior observation in primates of marked activation by progestins of surrogate biomarkers relevant to chemoprevention in the reproductive tract (6, 7, 37). Taken together, these findings provide a strong rationale for examination of progestins as potential reproductive tract chemopreventives. These data also further open the door to the development of a highly effective pharmacologic cancer preventive strategy for the reproductive tract in women. We have previously shown in women from the Cancer and Steroid Hormone Cohort that use of progestin-potent OCPs for as little at 18 months or less lowered ovarian cancer risk by more than 60%, and that progestin-potent OCPs also confer enhanced protection against endometrial cancer in women with a high body mass index (BMI; refs. 22, 38). It is interesting to speculate that it may be possible to identify the optimal progestin formulations, dosages, and schedules, leading to the development of a progestin-based pharmacologic strategy that is even more effective than routine OCP use, with the potential to prevent most reproductive tract cancers.

Disclosure of Potential Conflicts of Interest

J.M. Lancaster has honoraria and is a consultant/advisory board member of Amgen. No potential conflicts of interest were disclosed by the other authors.

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