HHS Public Access

Author manuscript

Proteomics Clin Appl. Author manuscript; available in PMC 2016 June 28.

Published in final edited form as:

Proteomics Clin Appl. 2014 October ; 8(9-10): 689–699. doi:10.1002/prca.201300135.

The Chicken Model of Spontaneous Ovarian Cancer

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Abstract

The chicken is a unique experimental model for studying the spontaneous onset and progression of ovarian cancer (OVC). The prevalence of OVC in chickens can range from 10–35% depending on age, genetic strain, reproductive history, and diet. Furthermore, the chicken presents epidemiological, morphological, and molecular traits that are similar to human OVC making it a relevant experimental model for translation research. Similarities to humans include associated increased risk of OVC with the number of ovulations, common histopathological sub-types including high-grade serous, and molecular-level markers or pathways such as CA-125 expression and p53 mutation frequency. Collectively, the similarities between chicken and human OVC combined with a tightly controlled genetic background and predictable onset window provides an outstanding experimental model for studying the early events and progression of spontaneous OVC tumors under controlled environmental conditions. This review will cover the existing literature on OVC in the chicken and highlight potential opportunities for further exploitation (e.g, biomarkers, prevention, treatment, and genomics).

INTRODUCTION

The World Health Organization (WHO) estimates that approximately 225,000 women are diagnosed with ovarian cancer (OVC) per year accounting for 4% of all cancer cases in the world on an annual basis[1]. An estimated 140,000 deaths[1] are attributed to OVC per year with the highest prevalence in Europe[1]. 5-year mortality rates for OVC are high due to advanced stage diagnosis and resistance to existing chemotherapeutic drugs.[2–4] More aggressive surgical procedures have resulted in slight improvements to survival rates over the past several years but early detection remains the most critical factor in determining patient survival[5]. For example, more than two thirds of OVC patients are diagnosed at Stage III or Stage IV where overall 5-year survival rate under optimal conditions is approximately 33%[6] whereas diagnosis at Stage I results in 5-years survival rates of greater than 90%. Progress in finding more accurate biomarkers and effective therapeutic strategies have been slowed by the heterogeneous nature of OVC tumors[3], paucity of early-stage biospecimens (i.e., primary tissues and plasma) from which to identify early molecular events of tumorigenesis, and few experimental models that faithfully recapitulate the pathophysiology of the human disease [7–10].

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The magnitude of OVC heterogeneity is summarized by the International Federation of Gynecological Oncologists (FIGO)[11] and WHO[12] having identified over 100 histopathological sub-types of OVC, 46 of which are epithelial types that constitute the majority of OVC cases (est. 90%). Further complicating the OVC phenotype is the fact that the tissue of origin remains unresolved. The ovarian surface epithelium (OSE), a monolayer of flat-to-cuboidal cells that line the surface of the ovary, has historically been viewed as the primary tissue of origin for epithelial ovarian cancer[13]. The small population OSE cells are difficult to obtain and then maintain in culture making it difficult to establish fundamental traits of healthy versus cancerous (transformed) OSE[14–16]. Furthermore, there is limited access to healthy ovary tissues for exhaustive molecular characterization. Thus, experimental models based on the 'OSE-to-OVC' hypothesis have been difficult to design with incomplete molecular characteristics of OSE transformation. An alternative to the OSE hypothesis that is gaining greater appreciation is that some histopathological sub-types of OVC, particularly high-grade serous adenocarcinomas, originate from the fallopian tube epithelium (FTE)[17]. Recent studies in mice have provided convincing functional evidence to support the 'FTE-to-OVC' hypothesis.[18–20] Although the development and wider availability of novel experimental systems with engineered genetic elements will undoubtedly improve our understanding of OVC, the emergence of the chicken as a natural experimental model of spontaneous OVC is providing an unbiased, complimentary system to studying OVC pathogenesis.

The chicken is rapidly emerging as an important experimental model for studying the spontaneous onset and progression of OVC.[10] Although reports of high rates of OVC in chickens have existed in literature for over 80 years[21–24], it is more recently that the animal has gained greater attention for OVC biomedical research. No other animal develops spontaneous OVC tumors at comparable rates to the chicken which can exceed 35% depending on the genetic background (i.e., strain[24, 25]), age, and number of eggs produced by the flock. Furthermore, the chicken OVC model displays similar histopathological subtypes[26] and multiple molecular level traits (e.g., CA-125) similar to human OVC. Fredrickson[24] provided the most detailed account of OVC in the laying hen by surveying over 400 birds from three genetic strains for up to 7 years. The quantitative evidence from this study combined with the sequencing of the chicken genome [27] has provided the foundation for the expansion of studies into the onset and progression of spontaneous OVC in the chicken. This review will cover epidemiological, histopathological, and molecular features of OVC in the chicken as well as potential opportunities for further exploration.

