Research Article

Cancer Prevention Research

Reduction of Ovarian and Oviductal Cancers in Calorie-Restricted Laying Chickens

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Abstract

Epithelial ovarian cancer (OVAC) remains a highly lethal malignancy. It is a leading cause of cancer deaths among women in the United States causing more deaths than all other gynecologic malignancies combined. The pathogenesis of OVAC is not completely understood, but the process of repeated ovulation is believed to lead to genetic damage in the ovarian epithelium. As part of a prospective trial designed to evaluate OVAC chemopreventive strategies using the chicken model, caloric restriction (55% less energy) was used to inhibit ovulation in groups of hens receiving chemopreventives, thereby minimizing the impact of ovulation on the incidence of reproductive tract cancer. A separate group of chickens was maintained concurrently in the same environment, and managed similarly, except that caloric intake was not restricted. Among birds not receiving chemopreventive agents, we compared caloric versus noncaloric restricted birds to determine the relations between calorie restriction and risk of developing adenocarcinoma of the reproductive tract. Mortality in the calorie-restricted group was almost half that of those on full feed. Calorie-restricted chickens maintained body weights averaging 1.423 kg compared with the full-fed birds at 1.892 kg. Ovulation rate varied with the full-fed group producing 64% more eggs than the calorie-restricted group. Total reproductive cancers occurred in 57 (33.3%) birds for the full-fed group and 26 (10.3%) birds for the calorie-restricted group. On the basis of histopathology, 45 (26.3%) birds in the full-fed group had ovarian adenocarcinoma compared with 16 (6.3%) birds in the calorie-restricted group. Calorie restriction in laying hens resulted in a near five-fold reduction in OVAC. *Cancer Prev Res; 4(4); 562–7. ©2011 AACR.*

Introduction

Epithelial ovarian cancer (OVAC) remains a highly lethal malignancy. It is the fifth leading cause of cancer deaths among women in the United States and causes more deaths than all other gynecologic malignancies combined (1). The pathogenesis of OVAC is not completely understood, but it is believed that the process of ovulation leads to genetic damage in the ovarian epithelium. OVAC risk correlates with the number of ovulatory cycles in a woman's lifetime, whereas factors associated with decreased ovulation, such as increased parity, breast feeding, and oral contraceptive use, have a protective effect (2–4). These observations have led to the "incessant ovulation" hypothesis which purports that repeated cycles of epithelial disruption and repair may facilitate neoplastic transformation of the ovarian epithelium in susceptible individuals and that the risk of OVAC may be proportional to the number of ovulatory cycles in a woman's lifetime (5). Repeated cycles of rupture and repair of the ovarian epithelium associated with ovulation may predispose the ovarian epithelium to DNA damage, inclusion cyst formation, and dysplastic changes which can lead to neoplastic transformation.

Lack of a valid OVAC animal model has been a major obstacle to OVAC research. To develop effective therapeutic strategies for OVAC in a timely fashion, animal models that closely mimic human OVAC are desperately needed. Human prevention and treatment trials are costly requiring large numbers of subjects and many years to complete. Development of an animal model for OVAC research would represent a significant breakthrough allowing the expedited evaluation of numerous agents. Ideally, this would lead to the rapid identification of a select number of agents with the greatest potential for OVAC prevention and treatment that could be evaluated in human trials.

Among the candidate OVAC animal models, the domestic fowl may have great potential with characteristics that

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make it especially attractive for chemoprevention research. The most relevant feature of the domestic fowl is its high incidence of *spontaneous* OVAC, which ranges from 11% to 35% between 4 and 6 years of life (6–9). This makes the chicken unique, relative to other animals that require either experimental induction or genetic engineering to induce ovarian tumors (10–33). In addition, the chicken has a high ovulatory rate (daily), raising the possibility that chicken and human OVACs have a common pathogenesis related to ovulation-induced genetic damage to ovarian epithelial cells. Importantly, we have recently shown that chicken ovarian adenocarcinomas have genetic or molecular features that are similar to those of human OVAC (34) and that the prevalence of alterations in p53 in chicken tumors correlated with the number of prior ovulatory events, These findings further support the concept that ovulation with repeated cycles of rupture and repair of the ovarian epithelium may increase the number of proliferative events and genetic errors.

