Mechanisms and pathobiology of ovulation

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The ovulatory process is extraordinary in that it constitutes a hormone-induced injury. Gonadotropin delivered via the follicular vascular wreath stimulates secretion of plasminogen activator by contiguous ovarian surface epithelial cells. A consequent elevation in interstitial plasmin activates collagenases and cleaves tumor necrosis factor α from its anchors on endothelium. Collagen fibril degradation and cellular death at the apex of the preovulatory follicle are hallmarks of impending ovulation. Follicular contractions rupture the weakened fabric at the apex, and the ovum, which has been disconnected from the underlying granulosa, is expelled; these components of the cascade are prostaglandin-mediated. Ovulation is required for fertility; unfortunately, it imparts a cancer risk to the ovarian surface epithelium. DNA-damaging reactive oxygen species are generated by inflammatory cells attracted into the vicinity of the ovulatory stigma. An ischemia-reperfusion flux coincident with ovulation and wound repair also contributes to genotoxicity. Potentially mutagenic lesions in DNA are normally reconciled by TP53 tumor suppressor-dependent cell-cycle arrest and base excision repair mechanisms; it is a unifocal escape that could be problematic. Epithelial ovarian cancer is a deadly insidious disease because it typically remains asymptomatic until it has metastasized to vital abdominal organs.

Introduction

A follicle recruited to ovulate emerges along the ovarian cortex and comes into apposition with the surface epithelium. The initiating signal for ovulation is the surge in secretion of luteinizing hormone (LH) that occurs during the late follicular phase. Proteolytic enzymes and inflammatory mediators liberated at the follicular-ovarian surface interface degrade collagen matrices and provoke cellular death. Follicular contractions facilitate rupture and ovum expulsion (Murdoch, 2000).

More than 90% of cancers of the ovary are thought to arise from the surface epithelium. Circumstances that avert ovulation protect against epithelial ovarian cancer (Bandera, 2005). Damages to DNA caused by oxyradicals are a major cause of cancer (Valko *et al.*, 2004). Indeed, reactive oxygen species produced during the periovulatory period compromise the DNA integrity of bystander ovarian surface cells. These cells proliferate and migrate to mend the void in the ovarian epithelium created by ovulation. It is conceivable that clonal expansion of a cell with unrepaired DNA is an initiating factor in the etiology of ovarian cancer (Murdoch, 2008). Early-stage disease is characterized by formation of an inclusion cyst which contains surface epithelial cells that have invaded the ovary (Feeley and Wells, 2001). Apparently the

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microenvironment of the inclusion is conducive to metaplastic changes that precede cancer. Malignant cells seed the abdominal cavity when a cyst ruptures. A mutant cell exfoliated during the mechanics of ovulation may account for cases of diffuse intraperitoneal disease in which the ovaries remain relatively uninvolved (Hamilton, 1992).

Objectives of this chapter are to provide overviews of the mechanisms and carcinogenic implications of ovulation with an emphasis on research using an ovine model.

Experimental paradigm

Mature western-range ewes were penned with vasectomized rams and observed for standing estrus (Day 0). Animals were treated on Day 14 with prostaglandin (PG) $F_2\alpha$ to synchronize luteal regression. An agonistic analog of gonadotropin-releasing hormone (GnRH) was administered 36 h after $PGF_2\alpha$ to elicit a preovulatory surge of LH. The dominant follicle within the pair of ovaries will consistently ovulate approximately 24 h after GnRH and form a normal corpus luteum. A translucent stigma develops within 2 h of ovarian rupture (Roberts *et al.*, 1985).

Ovulation: Proteolytic enzymes and tumor necrosis factor

Interstitial (type I) collagen constitutes the primary connective tissue component of the follicular theca and tunica albuginea. Basement membranes that circumscribe thecal capillary beds and support mural granulosa and ovarian surface cells are composed of type IV collagen (Luck, 1994). Fibrillar collagens are comprised of three polypeptide chains coiled into a helix; nascent α-chains consist of repeating triplets of glycine-X-Y, where X and Y are often proline or hydroxyproline (Shoulders and Raines, 2009). Type IV collagen forms a flexible mesh-like scaffold to which matrix constituents (laminin, entactin, perlecan) and epithelial cells attach (LeBleu *et al.*, 2007). Collagen breakdown within the apex of the preovulatory follicle is predictive of ovulation.