GENERAL OVERVIEW OF CHICKEN OVC STUDIES

The agricultural utility and OVC research potential of the chicken is summarized in Figure 1. A hen begins laying eggs once it reaches sexual maturity which typically starts at ~5 months of age.[28] Birds typically produce eggs for at least the next year up to ~ 2 years of age after which the research potential for OVC begins. Most studies reviewed herein cover birds 2–4 years of age as OVC presents itself in >5% of the birds providing sufficient numbers of biospecimens (assuming at least 200 birds at the start of a study). Pilot chemoprevention studies that inhibit egg production or reduce inflammation have

established key epidemiological similarities between chicken and human OVC[29–35]. More recent molecular characterizations of chicken OVC have found many notable similarities including CA-125 expression[36], p53 mutational frequency[37], and E-cadherin expression [38–41]. Furthermore, several oviduct-specific proteins have been identified in chicken OVC[42–44] suggesting potential links to oviductal involvement in chicken OVC pathogenesis. Efforts are underway to translate these molecular markers to humans in the hope that they will serve as early-stage biomarkers for OVC diagnosis. Unlike contrived mouse models, biomarker discovery studies in the chicken can be carried out in great detail with matched longitudinal plasma samples and tissue biospecimens which can be harvested for genomic, proteomics, or metabolomics signatures. Because the birds develop spontaneous tumors while maintained under identical conditions, traditional confounding factors in human studies (e.g., environment and diet) can be minimized making it easier to detect true biological changes related the onset and progression of OVC. It is expected the intra-individual variability, or personalized reference ranges, will be critical to an early-stage OVC screen in humans as previously demonstrated for CA-125.[45–49] The combined biomarker discovery and validation potential of longitudinal plasma repositories [43, 50, 51] provides a powerful model for OVC biomarker development and translation to humans.

EPIDEMIOLOGICAL CHARACTERISTICS OF CHICKEN OVC

A woman's risk for developing OVC has been associated with the number of ovulations in her lifetime[52] which can be affected by pregnancy and oral contraceptive use. A woman who has never been pregnant and did not use birth control could in theory ovulate at least 400 times over her lifetime. Women who use oral progestin-estrogen contraceptives can reduce their risk of OVC by as much as 50%.[53] Figure 2A (conceptually adapted from Noara *et al.*[54] and Silverthorn[55]) shows a cross sectional illustration of the human ovary. Ovulation in women occurs on average every 28 days with the promotion of primary follicles to egg release occurring over ~14 days.[55] The premise of the OSE-to-OVC is that the constant rupture and repair of the OSE during the course of ovulation gives rise to the majority of OVC cases. However, as previously noted there is increasing evidence to suggest that the fallopian epithelium contribute to the origin of OVC as they are in contact with the OSE. Figure 2B shows a cross-sectional illustration of the chicken ovary based on the ovulatory cycle of the bird[56]. Like in the human ovary, the chicken ovary has immature follicles and an OSE as shown in the H&E stained formalin fixed tissue images in Figure 2C and 2D from a healthy fully functional (egg-producing) bird. The mature follicle (Figure 2B: F1) is released into the oviduct where it forms into a mature egg in ~24–27 hours. During this time period, the F2 follicle moves into place where it can be released into the oviduct immediately after the F1 egg is produced. This cycle repeats itself every ~27 hours disrupting the OSE. The agricultural utility of a laying hen is typically limited to the first 1–2 years of the bird's life wherein a bird can lay between 200–400 eggs. After 2 years of age, birds are removed from the egg-producing flock but they can continue to live several more years and produce another ~200 eggs. Thus, the number of ovulations in chickens and humans is comparable but the compressed ovulation cycle of ~28 days in humans versus ~27 hours in the chicken in theory gives rise to high rates of OVC over a narrower window of time relative to humans. However, it was noted by Auersperg[57] that the mouse, which does

not typically develop spontaneous OVC at appreciable rates, can undergo multiple ovulations over a 2 year lifespan generating as many if not more insults to the ovary than in humans or chickens.

Fredrickson [24] monitored egg production from 2–7 years of age but found it was not associated with OVC prevalence. Barnes *et al.*[29] studied the effect of egg production on OVC prevalence in four hundred 3-year old birds where half were injected with the contraceptive medroxyprogesterone (Depo-Provera) three times over a 16 month period versus no injection for the control group. The treated birds produced fewer eggs and showed a 15% reduction in OVC risk. In a more recent study, Trevino *et al.*[34] looked at the prevalence of OVC in chickens as a function of progestin treatment. The results of the study showed that progestin treatment could reduce the risk of OVC in chickens by more than 90% compared with age-matched controls. Giles *et al.*[32] studied the prevalence of OVC in 33 mutant restricted ovulator (RO) birds and 31 wild-type (WT) siblings. The birds were studied over a 31-month period during which the WT birds produced an average of 422 ± 134 eggs and the RO birds produced 28 ± 66 eggs. Following necropsy, only one of the WT birds (3%) had developed OVC whereas 9 RO birds (27%) developed OVC strongly suggesting the lower ovulations gave rise to reduced risk of developing OVC. RO birds have lower progesterone levels but higher estrogen and gonadotropin levels in plasma compared with WT birds.[58] A higher progesterone level is protective whereas women with lower levels of estrogen and gonadotropins are at higher risk of developing OVC.[59]