As part of a prospective trial designed to evaluate OVAC chemopreventive strategies using the chicken animal model, caloric restriction was used to inhibit ovulation in groups of hens receiving chemopreventives, thereby minimizing the impact of ovulation as a confounding factor on reproductive tract cancer incidence. A separate group of chickens was maintained concurrently in the same environment and managed similarly, except that caloric intake was not restricted. Among birds not receiving chemopreventive agents, we compared caloric versus noncaloric restricted birds to determine the relation between calorie restriction and risk of developing cancer, specifically adenocarcinoma of the reproductive tract.

Methods

Rearing and husbandry

As part of a 2-year chemoprevention trial designed to evaluate candidate pharmacologic OVAC preventive strategies, 2,400 2-year-old leghorn-laying hens were randomized into 6 groups. Two groups of birds received no chemopreventives but were maintained on either a restricted calorie diet ($n = 394$) or a diet formulated for optimal egg production ($n = 393$). Birds were housed in a cage layer system typical of those used in the table egg layer industry. Birds were maintained with 16.5 hours of light and 7.5 hours of dark. Room temperature was maintained at 80°F ± 5°F. Water was provided *ad libitum*. Feed was supplied *ad libitum* with the full-fed group receiving an average of 1,300 kcal/lb of feed and the calorie-restricted group receiving 715 kcal/lb of feed. Birds remained on these diets for 2 years. Eggs were collected daily to determine ovulation rates for the 2 groups. To control for the contribution of mortality to the number of eggs produced, data are reported as number of eggs per bird per month. Mortality was monitored for the 2-year period. Birds found dead were examined grossly and categorized as either a cancer suspect or visibly cancer free. At 4 years of age, surviving birds were weighed, euthanized via cervical dislocation, and evaluated via necropsy and histopathology by an avian pathologist (H.J.B.) for adenocarcinoma involving the reproductive tract.

Necropsy and tissue collection

All birds were evaluated for ovarian and oviductal adenocarcinoma by gross necropsy. Histopathologic examination was performed on reproductive tracts in 97.7% of birds in the full-fed group and 98.8% of the birds in the calorie-restricted group. Ovarian tumors were staged as follows: stage 1 (confined to ovary), stage 2 (ovary and oviduct affected), stage 3 (ovary and/or oviduct with serosal metastasis), and stage 4 (stage 3 with distant metastasis to liver, spleen, lung, or kidney). Ovary and representative samples of oviduct from each bird were placed into 10% buffered neutral formalin for 72 hours, transferred to 70% ethanol, trimmed, processed by conventional paraffin embedding, sectioned at 5 μm, and stained with hematoxylin and eosin for microscopic examination. When present, lesions in other organs also were similarly processed.

Statistics

Continuous data were analyzed using the General Linear Model's T test in SAS. Dichotomous data were analyzed using the Frequency Procedure's Chi Square test in SAS. Outcome differences with a probability of less than 0.05 were considered significantly different.

Results

Birds in the full-fed group experienced greater overall mortality than birds in the calorie-restricted group (Table 1). Birds eating a calorie-restricted diet died at almost half the rate of those on full feed. By ingesting a diet with 55% less energy, the calorie-restricted group maintained body weights averaging 1.423 kg compared with 1.892 kg for the full-fed group (Table 2). This represents a 25% difference in body weight between the 2 groups. Overall egg production varied for the 2 groups with the full-fed group producing 8.67 eggs per bird per month at peak production compared with 4.47 eggs per bird per month peak production for the calorie-restricted group (Fig. 1). The full-fed group ovulated over 32,000 times during the 2-year period compared with 11,500 for the calorie-restricted group. Gross pathology revealed that

Table 1. Mortality

	Full-fed	Calorie-restricted
Mortality number	261a	143b
Percent mortality,[a] %	62.1	36.3

NOTE: Outcomes with different letters (a and b) were significantly different with $P \leq 0.001$.
[a]Percent was calculated by dividing the number that died by number of birds originally placed.

