A. **PROJECT TITLE**
The Development of New Tools to Study, Identify, and Prevent Ovarian Cancer

B. **PROJECT OFFICER(s)**
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C. **PROJECT DESCRIPTION**
Currently, it is possible at NCSU to stage large scale chemopreventive trials on birds. However, it is inefficient to screen chemopreventive candidates or therapeutic candidates using large scale animal trials. It is important to generate an in vitro model system because it can be useful to screen chemopreventive or potentially therapeutic drug candidates. Secondly, the cancer cell lines would make it possible to perform mechanistic studies in vitro that can be correlated with actual in vivo data. Objective 2 Elucidate the role of CA-125 in Ovarian Cancer CA-125 is a cell surface glycoprotein that is characteristic of ovarian cancer and it is used as a diagnostic marker for ovarian cancer in women. In women, CA-125 is only expressed on cells located on the periphery of the tumor, whereas preliminary data from our laboratory suggests that CA-125 is strongly expressed throughout the whole tumor. The up-regulation of CA-125 is well established and studies will be undertaken to understand any causative role of CA-125 in the cancer phenotype. Objective 3 Determine the origin of Avian Ovarian Cancer. It is presently unknown whether the majority of ovarian cancers actually arise in the germinal epithelium of the ovary or the epithelial cells of the oviduct because the tumors usually present at both locations when they are discovered at necropsy in the hen (Fredrickson, 1987). Therefore, it is important to understand the location of origin of the ovarian cancer to fully understand the avian model and understanding where the cancer arises in the hen may provide insight into the human disease process.

Approach Objective 1 Develop Ovarian Avian Cancer Cell Lines Brief Experimental Plan Briefly, tumors will be harvested from chickens that present the classic symptoms of ovarian cancer. The tumors will be gently digested with 0.2% trypsin, and seeded onto cell culture plates. The cells will be grown to confluency and split 1:10. When the mass cultures surpass 20 passages, clones will be established by limiting dilution or the use of cloning rings. Subsequently, clonal cell lines will be expanded and frozen in liquid nitrogen. Between 10 and 20 cell lines will be developed to meet Objective 1. If the cells do not spontaneously immortalize then cultures of ovarian cells will be immortalized using B95-8, Epstein-Barr virus suspension, ATCC. Approach Objective 2. The possibility that CA-125 expression plays a role in inducing the cancer phenotype will be evaluated by over-expressing CA-125 in primary ovarian cultures. Briefly, ovarian cultures will be established and the cells will be transfected with a retroviral expression construct encoding CA-125. The phenotype of the cells following over-expression of CA-125 will be evaluated as well as the expression of other cell cycle regulatory proteins. Objective 3 Determine the origin of Avian Ovarian Cancer NC State University has generated the first lines of experimental chickens that express reporter genes. We currently have transgenic chickens that carry the lacZ reporter gene
and express bacterial beta-galactosidase, and the founder lines of chickens are from a strain (Bovans) which is predisposed to ovarian cancer. The lacZ gene is a stable heritable marker, which will label all cells that arise from the initially infected cells. Therefore, we propose to transplant ovaries from the transgenic chickens into wild-type chickens. Subsequently, the chickens will be allowed to survive for 18 months post-surgery when they will be killed (24 months of age) and assayed for the presence of tumors. When any tumor is observed in the birds, the tumor and surrounding tissue will be harvested, stained with X-Gal (substrate for beta-galactosidase), histologically sectioned, and observed with a microscope. The transgenic nature of the tissue will also be confirmed by PCR and Southern blots. In a second series of studies, a concentrated retroviral vector (CXL Mikawa et al. 1991, Mozdziak et al., 2000) will be injected into the ovaries of 24 week old hens (Bovins). Subsequently, the chickens will be allowed to survive for 18 months post-surgery. The chickens will be killed and examined for the presence of tumors. When any tumor is observed in the birds, the tumor and surrounding tissue will be harvested, stained with X-Gal (substrate for beta-galactosidase), histologically sectioned, and observed with a microscope. The retroviral vector will provide a stable heritable marker that will label all mitotically active cells from the ovary that contacted the virus. In either case, tumors expressing beta-galactosidase will support the hypothesis that ovarian cancer arises from the ovary...]

D. PROJECT START AND END DATES 7/1/04-9/30/05

E. PROJECT ACTIVITIES / ACCOMPLISHMENTS
Firstly, Human and Chicken Ovarian tumors have been compared for CA125 (Generally Accepted Ovarian Cancer Marker) expression. It was discovered that CA125 was expressed only on the surface epithelial layers of the human tumors, while it is expressed throughout the entire chicken tumor.
Secondly, ovarian tumors were harvested and their growth was monitored in vitro. Growth curves for the cultures have been generated for the first and second passages. Currently, CA125 staining for the cultures is being evaluated coincident with the growth curves to determine the changes in the proportion of CA125 positive cells with time in culture.
Lastly, cells are still being cultured in an effort to establish immortalized cell lines. Ovarian cultures have been maintained in vitro for several months and they are beginning to show signs of immortalization.