Use of Genetic Strains of Chickens in Studies of Ovarian Cancer

P. A. Johnson and J. R. Giles

Department of Animal Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Ovarian cancer is a deadly disease often diagnosed late in development when there is little chance for a successful recovery. Although ovarian cancer is a rare occurrence in most animals, the domestic hen has been shown to spontaneously develop the disease with an age-related incidence. Two strains of hens derived from a similar genetic background and maintained at Cornell University have been shown to differ in the incidence of the disease. At 2 yr of age, the C strain hens have a greater incidence of ovarian neoplasms than do K strain hens. Interestingly, levels of plasma estradiol are elevated in the C strain compared with K strain hens. In addition, plasma immunoreactive inhibin is lower in the C strain than in the K strain. Finally, mRNA expression of the α-subunit of inhibin in the granulosa cell layer of the large yellow follicles is lower in the C strain compared with the K strain hens. Further studies using these as well as other strains of hens may be useful in learning more about the etiology of this disease.

Key words: ovarian cancer, hen, animal model, genetic strain, adenocarcinoma

INTRODUCTION

Ovarian cancer is the fifth leading cause of death from all cancers among women and is the leading cause of death from gynecological malignancies. It is estimated that nearly 25,000 new cases of ovarian cancer will be diagnosed this year; more than half that many women die from ovarian cancer each year (National Cancer Institute, http://seer.cancer.gov/csr/1975_2002/results_single/sect_01_table.01.pdf). Ovarian cancer occurs with an approximate incidence of 1 in 57 women. The estimated percentage of survival to 5 yr is strongly influenced by the stage at which the cancer is diagnosed. The 5-yr survival for early stage tumors (stages I and II) is approximately 80 to 90%, whereas for later stage tumors (stages III and IV) is much less—5 to 40% (Chi and Hoskins, 2000). Unfortunately, a large study (Pettersson, 1991) has indicated that the majority (approximately 65%) of tumors are not discovered until stages III or IV. This is likely related to the few symptoms experienced by women with ovarian cancer in the early stages.

Ovarian tumors are believed to arise from several sites on the ovary. A low percentage is termed nonepithelial and accounts for 7 to 10% of cases. These cancers arise from the germ cells or stroma of the ovary. In contrast, most cases of ovarian cancer are termed epithelial and are believed to arise from the surface epithelium. Family history, including genetic mutations such as those involving the BRCA genes (Auersperg et al., 2001), accounts for only about 5% of cases; most cases are sporadic. The strongest risk factor for ovarian cancer is age, in which the risk is low for young women and increases throughout reproductive life to plateau at about age 55 (Banks, 2000).

Other factors have been associated with alteration of risk for ovarian cancer. An important factor is pregnancy. A full-term pregnancy is associated with a risk reduction of approximately 40% (Banks, 2000). Moreover, each subsequent pregnancy confers an additional 10 to 15% reduction of risk. Use of the oral contraceptive pill for 3 yr is associated with a 40% reduction of risk for ovarian cancer. Each additional year of use reduces the risk by 5 to 10% (reviewed by Banks, 2000). Fathalla (1971) proposed that frequent ovulation contributes to increased risk for ovarian cancer. His theory was termed “the incessant ovulation hypothesis.” He proposed that repeated rupture and repair of the ovarian surface epithelium provides the opportunity for genetic aberrations. The single cell layer surrounding the ovary must be repaired after each ovulation. It is possible that areas of the surface epithelium become detached from the surface and are invaginated in so-called inclusion cysts (reviewed by Auersperg et al., 2001). These inclusion bodies may provide an abnormal environment for the epithelial cells and are implicated in the origin of ovarian cancer. One study indicated that women with ovarian cancer have been observed to have an increased incidence of inclusion cysts in the contralateral ovary (Salazar et al., 1996). The incessant ovulation
hypothesis is supported by epidemiological data relating
to pregnancy and oral contraceptive use. It is also possible
that endocrine factors are involved in the genesis or pro-
gression of ovarian cancer.

Figure 1. Ovarian cancer in the hen. Gross appearance is characterized
by firm, white nodules. Cystic or hemorrhagic follicles are also often
present.