A large-scale chemoprevention study at North Carolina State University investigated OVC prevalence in 2400 two-year old birds as a function of different treatment conditions over a 2 year period.[33, 35] Carver *et al.*[33] reported a 5-fold reduction in OVC prevalence for birds on a calorie-restricted diet which inhibited egg production. Control birds fed a full calorie diet produced 64% more eggs compared with birds fed a calorie-restricted diet. A second report by Rodriguez *et al.*[35] investigated the OVC prevalence as a function of progestin (levonorgestrel and Provera) and Vitamin D treatments. The Vitamin D treated birds showed no reduction in OVC risk whereas the progestin treated birds showed a 60% risk reduction. Importantly, the authors concluded that progestins and not the reduction in ovulations provided the reduced OVC risk.

Inflammation is a critical factor in the onset and progression of cancer.[60] Urick *et al.* studied the effect of non-steroidal anit-inflammatory drugs (NSAID) on the proliferation and prevalence of OVC in the chicken.[30, 31] Primary cultures of ascites-derived cells were treated with aspirin and cyclooxygenase enzyme (COX-1 and COX-2) inhibitors to study cell growth, VEGF expression, and prostaglandin E_2 (PGE_2) concentration[30]. Aspirin and the COX-1 inhibitor SC-560 were effective at reducing cell proliferation whereas the COX-2 inhibitor NS-398 was ineffective. A second study by the same group looked at the effect of dietary aspirin on the prevalence of OVC in 200 chickens over different age-groups.[31] The aspirin-treated birds showed reduced PGE_2 levels in liver tissues but the overall prevalence of OVC was not affected versus the control group. Although OVC prevalence was not affected by aspirin diet, the severity of OVC (i.e., Stage) was reduced in aspirin-treated birds versus the control birds. Eilati *et al.*[61] investigated the effect of chronic inflammation on the onset and progression of OVC 600 birds from 1.5−3.5 years of age. The concentrations

of COX-1 and COX-2 and their downstream pro-inflammatory lipid product PGE_2 were measured in ovarian tumors and healthy tissues. The study found that all three targeted analytes increased in concentration with age concurrent with the onset and progression of OVC.

These studies underscore the potential of the chicken as a model for OVC chemoprevention studies. Molecular signatures in chickens are beginning to emerge that will support such studies as diagnostic and prognostic biomarkers that could potentially translate to humans. These markers, potentially in concert with transvaginal sonagraphy[62], could facilitate experimental chemotherapeutic intervention studies in chickens with translational potential in humans.

MOLECULAR CHARACTERISTICS OF CHICKEN OVC

The sequencing of the chicken genome[27] has enabled researchers to begin studying OVC in the chicken making comparative observations with humans. Table 1 gives a summary of studies that have identified biomolecular characteristics in the chicken that either corroborate previous observations in human OVC or identify new gene products that have translational potential. Many of these reports have occurred in the past 5 years suggesting the model is gaining more widespread appreciation and acceptance. One of the limitations of the model that has restricted progress is the lack of commercially available antibodies for immmunohistochemcial staining (IHC) and Western blot analysis. Nonetheless, antibodies against human proteins related to OVC have been used with success in the chicken (vide supra). The development of chicken-specific reagents combined with advanced systems biology technologies (i.e., genomics, proteomics, and metabolomics) will further expand the potential utility of the model for translation OVC research.

CA-125 expression in chicken OVC tumors

Cancer antigen 125 (CA-125), also referred to as mucin 16 (MUC-16), was discovered by Bast *et al.*[63, 64] and has emerged as the most widely used biomarker for OVC detection in the world. Although it does not have the predictive power to be used as a population-based screen, it remains the most accurate marker for OVC to date. Two studies have investigated the presence of CA-125 in chicken OVC using commercially available human antibodies with conflicting results. Rodriguez-Burford *et al.*[65] used IHC to measure the expression levels of several OVC-related proteins in chicken OVC tumors. Positive IHC staining was observed for EGFR, Her2, AE1/AE3, Lewis Y, CEA, and Tag 72 and negative IHC staining was observed for CA-125, PCNA, p27, and TGF-alpha. Jackson *et al.*[36] measured CA-125 expression in chicken OVC tumors and primary cultures of OVC cells by both IHC and Western blot (WB). CA-125 was positive in tissues specimens and in 90% of primary ovarian cancer cultures after 28 hours of growth. The closest CA-125 homologue in chickens based on BLAST analysis is a predicted form of MUC-16 (NCBI RefSeq XP_0012330006.3). The predicted chicken isoform is found on Chromosome 28 and has 726 amino acids with 38% sequence to human CA-125. Additional research and chicken-specific antibodies are needed to establish and confirm the existence of CA-125 in chicken OVC.