Two principal families of enzymes, plasminogen activators/plasmin and matrix metalloproteinases (MMPs), govern tissue dissolution during ovulation. Plasmin (fibrinolysin) is a pleiotropic serine protease that is derived from its zymogen by enzymatic activation. Two forms of plasminogen activator (PA) have been characterized in vertebrates: urokinase (u) and tissue (t) types. Most studies indicate that uPA mediates tissue degradation and carcinogenesis, whereas tPA (that has an affinity for fibrin) modulates thrombolysis (Andreasen *et al.*, 2000). Collectively the zinc/calcium-dependent MMPs ($n \geq 28$) degrade collagens, elastin, proteoglycans, and adhesion molecules. Classical mammalian collagenases truncate each of the polypeptide chains of fibrillar collagens at sites near the carboxyl end (3/4, 1/4 fragments). Matrix metalloproteinases share many structural and functional attributes but differ somewhat in substrate specificities. Types I and IV collagens are the prototype substrates for types I (MMP-1) and IV (MMP-2, gelatinase A; MMP-9, gelatinase B) collagenases. Matrix-degrading effects of metalloproteinases depend upon de novo production, proteolytic activation, and endogenous tissue inhibitor concentrations. Excision of latent collagenases (e.g., by plasmin), permitting a second autolytic cleavage of the Cys-Zn^{2+} bond that stabilizes the propeptide, exposes the catalytic domain of the enzyme. Tissue inhibitors of MMPs (TIMPs 1-4), which noncovalently interact on a 1:1 stoichiometric basis with enzymatic substrate-binding sites, limit the degree of extracellular damage that otherwise would be inflicted by untempered proteolysis (Nagase and Woessner, 1999).

Sheep ovarian surface epithelial cells secrete uPA toward the tunica albuginea and apical follicular wall in response to LH. Receptors for LH on ovarian surface cells are up-regulated

at proestrus by estradiol of preovulatory follicular origin (Murdoch *et al.*, 1999a). Ovarian epithelium in close proximity to the preovulatory follicle is exposed to surge concentrations of LH due to an acute histamine-mediated increase (4-12 h after GnRH) in permeability of and blood flow through the thecal vascular wreath (Halterman and Murdoch, 1986; Cavender and Murdoch, 1988).

An increase in plasmin within the apical hemisphere of preovulatory ovine follicles at 12 h after GnRH was attributed to secretion of uPA by ovarian surface epithelial cells (tPA was undetectable). When ovarian surface epithelium was removed surgically at 8 h following GnRH treatment, the follicular rise in uPA and ovarian rupture were negated. Furthermore, ovulation was suppressed by intrafollicular injection of uPA (but not tPA) antibodies at 8 h (Colgin and Murdoch, 1997) or α_2-antiplasmin at 16 h (Murdoch, 1998a) after GnRH. Plasminogen activators also were increased within the apices of preovulatory porcine (Smokovitis *et al.*, 1988) and rat (Peng *et al.*, 1993) follicles. Both uPA and tPA contribute to ovarian plasmin production and ovulatory efficiency in rodents (Hagglund *et al.*, 1996).

Collagenolysis was associated with apical accumulation of plasmin in preovulatory ovine follicles (Murdoch and McCormick, 1992). Explants of follicular wall released hydroxyproline-containing peptides (degraded collagen) after exposure to plasmin and injection of antiplasmin into preovulatory follicles repressed collagenase bioactivity of tissue extracts. The effect of plasmin on collagen breakdown was not transcription-dependent, but rather was related to proenzyme activation (Murdoch, 1998a). Collagenolysis also was elevated preferentially within the apex of preovulatory human follicles (Fukumoto *et al.*, 1981). Morphological observations indicate that preovulatory connective tissue disruption begins at the ovarian surface and advances inward to encompass the apical follicular wall (Bjersing and Cajander, 1975; Talbot *et al.*, 1987). Tunica/thecal fibroblasts and follicular steroidogenic (theca, granulosa) cells are sources of procollagenases (Tadakuma *et al.*, 1993). General chemical inhibitors of collagenases suppressed ovulation in rodents (Reich *et al.*, 1985; Butler *et al.*, 1991). Mice with a mutation in the type I collagen gene, conferring resistance to collagenase, are infertile due to anovulation (Liu *et al.*, 1995).