CHICKEN AS A MODEL

Most animals do not spontaneously develop ovarian
cancer, which has made the study of the tumors difficult
(MacLachlan, 1987). A variety of rodent models (Orsulic
et al., 2002; Connolly et al., 2003) have been used as well
as cell lines from human tumors (Langdon and Lawrie,
2000) and normal ovarian surface epithelial cells (Auer-
sperg and Maines-Bandiera, 2000). These models have
been useful but study of the origin and development
of early tumors is limited. Generally, among domestic
animals, the desired state is pregnancy or lactation and
most wild animals are pregnant, lactating, or seasonally
anestrous. These physiological states are not associated
with frequent ovulation or ovarian cancer. The one model
that does exhibit ovarian cancer with a high incidence is
the domestic hen (Campbell, 1951; Wilson, 1958; Fred-
rickson, 1987). This observation, along with the fact that
the hen is a persistent ovulator, makes the hen a good
model for the disease. Many commercial strains of laying
hens ovulate almost daily through 1 or 2 yr of egg produc-
tion. This is similar to the pattern experienced by many
contemporary women who ovulate monthly for 10 to 20
yr, have 1 or 2 closely spaced pregnancies, and then re-
sume ovulation for 10 to 20 more yr.

Fredrickson (1987) conducted a 3.5-yr study in which
he evaluated the incidence of reproductive tract neoplasia
in 466 White Leghorn hens ranging from 2 to 7 yr of age.
He found that 24% of all hens developed age-dependent
malignant ovarian adenocarcinomas. He also observed
that these tumors were uncommon in hens less than 2 yr
of age and that ovulation rate was not associated with
incidence. Hormonal imbalance did not appear to be a
factor although the hormone levels were very variable.
Genetic differences were observed in susceptibility to
ovarian tumors among selected lines of hens, with 1 flock
having about a 5-fold greater tumor incidence than an-
other flock (Fredrickson, 1987).

CHICKEN OVARIAN TUMORS

In the normal laying hen, the ovary contains a hierarchy
of large, yolk-filled follicles in addition to the growing
population of small yellow and large white follicles. Ad-
vanced cases of ovarian cancer in the hen are observed
as very firm, white, cauliflower-like nodules on the ovary.
Often, fluid-filled cysts and hemorrhagic follicles are also
observed (Figure 1). Ascites is frequently present and
there are usually metastases to the serosal surfaces of
the oviduct, mesentery, and intestines. The cancers are
characterized by a glandular growth pattern and the
glands are lined by simple, columnar epithelium (Figure
2). A previous study showed that hen ovarian tumors are
cross-reactive with many antibodies used to detect several
antigens in human ovarian cancers (Rodriguez-Burford
et al., 2001). We have found that progesterone receptor

Figure 2. Hematoxylin and eosin photomicrographs of ovarian cancer
in the hen. The cancer is characterized by a glandular growth pattern.
The glands are generally lined by a simple columnar epithelium. Scale
bar = 50 μm.
is expressed in the cells lining the glands of hen ovarian cancers (Figure 3) and have shown that hen ovarian cancers are positive for expression of ovalbumin, an oviductal protein (Giles et al., 2004). Ovalbumin expression is particularly prominent in the glandular areas of the tumor, which are also positive for proliferating cell nuclear antigen. The finding of ovalbumin expression suggested that hen ovarian cancer was similar to the most common type, serous (oviduct-like), found in women.

**GENETIC STRAIN DIFFERENCES**

Two closed strains of White Leghorn hens (Cornell C and K) have been maintained at Cornell University since 1935 and 1936, respectively (Cole and Hutt, 1973). These strains were derived from a similar genetic background and selected for resistance to avian leucosis complex combined with selection for other important economic traits. In recent years, they have been maintained with random breeding. Cole and Hutt (1973) observed differences in rate of reproductive adenocarcinoma between the strains. During a 10-yr period, 6.6% of 2-yr-old C strain hens displayed adenocarcinoma of reproductive organs when necropsied, whereas similar findings were observed in only 0.3% of K strain hens (n = 1,300 hens of each strain). A genetic association in human epithelial ovarian cancer is well documented; however, this accounts for only a small proportion of those with the cancer. Most cases of epithelial ovarian cancer occur in women with no family history of the disease.

To confirm that genetic drift had not occurred to a significant degree since the original observations of Cole and Hutt (reported in 1973), we recently examined a smaller number of C and K strain hens (77 of each strain) for incidence of adenocarcinoma. A collaborating avian pathologist (Jarra Jagne, Cornell University) worked on this study with us. We found that 2-yr-old C strain hens had a significantly (P < 0.02) increased rate of ovarian cancer compared with 2-yr-old hens of the K strain. As part of our ongoing work on this project, we have maintained hens of both strains for a number of years. Any hens that become sick or die are examined at necropsy, and tissues are evaluated for tumor lesions. As seen in Figure 4, among hens that were obviously ill or found dead, we found that C strain hens had a significantly higher incidence (P < 0.05) of ovarian cancer compared with the K strain hens.