Table 2. Body weight

	Full-fed	Calorie-restricted
Body weight, kg	1.896a	1.423b
Caloric intake, average, kcal/bird/d	663	365

NOTE: Outcomes with different letters (a and b) were significantly different with $P \leq 0.001$.

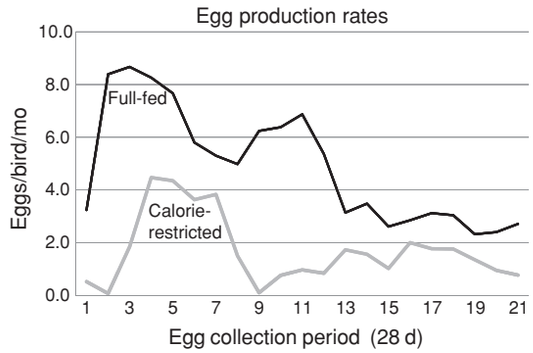

Figure 1. Ovulation rate.

71% of birds in the full-fed group were actively producing eggs at termination of the study, whereas only 29% of birds in the calorie-restricted group had fully functioning ovaries. Gross pathology results for birds that died prior to termination of the study, revealed 69 (26.4%) cancer suspects in the full-fed group compared with 5 (3.5%) cancer suspects in the calorie-restricted group.

Necropsy of birds remaining at the completion of the 2-year trial revealed that birds in the full-fed group had extensive abdominal fat involving the viscera and oviduct and a higher prevalence of cancers involving the reproductive tract, including cancers of the ovary and oviduct compared with birds that were calorie restricted (Table 3).

Table 3. Cancer prevalence (based on histopathology)

	Full-fed	Calorie-restricted
Number of birds	171	253
OVAC	45	16
% Prevalence	26.3a	6.3b
OVDC	52	17
% Prevalence	30.4a	6.7b
Total carcinoma involving the reproductive tract[a]	57	26
% Prevalence	33.3a	10.3b

NOTE: Outcomes with different letters (a and b) across rows were significantly different with $P \leq 0.001$.
Abbreviation: OVDC, oviductal cancer.
[a]Often hens had lesions in both the ovary and oviduct so the total with reproductive adenocarcinomas is not the sum of ovarian and oviductal cancers.

On the basis of histopathology, 45 (26.3%) birds in the full-fed group had ovarian adenocarcinoma compared with 16 (6.3%) birds in the calorie-restricted group. Cancers involving the oviduct were also more prevalent in the full-fed birds (52 birds or 30.4%) than in the calorie-restricted birds (17 birds or 6.7%). Tumor stages were similar irrespective of diet group (Table 4) with most tumors metastatic beyond the ovary. Overall adenocarcinomas involving the reproductive tract were more prevalent in full-fed birds (57 birds or 33.3%) than calorie-restricted birds (26 birds or 10.3%).

Discussion

Birds, like mammals, have a lower incidence of cancer and improved life expectancy when given a diet restricted in calories (35–45). Mortality rates were significantly higher in birds on the calorie-dense diet than for those fed a calorie-restricted diet. Histologic examination of tissues collected at the termination of the study clearly showed the prevalence of adenocarcinomas of the reproductive

Table 4. Cancer staging (ovarian adenocarcinoma), tumor numbers, and percentage of all tumors by tumor stage

Tumor stage	Full-fed (birds examined $n = 171$, tumor $n = 45$)	Calorie-restricted (birds examined $n = 253$, tumor $n = 16$)
1 (ovary only)	2 (4.4%)	3 (18.8%)
2 (ovary + oviduct)	7 (15.6%)	1 (6.3%)
3 (ovary ± oviduct with serosal metastasis)	10 (22.2%)	7 (43.8%)
4 (stage 3 + distant metastasis to liver, spleen, lung, kidney)	26 (57.8%)	5 (1.3%)