p53 is commonly mutated in chicken OVC

The tumor suppressor p53 is mutated in over 95% of high-grade serous ovarian cancers (HGSOV), the most common and lethal form of OVC.[66] Hakim *et al.*[37] found that the overall frequency of p53 mutation in OVC tumors from a total 172 4-year old birds from two different flocks was 48% (81/172). The birds (Flock A and B) were age-matched but raised under different conditions. Flock A had reduced light and calorie restriction that limited the number of ovulations whereas Flock B received normal light and diet. The p53 mutations in Flock A were exclusively found in the p53-DNA binding domain (14/14) whereas the Flock B results showed the large majority of mutations occurred in the proline-rich region (68/76) with few in the transactivation domain (8/76) and none in the DNA binding domain. The types of mutation for Flock A were largely deletions (13/14) with one missense whereas missense dominated in Flock B (71/76) in agreement with studies in human HGSOVC[67]. The mutation and expression of H-/K-ras and Her2, respectively, were also investigated.[37] H-ras was not mutated in any of the OVC tumors and only two tumors showed K-ras mutations which agrees with findings in human HGSOVC[68, 69]. Her2 was overexpressed in 53% of OVC tumors by IHC staining which is high but not outside reported ranges found in the human OVC literature (8–66% depending on Stage and sub-type)[70]. Overall these molecular features of chicken OVC, in particular p53 mutation, provide a strong link to common traits in human OVC.

E-cadherin is up-regulated in chicken OVC

E-cadherin is a 120 kDa calcium-dependent transmembrane protein that plays a critical role in epithelial cell-cell adhesion.[71] E-cadherin is constitutively expressed in human endometrial and oviductal epithelium, not expressed in normal human OSE except for rare instances where OSE cells have committed to metaplastic and neoplastic phenotypes, and overexpressed in the majority of human OVC tumors.[13] Thus, E-cadherin is a biomarker for epithelial differentiation in human reproductive tissues and OVC tumors. Ansenberger *et al.*[38] studied the expression of E-cadherin in normal and cancerous chicken ovaries using a combination of IHC, WB, and mRNA analysis. Positive E-cadherin staining was observed in the granulosa cells of healthy fully functioning ovaries, glandular regions in early-stage (confined to the ovary) OVC tumors, and throughout late-stage OVC tumors. WB and mRNA analysis confirmed the presence and up-regulation of of E-cadherin in OVC tumors relative to healthy chicken ovaries. Two independent global gene expression studies found up-regulation of E-cadherin in chicken OVC tumors.[39, 40] Gonzalez-Bosquet et al.[40] examined early-stage (confined to ovary) OVC, metastatic OVC, and oviductal carcinomas and found 1.28, 2.64, and 3.39 (log-expression) higher levels of E-cadherin, respectively. Tiwari *et al.*[41] characterized ascites-derived primary OVC cells and normal OSE cells in culture and found E-cadherin was expressed in all cell types. Collectively, these reports support the use of E-cadherin as a tissue marker for chicken OVC pathogenesis and provide additional evidence for its similarity to humans.

Oviductal proteins and chicken OVC pathogenesis

The OSE has traditionally been considered the tissue of origin for human OVC[13] but emerging data supports the alternative hypothesis that some histopathological subtypes of

OVC, most notably high-grade serous (HSOVC), originates in the fallopian tube epithelium.[18–20] The majority of late-stage OVC cases in the chicken have adenocarcinomas on the oviduct. At present, there is no way to determine the tissue of origin when tumors are present on both the ovary and oviduct although molecular signatures suggest the latter may be the initiation site. Giles *et al.*[42] showed that ovoalbumin, a protein primarily expressed in the oviducts of fully functional birds and the most abundant protein in egg whites, was over-expressed in chicken OVC tumors. This study provided the first molecular evidence of oviductal involvement in the chicken OVC pathogenesis. Hawkridge *et al.*[43] characterized the intra-individual variability of the plasma proteome by LC-MS/MS and identified a predicted form of ovostatin (ovostatin 2). This unique protein is a member of the I39 MEROPS I39 protease inhibitor family which includes alpha-2 macroglobulin and ovostatin 1, proteins primarily synthesized in the liver and oviduct, respectively. Trevino *et al.*[39] identified several oviduct-specific proteins in chicken OVC including paired box 2 protein and estrogen receptor 1 in a global gene expression study. Lim et al.[72] studied the tissue distribution and expression levels of WNT4 in healthy and OVC tumor tissues. WNT4 was expressed primarily in healthy chicken oviducts, over-expressed in glandular epithelium of OVC tumors, and not expressed in healthy ovaries suggesting it may be a marker for oviductal involvement. The increasing list of oviduct-specific proteins over-expressed in chicken OVC tumors reinforces the hypothesis that the oviduct contributes to the onset of OVC in the hen.