The main thrust of our research is reproductive endocrinology and therefore, our interest relates to possible hormonal correlates of ovarian cancer. Estrogen-only replacement therapy has been indicated as a risk factor for ovarian cancer (Lacey et al., 2002) and this has led us to examine plasma estradiol in our 2 strains of hens. Preliminary analysis showed that basal plasma estradiol was elevated in the C strain of hens compared with the K strain hens (D. Davignon and P. A. Johnson, Cornell University unpublished data). Furthermore, plasma estradiol was higher at all ages examined (1, 2, and 3 yr of age) in C strain hens compared with K strain hens, suggesting that the C strain may be exposed to chronic, higher levels of estradiol. Egg production (as a reflection of ovulation rate) and plasma progesterone were not different between the strains. The biological basis for the difference in plasma estradiol may be due to a significantly larger ovary in C strain hens (P < 0.02), resulting in more small follicles capable of estradiol production (Robinson and Etches, 1986).

Another hormone that has been implicated in the development or progression of ovarian tumors is the gonadal hormone inhibin. Inhibin is a protein hormone composed of 2 subunits (α and β) with the α-subunit unique to inhibin (Vale et al., 1988). Although most studies that
have examined plasma levels of inhibin in humans as a marker for ovarian epithelial cell cancer have not found a direct relationship, Matzuk and coworkers (1992) demonstrated that inhibin or its α-subunit may be a tumor suppressor factor in mice. These findings were based on the observation that 100% of transgenic mice deficient in the inhibin α-subunit gene developed gonadal tumors (Matzuk et al., 1992). The tumors that resulted in this transgenic mouse model were stromal in nature. If the inhibin α-subunit has a tumor suppressor role as indicated in the mouse model (Matzuk et al., 1992), the different biology of the ovary of the mouse compared with that of human and hen may lead to the occurrence of tumors in different cell types of the ovary when the inhibin α-subunit is deleted or reduced. We hypothesized that inhibin may be expressed at a lower level in the hens more prone to ovarian cancer (C strain) compared with the K strain hens. For this reason, we examined immunoreactive inhibin in the plasma and messenger RNA expression in the granulosa layer of the C and K strain hens. We measured plasma inhibin using a radioimmunoassay system previously validated for use in the chicken (Johnson et al., 1993). In 2 separate trials (Figure 5), plasma immunoreactive inhibin was significantly lower (P < 0.02 and P < 0.05 for trials 1 and 2, respectively) in C strain hens compared with K strain hens. We also isolated RNA from the granulosa layer of the 5 largest follicles from the C and K strain hens (n = 5 hens of each strain) and assessed the mRNA expression of the α-subunit by Northern blot analysis. We found that inhibin α-subunit was expressed at a lower level (P < 0.02) in the C strain compared with the K strain and that this difference was primarily due to decreased expression in the fifth largest follicle (P. A. Johnson, Cornell University, unpublished data). These results suggested that differences in granulosa layer expression of the inhibin α-subunit mRNA may account for the difference in plasma levels of inhibin between the 2 strains of hens. The role of inhibin in ovarian cancer in the hen warrants further study.

**Figure 5.** Plasma levels of immunoreactive inhibin (mean ± SE) in C and K strain hens in 2 separate trials. Inhibin was significantly lower in the C strain compared with the K strain in trial 1 (P < 0.02) and trial 2 (P < 0.05).

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**CONCLUSIONS**

The etiology of ovarian cancer in humans is poorly understood, in part due to the lack of animal models. One animal that has been shown to spontaneously develop the disease is the domestic hen. Similar to that in women, the incidence of ovarian tumors in hens increases with age and exhibits similar metastases to abdominal tissues and production of ascites. A genetic component has been shown in hens with incidence influenced by strain. Ovarian tumors are more common in the C strain of White Leghorn hens compared with the K strain. Interestingly, C strain hens also have greater plasma estradiol levels and larger ovaries than K strain hens. Furthermore, blood plasma inhibin levels were reduced for C strain hens compared with K strain hens, which may be explained by the reduced granulosa layer expression of the inhibin α-subunit mRNA. In conclusion, analysis of genetic differences in hens and the relationship of these factors to the incidence of ovarian cancer may be very helpful in learning more about the etiology of the disease in the chicken, with application to this very lethal disease in humans.

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**REFERENCES**