Tumor necrosis factor (TNF) α, by promoting collagenase gene expression and (at relatively higher concentrations) cellular death, is an intermediary of ovulation. The precursor cytokine is a transmembrane protein that upon cleavage yields a bioactive extracellular subunit. Mature (soluble) TNFα is a noncovalent homotrimer. Target tissue effects of TNFα are receptor subtype- and concentration-dependent. Plasma membrane glycoprotein receptors for TNFα (RI, RII) are present on virtually all nucleated cells. Receptors bind trimeric ligand through a homologous extracellular amino terminal motif. The cytoplasmic segment of TNFRI contains a death domain that upon receptor aggregation can evoke a caspase cascade leading to apoptotic (internucleosomal) DNA fragmentation and pyknosis. Nonlethal transcriptional events (to include collagenase gene up-regulation) also can be activated by TNFRI and TNFRII ligation. It remains unclear what mechanisms dictate pathways of signal transduction outcome - toward genomic stimulation with or without programmed death. At high tissue concentrations, TNFα initiates microvascular coagulation associated with necrotic cellular death and acute inflammation (Larrick and Wright, 1990; Baker and Reddy, 1996).

Tumor necrosis factor α was localized to thecal endothelial cells of preovulatory ovine follicles by fluorescence microscopy. Immunostaining of endothelium within the follicular apex declined abruptly with the approach of ovulation (cells within the counterpart basal wall were unaffected). Plasmin truncated TNFα, releasing it into the site of rupture (Murdoch *et al.*, 1997, 1999). Preovulatory follicles of other species (rat, bovine, human) also secrete TNFα (Terranova, 1997; deMola *et al.*, 1998). Urokinase and basement membrane-degrading MMPs were secreted

from human ovarian surface epithelial cells stimulated by TNFα (Yang *et al.*, 2004). Human TNFα is liberated from cells by a metalloproteinase disintegrin (Black *et al.*, 1997).

Types I and IV collagenases were induced in sheep follicular tissues by TNFα; these responses were abrogated by the transcriptional inhibitor actinomycin D (Johnson *et al.*, 1999; Gottsch *et al.*, 2000). Therefore, it appears that TNFα potentiates ovulatory collagenolysis by assuring that sufficient quantities of (pro) MMPs are synthesized. Intrafollicular injection of TNFα antibodies at 10-12 h after GnRH inhibited collagen degradation (Johnson *et al.*, 1999) and blocked ovulation (Murdoch *et al.*, 1997). Moreover, progressive (16-24 h post-GnRH) increases in apoptotic and necrotic cells within the ovarian surface epithelium, tunica albuginea, and apical follicular wall were nullified by immunoneutralization of TNFα (Murdoch 1994, 1995a,b; Murdoch *et al.*, 1997, 1999b). Secretion into the follicular fluid of low (nonlethal) concentrations of TNFα by the oocyte-cumulus complex facilitated collagen breakdown throughout the follicular wall (Johnson *et al.*, 1999). Microinjection of high concentrations of TNFα into the apical wall of explanted sheep follicles (to mimic the local extracellular milieu near ovulation) provoked stigma development (Murdoch *et al.*, 1999b). Addition of TNFα to perfusates of rat ovaries enhanced ovulation rates elicited by LH (Brannstrom *et al.*, 1995). Ovulation occurred in TNFα RI knockout mice during the peripuberal period; however, ovarian cyclicity was disrupted by 6 months of age (the peak in reproductive performance) (Roby *et al.*, 1999). Tumor necrosis factor α-induced hyaluronate-binding protein-6 was expressed in preovulatory rat (Yoshioka *et al.*, 2000), equine (Sayasith *et al.*, 2007), and porcine (Nagyova *et al.*, 2009) follicles.

Net proteolysis during ovulation is controlled by relative balances of enzymes to inhibitors. In theca of periovulatory rat follicles membrane type 1-MMP activates pro-MMP-2 by complexing with TIMP-2 (Jo *et al.*, 2004). Increased production of TIMP-1 and α_2-macroglobulin by granulosa cells serves to confine the extent of ovulatory tissue destruction and assure that a viable corpus luteum can be formed (Curry and Smith, 2006).