FUTURE OPPORTUNITIES

There remain significant opportunities for the continued development of the chicken as an experimental model for spontaneous OVC. The epidemiological and molecular characteristics strongly support the chicken as a viable model that recapitulates many of the significant hallmarks of human OVC. Figure 1 provides a general overview of the chicken's lifespan as it relates to OVC in addition to the existing and potential uses for translation biomedical research. An area that has not been developed thus far is identifying genetic susceptibilities of OVC in the chicken. Genome wide association studies could provide critical markers for selecting specific populations for future studies. Targeted studies that focus on known susceptibility genes such as BRCA1[73] and BRCA2[74], both of which have been identified in the chicken, could also further support the use of the chicken model. Furthermore, the measurement of intra- versus inter-individual variability of plasma proteins and metabolites with LC-MS/MS as a function of genotype and phenotype (healthy vs. OVC) and genotype provides the opportunity to develop tools and strategies for a truly systems-level experimental model with a heterogeneous genetic background. Despite extensive breeding of chickens in the agricultural industry, their genetic background remains similar in diversity to the wild jungle fowl.[27] Thus, the chicken is complimentary to genetically engineered mouse models with defined genetic elements. In summary, the chicken fills an unmet need in the OVC research community that lacks a true natural experimental model for OVC. Through careful experimental design and advanced systems-level 'omics' technologies, it will be possible to explore and discover in an unbiased way the epidemiological and molecular characteristics that contribute to the spontaneous onset and progression of ovarian cancer.

ACKNOWLEDGEMENTS

This work was financially supported in part by NIH-NCI through award K25CA128666 to A.M.H. and Virginia Commonwealth University.

ABBREVIATIONS

OVC	ovarian cancer
WHO	world health organization
FIGO	international federation gynecology and obstetrics
OSE	ovarian surface epithelium
FTE	fallopian tube epithelium
RO	restricted ovulator
WT	wild type
NSAID	non-steroidal anti-inflammatory drugs
IHC	immunohistochemistry
LC-MS/MS	liquid chromatography tandem mass spectrometry
H&E	hematoxylin and eosin
HGSOV	high-grade serous ovarian cancer

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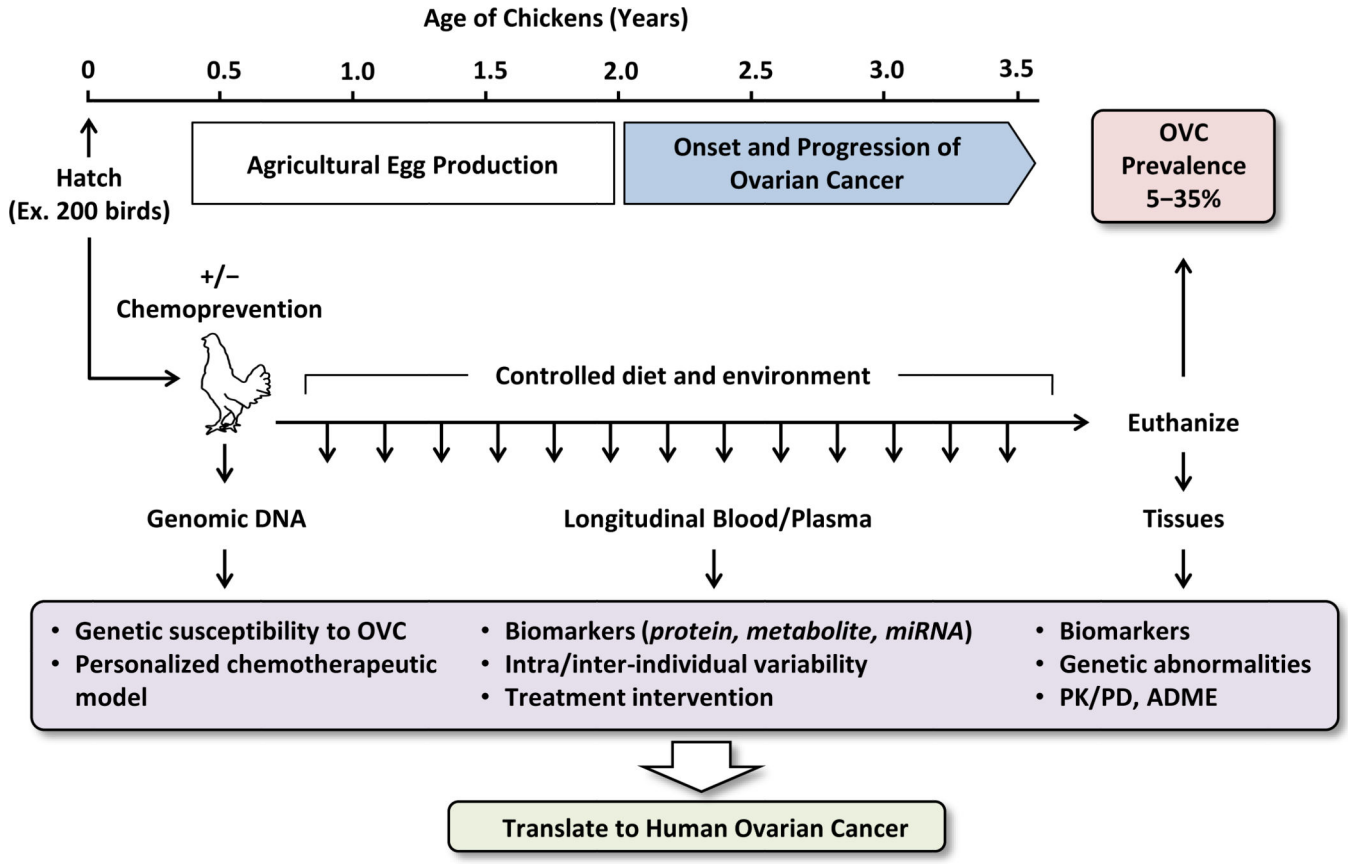