NOTE: Values are represented as number (percentage). Outcomes were not statistically significant with $P \leq 0.0711$.

tract was higher for birds fed a more calorie-dense diet than those on the calorie-restricted diet (33.3% vs. 10.3%, respectively). A weakness in our study is the lack of accurate classification of tumors in birds that died prior to the terminal necropsy. For these birds, gross pathology suggested a much higher rate of suspect cancers in the full-fed group than in the calorie-restricted group (26% vs. 3%, respectively). Although some tumor misclassification may have occurred, the trend toward greater tumor presence in birds that died in the full-fed group is unmistakable and is comparable to rates found by histopathology in the both groups of birds.

Egg production in both groups of hens was well below the normal 0.8 eggs per day. The hens were 2 to 4 years of age during this study, which is older that typical layers. In addition, the reproductive tracts of birds had become refractory to photostimulation due to intense lighting during the first 2 years of their lives. Even with lower egg production, records show a dramatic decrease in ovulation rate for the calorie-restricted group compared with the full-fed group. The full-fed group ovulated at a rate approximately 3 times that of the feed-restricted group. This reduction in ovulation rate correlates well with the reduction of adenocarcinoma in the ovary, which was reduced nearly 5-fold. The correlation between calorie restriction, decreased ovulation rate, and decreased ovarian adenocarcinoma demonstrated by this study is notable and analogous to that observed in women. The risk of OVAC in women is proportional to the number of lifetime ovulatory events. There is epidemiologic evidence that factors associated with fewer lifetime ovulations, such as pregnancy, lactation, or use of oral contraceptives, is associated with a marked decrease in OVAC risk. (2, 46–50). Although the mechanism responsible for this protective effect is not fully understood, the findings are supportive of the "incessant ovulation" hypothesis which states that the ovarian epithelium is subject to genetic damage during the wound and repair processes associated with ovulation (5).

The design of our study does not allow us to definitely conclude that the lower incidence of OVACs in the feed-restricted group was due to decreased ovulation, in that both caloric restriction and weight loss may beneficially impact OVAC risk and mortality. The reduction in the ovulation rate in the feed restriction group was associated with a 25% reduction in body weight. A positive association between excess body weight and mortality has been shown in both humans and animals. (35–39). Human mortality due to cardiovascular disease for example has long been associated with excess body weight (40). With regard to malignancy, there is a growing body of evidence that consistently shows an association between higher body weight and increased risk of cancers in various tissues (41–47). There is evidence that human death from OVAC is also influenced by body weight (38). Effects of body weight on tumor development and proliferation have been demonstrated in short-lived and long-lived mammalian species. Laboratory rodents and nonhuman primates have been used to demonstrate the effects of caloric restriction on life span and carcinogenesis. Moreschi and Rous observed that tumors transplanted into underfed mice did not grow as well as those transplanted into mice fed *ad libitum* (51, 52). In addition, free radicals generated by metabolism of some foods and environmental contaminants may promote establishment and proliferation of tumors (53, 54). Free radicals cause damage to DNA which, if not repaired, can facilitate carcinogenesis. Calorie restriction may be associated with a reduction in oxidative damage to DNA (53) as well as enhanced apoptosis. Apoptosis, which regulates cell death and is an important mechanism *in vivo* for elimination of genetically damaged cells, has been shown to be enhanced in animals fed a calorie-restricted diet (55). Apoptosis is important in the identification and self-destruction of cells which are old or damaged by toxins, ultraviolet light, or free radicals.

There are no previous studies that address the effects of caloric restriction on cancer prevalence and mortality in chickens. Although the mechanism of OVAC reduction in calorically restricted laying hens remains unknown, the dramatic difference in ovulation rates in this study suggests that ovulation or a combination of ovulation and other factors contributed to the reduction of adenocarcinoma in the calorie-restricted hens. Because calorie restriction directly affects ovulation rate in the chicken, it is unclear which factor has the greatest effect on reducing the risk of cancer development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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