Ovulation: Progesterone and prostaglandins

During the preovulatory period, steroidogenic function of the follicle shifts from an estrogen- to progesterone-producing gland. It has been recognized for many years that the rise in progesterone is more than just an index of transition toward the luteal phase. Inhibitors of progesterone biosynthesis/action suppress ovulation (Zalanyi, 2001). That inhibition of follicular prostaglandin (PG) production (by nonsteroidal antiinflammatory agents, most notably indomethacin) inhibits ovulation also is well established. Progesterone and prostaglandins have been implicated as effectors of collagenase production (Murdoch *et al.*, 1986, 1993).

A marked increase in progesterone in preovulatory ovine follicles occurred at 16 h after GnRH. The initial rise in prostaglandin production at 8 h favored PGE_2. Prostaglandin E_2 mediates losses in contacts among mural granulosa and cumulus cells (Murdoch, 1988). Progesterone subsequently induces 9-ketoreductase, which converts PGE_2 to $PGF_2\alpha$ (Murdoch and Farris, 1988). Prostaglandin $F_2\alpha$ stimulates contractile elements within the theca externa (Murdoch *et al.*, 1993). Expressions throughout the bovine follicular wall of prostaglandin receptors predict diverse actions in the ovulatory cascade (Bridges and Fortune, 2007).

Ovulatory mechanisms: Model and additional considerations

An integrative scheme is presented (Figure 1) whereby: 1) gonadotropic stimulation of uPA secretion by ovarian surface epithelial cells conjoined with the preovulatory follicle elicits a localized increase in tissue plasmin, which activates latent collagenases and releases TNFα from its anchors along thecal endothelium; 2) soluble TNFα augments collagenolysis by induction of MMP gene expression and

causes epithelial/vascular dissolution; 3) progesterone and prostaglandins facilitate collagenolysis and mediate oocyte delivery to the ovarian surface; 4) weakening of the apical follicular wall leads to stigma formation and ovarian rupture; 5) collagen remodeling, migration of interstitial cells, and neovascularization during differentiation of the sheep corpus luteum were dependent upon TNFα-induced MMP-2 production (Gottsch *et al.*, 2000).