Figure 1.

Lifespan of chickens summarized in the context of the agricultural industry and ovarian cancer onset window overlayed with potential research opportunities afforded by the animal. PK/PD = pharmacokinetics/pharmacodynamics, ADME = absorption, distribution, metabolism, and excretion.

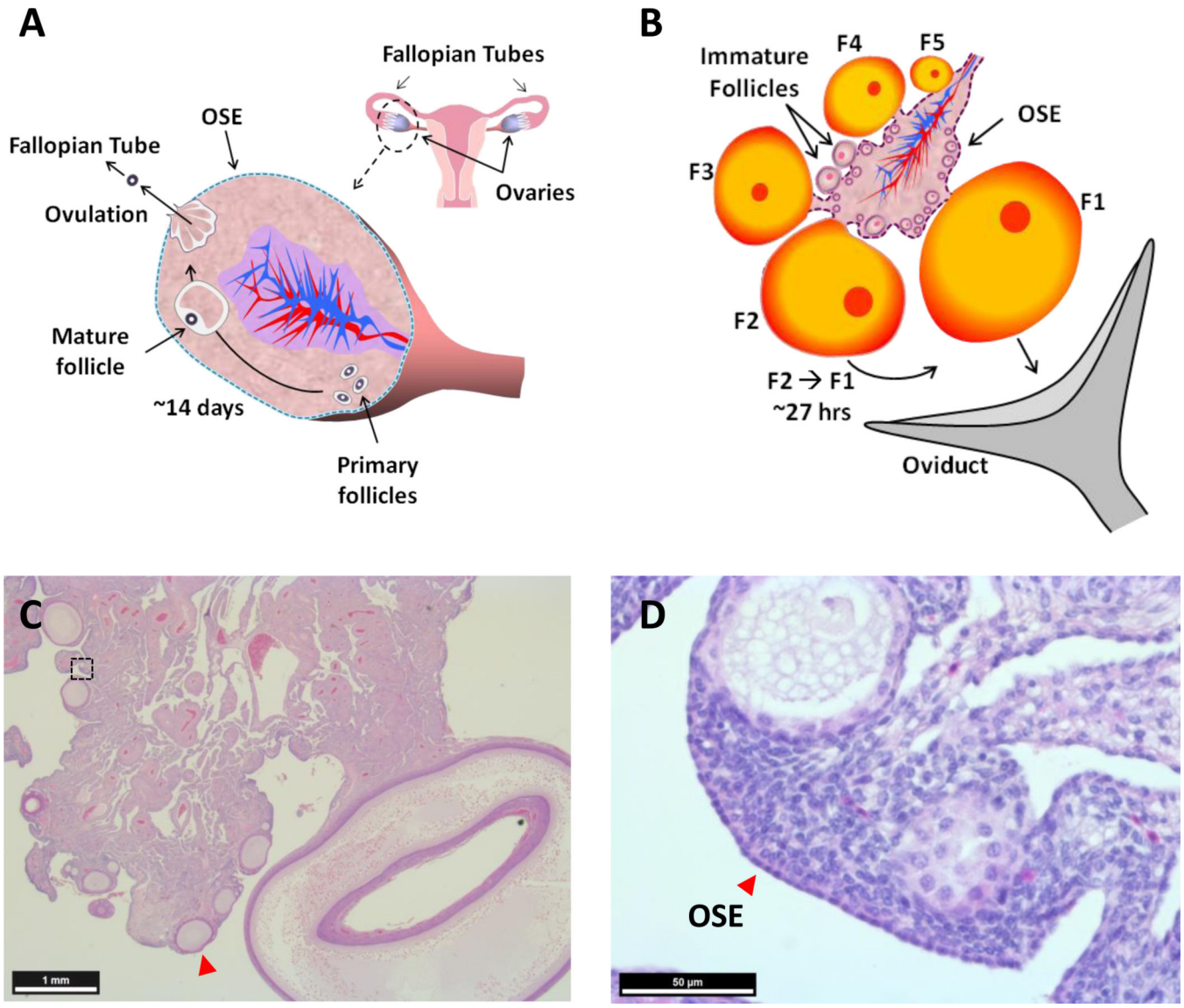

Figure 2.

Cross sectional illustration of a human ovary with an emphasis on the process of ovulation over ~14 days. (A) Cross sectional illustration of a chicken ovary with an emphasis on the process of ovulation over ~27 hours.(B) Formalin fixed hematoxylin and eosin stained tissues of a fully functional healthy ovary showing immature (arrow) and F-series follicles at 1.25× magnification (C) and the ovarian surface epithelium (OSE) at 40 × magnification (D).

Table 1

Summary of the biomolecular characteristics identified in chicken ovarian cancer. Evidence notes: IHC = immunohistochemical staining, mRNA = targeted messenger RNA analysis, Global mRNA = global (chip-based) messenger RNA analysis, WB = Western blotting, ELISA = enzyme-linked immunosorbent assay, Flow Cyt. = flow cytometry, LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry.

Reference (year)	Proteins/Genes of Interest	Notes	Evidence
Rodriguez-Burford *et al.* [65] (2001)	CA-125 CEA cytokeratin AE1/AE3 cytokeratin 34βE1/E2 EGFR erbB-2 (Her2) Ki-67 Lewis Y p27 PCNA Tag 72 TGF-α MUC-1 and MUC-2	CA-125, PCNA, p27, and TGF-alpha IHC staining was negative in OVC tumors EGFR, Her2, AE1/AE3, 34βE1/E2, Lewis Y, CEA, and Tag 72 IHC staining was positive in OVC tumors	IHC
Giles *et al.*[42] (2004)	Ovalbumin	Ovalbumin is expressed primarily in healthy oviducts and it is the most abundant protein in egg white. Not normally expressed in healthy ovaries but was found to be up-regulated in OVC tumor tissues.	IHC