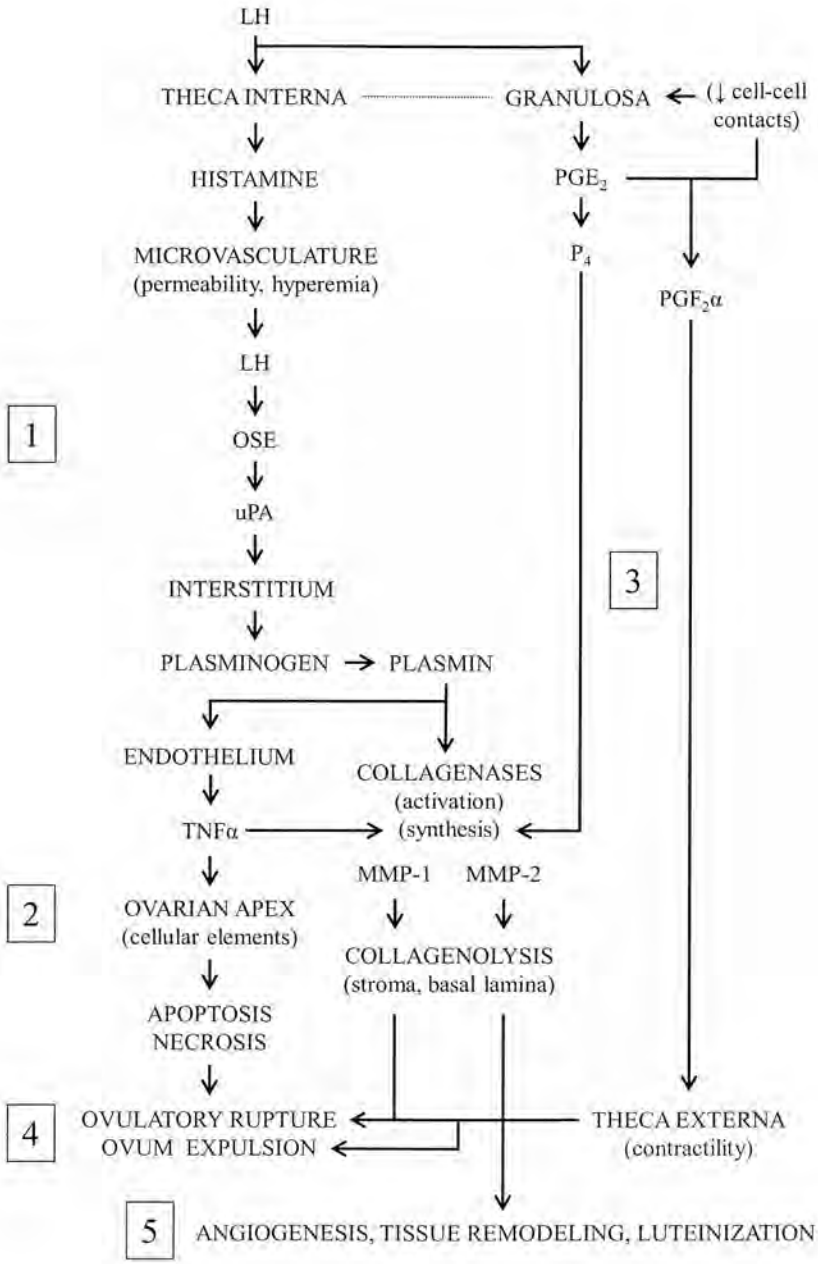

Fig. 1. Sequences of events dictating the ovulatory folliculo-luteal transition in the ewe. OSE, ovarian surface epithelium. P_4, progesterone. See text for explanations (1-5).

Proteases other than PAs/plasmin, MMP-1, and MMP-2 are potential regulators of ovulation. Matrix metalloproteinase-9 was elevated in fluid of preovulatory follicles of pigs (Driancourt *et al.*, 1998) and horses (Riley *et al.*, 2001). Collagenase-3 (MMP-13), which degrades collagens I-III, was increased in preovulatory rat follicles (Balbin *et al.*, 1996; Komar *et al.*, 2001). Cathepsin L, a lysosomal cysteine protease member of the papain family, and ADAMTS-1, a disintegrin and metalloproteinase with thrombospondin-like motifs, were induced in preovulatory follicles of rodents. Mice with a mutant progesterone receptor gene, which failed to ovulate, expressed MMP-2, -9, and -13 upon gonadotropic stimulation, however, mRNAs encoding cathepsin L and ADAMTS-1 were reduced (Robker *et al.*, 2000). Anovulation in a rat polycystic ovarian disease model has been related to increased expression of a lysyl oxidase that initiates cross-link formation of collagen and elastin (Endo *et al.*, 2001).

Thromboxanes and lipoxygenase products of arachidonate catabolism also have been implicated in the mechanisms of ovulation (Carvalho *et al.*, 1989; Wilken *et al.*, 1990). Functional cyclooxygenase and lipoxygenase pathways overlapped in a murine model of ovulation (Kurusu *et al.*, 2009).

Ovulation-cancer connection: Circumstantial evidence

Common (surface epithelial) cancer of the ovary is an ovulation-related disease. Inhibition of ovulation conferred by oral contraceptive use, pregnancy, and lactation safeguard against ovarian cancer (Bandera, 2005).

An ovulation-cancer hypothesis was first proposed by Fathalla in 1971, who suggested that "incessant" ovulations (without intervening dormant periods afforded by pregnancy) caused transformation of the ovarian epithelium. Exposure to estrogen-rich follicular fluid and injury were suspected causes.

Positive correlations among lifetime ovulations, ovarian precursor lesions, and carcinoma have been documented for women. In one analysis (Purdie *et al.*, 2003) there was a 6% increase in cancer risk with each ovulatory year. It appears that the most aggressive/damaging ovulations occur during the peak reproductive years and that ovulations have a more significant impact in premenopausal- than postmenopausal-onset ovarian cancer (Tung *et al.*, 2005).

Assisted reproductive programs that implement ovulation-inducing protocols would presumably increase the carcinogenic risk. Yet data relating use of fertility drugs to ovarian cancer have been inconclusive. Some have concluded that women who do not become pregnant and are subjected to multiple treatments are at an elevated risk, while others suggest weak or no significant correlations (Mahdavi *et al.*, 2006; Chene *et al.*, 2009). Overall rates of ovarian cancer have remained relatively constant despite the widespread application of ovulatory stimulants. Because the latency between initiation (at ovulation) and manifestation of established disease can be quite long (30-40 years or more), it will be important to continue to monitor recipients of superovulation regimens.

Support for the ovulation-cancer concept comes from observations of intensive egg-laying hens. These animals ovulate nearly every day and progress to carcinomatosis at a high frequency (4-40% depending on reproductive history and age) (Fredrickson, 1978; Damjanov, 1989; Rodriguez-Burford *et al.*, 2001). Inhibition of ovulation with a progestin protected hens from ovarian cancer (Barnes *et al.*, 2002).

There are essentially no published data on spontaneous rates of ovarian cancer among nonhuman mammals. One would expect incidences to be low because females of most species are either pregnant, lactating, or seasonally-anestrus for most of their reproductive lives. Inclusion bodies of surface epithelium have been reported in ovaries of ewes (Murdoch, 1994). In rodents, ovarian

surface epithelial stratifications, invaginations, and cysts were related to lifetime ovulations (Clow *et al.*, 2002; Tan *et al.*, 2005) and cycles of ovulation induction or estrogen administration (Celik *et al.*, 2004; Burdette *et al.*, 2006, 2007; Gotfredson and Murdoch, 2007). Advancement to cancer occurred in superovulated rats whose ovaries were exposed to a mutagen (Stewart *et al.*, 2004).

Ovulation has been likened to an acute inflammatory reaction (Espey, 1994). Whether inflammation is involved in ovarian carcinogenesis is a subject of recent attention (Shan and Liu, 2009).

Carcinogenic implication of ovulatory genotoxicity

Base damages to DNA caused by reactive oxygen species are an inevitable by-product of physiological metabolism. To combat this predicament, animals have evolved elaborate enzymatic antioxidant defense mechanisms (superoxide dismutase, glutathione perioxidase, catalase); however, these are less than perfect, and toxic oxidants find their way to DNA targets (Collins, 1999).

The N7-C8 bond of guanine is particularly susceptible to attack by the unpaired electron of hydroxyradical. Arguably, 8-oxoguanine is the most important mutagenic lesion in DNA; mispairing with adenine during replications can yield GC-to-TA transversions often detected in tumor cells (Grollman and Moriya, 1993; Cunningham, 1997; Fortini *et al.*, 2003). Ovarian surface epithelial cells isolated from the perimeters of ovulated sheep, human, and hen follicles contained concentrations of 8-oxoguanine that exceeded those of cells obtained from extrinsic areas not affected by ovulation (Murdoch *et al.*, 2001, 2005; Murdoch and Martinchick, 2004). Reactive oxidants are generated by leukocytes which are attracted (by fragments of collagen) into the vicinity of the ovulatory stigma and undergo a respiratory burst (Murdoch and McCormick, 1993). Another contributing determinant of genotoxicity is the ischemia-reperfusion flux that coincides with ovulation and wound reparation (Murdoch et al., 1983; Cavender and Murdoch, 1988). Challenges to the genetic integrity of the ovarian surface epithelium were negated by pharmacological ovulation blockade (Murdoch *et al.*, 2001).

A defective tumor suppressor gene, such as those that overexpress competitive mutant forms of the growth-inhibitory BRCA1/2 or TP53, is a probable basis for developing ovarian neoplasia as a result of ovulation (Aunoble *et al.*, 2000; Cvetkovic, 2003). Oxidative damages to guanine persisted in ovine ovarian surface epithelial cells that were affected by ovulation in vivo and in which synthesis of TP53 was then negated in culture by an antisense oligonucleotide; this was related to discordant cellular growth rates and expression of the cancer antigen CA-125 (Murdoch, 2003). Chromosomal anomalies and metaplasia have been detected in repetitive subcultures (to mimic recurrent ovulation-wound repair) of ovarian surface epithelial cells of rodents (Godwin *et al.*, 1992; Roby *et al.*, 2000).