Giles et al.[75] (2006)	PCNA Cytokeratin Progesterone receptor Vimentin	Cytokeratin, PCNA, and progesterone receptor IHC staining was positive in primary OSE cells and OVC tumors. Vimentin showed negative/weak IHC staining in OSE cells and OVC tumors	IHC
Urick *et al.*[76] (2006)	COX-1 COX-2	COX-1 was up-regulated in chicken OVC tumors vs. healthy ovaries but no difference was detected for COX-2 levels.	mRNA, IHC
Jackson *et al.*[36] (2007)	CA-125	Up-regulated in OVC tumors and 90% of ascites-derived primary OVC cells after 28 hrs in culture	WB, IHC
Stammer *et al.*[77] (2008)	Selenium-binding protein 1	Expressed in both healthy and OVC tissues but lower levels observed in OVC tumors	mRNA, WB, IHC
Urick *et al.*[30] (2008)	VEGF COX-1 COX-2 Prostaglandin E_2	VEGF levels were measured in ascites-derived primary OVC cells from chickens as a function of COX-1 and COX-2 specific non-steroidal anti-inflammatory inhibitors.	mRNA, IHC, ELISA
Ansenberger *et al.*[38] (2009)	E-cadherin	Up-regulated in OVC tumors relative to healthy ovarian tissues	mRNA, IHC, WB
Hakim *et al.*[37] (2009)	p53 H-ras K-ras Her2	Mutational frequencies in chicken OVC tumors for p53 (48%), H-ras (0%), and K-ras (3%) were similar to human OVC. IHC staining for Her2 was positive in 53% of chicken OVC tumors	DNA seq., IHC
Urick *et al.*[31] (2009)	Prostaglandin E_2	Decreased levels as a function of aspirin treatment	ELISA
Zhuge *et al.*[78] (2009)	CYP1B1	P450 enzyme up-regulated in chicken OVC tumors	mRNA
Ahn *et al.*[79] (2010)	Cysteine cathespins	Over-expressed in OVC tumors relative to normal ovaries	mRNA
Hawkridge *et al.*[43] (2010)	Ovostatin 2	Predicted member of the MEROPS I39 protease inhibitor family; up-regulated in OVC plasma, tumors	LC-MS/MS proteomics
Seo *et al.*[80] (2010)	Claudin 10	Up-regulated in OVC tumors	mRNA