Fortunately, corruptions to DNA instigated by ovulation are normally conciliated by housekeeping cell-cycle arrest and base-excision repair mechanisms. TP53 allots the time required for repair and proof-reading (Vousden and LU, 2002). Polymerase ß performs the penultimate gap-filling function in the short-patch pathway (Fortini *et al.*, 2003; Sung and Demple, 2006). TP53 and polymerase ß were up-regulated in response to the oxidative stress of ovulation imposed upon the ovarian surface epithelium of sheep (Murdoch *et al.*, 2001). Production of TP53 and polymerase ß were enhanced by progesterone (Murdoch an Van Kirk, 2002). Progesterone also stimulated poly(ADP-ribose) polymerase (PARP) in ovine cells (Murdoch, 1998b). Poly(ADP-ribose) polymerase serves as an adjunct in DNA repair. Binding of PARP and synthesis of branched polymers of ADP-ribose in areas adjacent to a single-strand interruption functions as an antirecombinogenic element (Lindahl and Wood, 1999). Progesterone inhibited

proliferation (Wright *et al.*, 2002) and induced apoptosis (Rodriguez, 2003) in cultures of ovarian surface epithelial cells of macaques. The cells of the ovarian epithelium bordering postovulatory follicles of hens (which do not form a corpus luteum) undergo apoptosis and are resorbed during follicular atresia (Murdoch *et al.*, 2005). Ovarian inclusion bodies of surface epithelium can evidently be eliminated via the Fas apoptotic system (Ghahremani *et al.*, 1999).

Antioxidants and ovarian cancer prophylaxis?

There is epidemiological evidence suggesting an inverse relationship between consumption of the antioxidant vitamin E and risk of ovarian carcinoma (Fairfield *et al.*, 2001; McCann *et al.*, 2001). Similar reports have advocated protective effects of vitamin E against cancers of the lung, colorectum, cervix, and prostate gland (Tamini *et al.*, 2002). It appears that in general incidences of oxidative DNA lesions and susceptibility to cancer are potentiated by micronutrient (e.g., antioxidant vitamin) deficiencies (Ames and Wakimoto, 2002). The circulatory antioxidant status of ovarian cancer patients was reduced compared to age-matched controls (Senthil *et al.*, 2004).

DNA of ovarian surface epithelial cells associated with the ovulation stigma of ewes was protected from oxidative base damage by pretreatment with natural-source vitamin E; programmed death within the surface epithelium, ovulation, and pregnancy outcome were not affected (Murdoch and Martinchick, 2004). Oxoguanine-positive surface cells of gonadotropin-primed/superovulated ewes treated with the tumor suppressor disruptor dimethylbenzanthracene and the mitogen estradiol-17ß hypersecreted uPA and formed ovarian inclusion cysts containing proliferative epithelium; these responses were circumvented by pretreatment with vitamin E (Murdoch *et al.*, 2008). Ischemia-reperfusion injury to grafts of ovarian tissues was reduced by vitamin E (Nugent *et al.*, 1998).

Vitamin E is an effective chain-breaking antioxidant in cellular membranes and thereby contributes to membrane phospholipid stability and safeguards intracellular molecules against damage caused by free radicals (Herrera and Barbas, 2001). Vitamin E also can act via mechanisms beyond its oxidant-quenching properties (Kline *et al.*, 2001; Azzi *et al.*, 2002). Nitric oxide production by endothelial cells and superoxide release by leukocytes were suppressed by vitamin E. Nonredox modes of α-tocopherol action include inhibitory and stimulatory effects on rates of mitosis and removal of damaged DNA, respectively. Therefore, vitamin E could act during the postovulatory period to impede untoward proliferative responses of ovarian surface epithelial cells until repairs to DNA can be accomplished.

Ovulation and ovarian cancer: Model and additional considerations

The DNA of ovarian surface epithelial cells contiguous with the site of ovulation is compromised by oxyradicals. It is proposed that this constitutes a first step in the etiology of ovarian tumorigenesis (Figure 2). To fend off accumulations of potentially harmful mutations it is essential that accurate restoration or proficient removal of anomalous cells occurs. The level of danger hence escalates when a cell (as a prelude to mutation) averts (due to a malfunctional tumor suppressor) repair or death. Perhaps the ovarian epithelium is vulnerable to genetic damages that are not reconciled because it has not been under evolutionary pressure to respond to superfluous ovulations (Auersperg *et al.*, 1998). Lifetime ovulations in most animals are kept to a minimum by pregnancy and anestrus.

It remains uncertain why, in particular, the ovarian surface epithelium is so prone to neoplastic transformation; after all, it represents only a small fraction of the diverse cell-types that populate

the ovary. Susceptibility may hinge on the fact that (normal) ovarian surface epithelial cells are of an uncommitted phenotype. Unlike the Mullerian epithelia of the female reproductive tract, development of ovarian surface cells is arrested at an immature pluripotent/stem stage (Auersperg *et al.*, 2001).

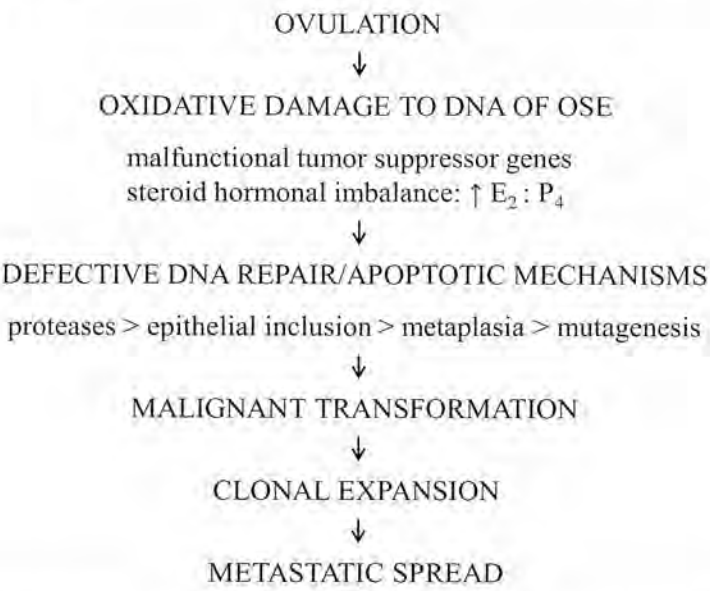

Fig. 2. Hypothetical role of ovulation in the chronology of epithelial ovarian cancer. E_2, estradiol.

The sequences of events that lead to common ovarian cancer are multifactorial. Several aberrant phases are undoubtedly required to yield a malignant phenotype with distinct growth and metastatic advantages (Figure 2). Ovarian cancer is generally considered to have some level of hormonal involvement; progestins are protective and gonadotropins, androgens, and estrogens are facilitative (Salehi *et al.*, 2008). Paracrine-autocrine modulators (growth factors and cytokines) can also influence ovarian cancer cell behaviors (Auersperg *et al.*, 2001). Metastatic spread is protease-dependent; urokinase and downstream matrix metalloproteinases, that digest basement membranes and interstitial connective tissues, are of particular importance (McDonnel Smedts *et al.*, 2005). Vascular endothelial growth/permeability factor is secreted by ovarian cancer cells and has been related to ascites formation and metastasis (Bamberger and Perrett, 2002).

Because the prognosis for ovarian cancer patients with metastatic disease is so poor, and early detection has proven elusive, it is imperative that methods of chemoprevention be explored. Perhaps supplemental vitamin E could be of value to individuals at risk for the development of ovarian cancer (e.g., those with a genetic predisposition who are not using a contraceptive that inhibits ovulation).

It is important to emphasize in closing that a correlative association between ovulation and the initiation of common ovarian cancer does not prove causal effect and that an "ovulation model" is not absolute and does not explain the genesis of all epithelial ovarian tumors. For example: protection is conferred by tubal ligation or hysterectomy in spite of uninterrupted ovulation; protection provided by one gestation with breast feeding or short-term oral contraceptive use is superior to the predicted benefits of those missed ovulations that would have occurred; reduced numbers of ovulatory cycles due to menstrual irregularities and infertility (e.g.,

polycystic ovarian syndrome) are independent risk factors for ovarian cancer; and in addition to ovulation, other inflammatory responses (endometriosis and exposure of the ovarian surface to exogenous irritants such as talc, pesticides, or viruses) have been linked to ovarian cancer (Holschneider and Berek, 2000; Ness *et al.*, 2000; Salehi *et al.*, 2008; Shan and Liu, 2009).

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