Reference (year)	Proteins/Genes of Interest	Notes	Evidence
Trevino *et al.*[39] (2011)	Paired box 2 protein E-cadherin	Identified several oviduct-specific proteins suggesting oviductal involvement in chicken OVC	Global mRNA, IHC
Choi *et al.*[81] (2011)	Matrix metalloprotease 3 (MMP-3)	MMP-3 was over-expressed in OVC tumors	mRNA
Dixon *et al.*[50] (2011)	Plasma N-linked glycome	Pilot study investigating the potential for N-linked glycans from plasma proteins to serve as OVC biomarkers	LC-MS/MS
Gonzalez-Bosque *et al.* [40] (2011)	E-cadherin	Metastatic chicken OVC tumors showed higher E-cadherin levels than localized OVC tumors.	Global mRNA
Lim *et al.*[44] (2011)	Alpha-2 macroglobulin	Up-regulated in serous OVC tumors	mRNA
Seo *et al.*[82] (2011)	CASP-1,2,3,6, and 9 BCL2	Apoptosis gene expression signature of chicken OVC was studied. Cysteine aspartate-specific protease 1 was up-regulated in OVC	mRNA
Yu *et al.*[83] (2011)	Mesothelin Mesothelin autoantibodies	Investigated the presence of mesothelin in OVC tissues and mesothelin autoantibodies in sera	mRNA ELISA
Eilati *et al.*[61] (2012)	COX-1 COX-2 Prostaglandin E_2	COX-1, COX-2, and PGE_2 were all up-regulated in OVC vs. healthy ovaries.	mRNA, IHC
Jeong *et al.*[84] (2012)	AHCYL1	S-adenosylhomocysteine hydrolase-like protein 1 levels were associated with OVC	mRNA
Lee *et al.*[85] (2012)	Cyclins Cyclin dependent kinases Cylcin dependent kinase inhibitors	Cell cycle-related gene regulation in OVC tissues was investigated at the transcriptional and post-transcriptional level	mRNA
Lee *et al.*[86] (2012)	Pleiotrophin	Estrogen regulated protein expressed primarily in the oviducts of healthy birds. Expressed in OVC tumors	mRNA, IHC
Lim *et al.*[87] (2012)	Secreted phosphoprotein 1 (SPP1)	Down regulated in oviductal epithelium upon exposure to estrogen but up-regulated in chicken endometrial OVC	mRNA
Lim *et al.*[88] (2012)	SERPIN B3	Member of the serpin protease family; up-regulated in chicken OVC tumors with proposed potential as a prognostic marker for platinum resistance	mRNA
Lim *et al.*[89] (2012)	SERPIN B11	Up-regulated in endometrial OVC tumors of the chicken	mRNA
Andrews Kingdon *et al.* [51] (2013)	Ovostatin 2 TTR	Validation and quantification of Ovostatin 2 and transthyreitin in plasma by selected reaction monitoring LC-MS/MS assay	LC-MS/MS proteomics
Bae *et al.*[90] (2013)	Beta-catenin	Associated with cadherins and involved with intercellular adhesion. Over-expressed in OVC tissues	mRNA
Braderic *et al.*[91] (2013)	T cells B cells	T and B cells were measured following sonography levels were found to correlate with the presence and stage of OVC	Sonography, Flow Cyt., IHC
Lee *et al.*[92] (2013)	DNMT1 DNMT3A DNMT3B	Expression patterns and cellular location of DNA methyltransferases in OVC and healthy chicken ovaries	mRNA
Lim *et al.*[72] (2013)	WNT4	Oviduct-specific protein over-expressed in OVC tumors	mRNA
Lim *et al.*[93] (2013)	Beta-defensin 11	Differentially expressed in OVC tumors versus healthy ovarian tissues.	mRNA, IHC
Tiwari *et al.*[41] (2013)	E-cadherin N-cadherin Cytokeratin	Characterization of several ascites-derived chicken ovarian cancer cell lines.	mRNA, WB

Reference (year)	Proteins/Genes of Interest	Notes	Evidence
	Vimentin Mesothelin Inhibin Estrogen receptors (ER-α and ER-β) Progesterone receptor Ovalbumin	Chicken OVC cells showed higher expression levels of E-cadherin, N-cadherin, and vimentin and lower expression levels of cytokeratin compared to normal chicken OSE. Ovalbumin was up-regulated in OVC tumors in agreement with earlier studies but it was not detected in any of the chicken OVC cell lines.	