Endogenous Hormones and Ovarian Cancer: Epidemiology and Current Hypotheses

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Abstract
The effect of major epidemiologic risk factors for ovarian cancer has been reviewed in the light of several hormonal hypotheses, including the gonadotropin, androgens, progesterone, estrogens, insulin-like growth factor-I, and insulin hypotheses. The role of inclusion cyst formation and Mullerian epithelium differentiation in the pathophysiology of the disease are also briefly outlined. Although based on limited data, the observed tendency in recent evidence suggests possible etiologic roles for elevated androgens and estrogens and decreased progesterone in the pathogenesis of ovarian cancer. A direct effect of gonadotropins cannot be entirely ruled out, but it is plausible that their effect on ovarian cancer risk is mediated by stimulation of ovarian steroidogenesis. Insulin-like growth factor-I also emerges as a hormone that may be directly involved in the pathogenesis of the disease, but thus far only one prospective study has examined this association. Hyperinsulinemia is an unlikely risk factor for ovarian cancer. The observed tendency for an increased risk with androgens from ovarian origin (in premenopausal women), the lack of association with adrenal androgens, and the relatively weak associations observed with obesity, hormonal replacement therapy use, and endogenous hormones after menopause suggest that ovarian synthesis of sex steroids rather than their circulating levels may be etiologically important. More data from prospective studies will be crucial to improve our understanding of the etiologic role of endogenous hormones in the pathogenesis of ovarian cancer. Such data will ultimately provide opportunities for research targeted; at early detection and preventive interventions. (Cancer Epidemiol Biomarkers Prev 2005;14(1):98–107)

Introduction
Ovarian cancer is the fourth most frequent cause of cancer death and the most lethal of all gynecologic tumors in women from North America and northern and western Europe (1). The lifetime risk of ovarian cancer for women in industrialized countries is ~2% (2). Women carriers of BRCA1 or BRCA2 mutations are at higher risk, with average cumulative risks by age 70 of ~40% and 10%, respectively (3).

According to the cell types from which ovarian tumors are presumed to originate, they are divided into three major groups: surface epithelial-stromal tumors, germ cell tumors, and sex cord-stromal tumors, each having distinct clinical and epidemiologic characteristics (Fig. 1; refs. 4-7). The epithelial tumors account for 80% to 90% of ovarian malignancies in the United States and western Europe (8) and usually dominate the data from cancer registries. They are further subdivided into serous (~50% of all ovarian malignancies), mucinous (5-10%), endometrioid (10-25%), clear cell (4-5%), Brenner, undifferentiated (5%), and mixed subtypes (5, 9). Most epidemiologic studies have focused on the group of epithelial ovarian cancers as a whole, although some studies were able to investigate and show possible differences in risk factors according to histologic subtype (6, 10-13). Fifteen percent of epithelial ovarian neoplasms are classified as “borderline tumors,” which are mostly of serous and mucinous subtypes (5, 14-16). They comprise a separate entity, usually with epidemiologic characteristics similar to the frankly invasive tumors, but occur in younger women, present at an earlier stage, and have a favorable prognosis (5, 15, 17).

In this article, we summarize the epidemiologic evidence with respect to the etiology of epithelial ovarian cancer, with a special emphasis on the role of endogenous hormones. An outline of well-established risk factors will be followed by a brief review of the possible role of inclusion cysts and Mullerian epithelium differentiation in the pathology of the disease. We shall then discuss the involvement of endogenous gonadotropins, sex steroids, insulin, and insulin-like growth factor-I (IGF-I) in the light of the available epidemiologic evidence and some experimental data.

Basic Epidemiologic Risk Factors
The highest age-standardized incidence rates of ovarian cancer are observed in countries from northern Europe, North America, and western Europe, whereas rates are severalfold lower (up to 10-fold lower) in most parts of Africa and Eastern Asia (1, 2). Within a particular country, incidence rates may differ among ethnic groups (2). In the United States, the highest rates are observed among non-Hispanic white women, rates are intermediate in Hispanic White women, and rates are lowest in African American and Asian women (2). Migration studies have shown that ovarian cancer incidence rates tend to approach those of the country of adoption (18-20).

Well-established risk factors for ovarian cancer are age, family history of ovarian cancer, and infertility, whereas increasing parity, oral contraceptive use, hysterectomy or tubal ligation decrease risk (4, 13, 21-23). The protective effect of pregnancy has been uniformly shown in North American, European, and Asian populations (22), with a 10% to 16%...
decrease in risk for each additional pregnancy (10, 23, 24). Some studies (10, 11, 25), but not all (6, 12, 13, 26), have indicated that the protection may be restricted to nonmucinous tumors. Several studies have reported a significant trend of decreased ovarian cancer risk with increasing age at first birth (13, 23, 27-30) and two studies reported a decrease of risk with greater age at last birth (28, 30).

It has been estimated that ≥5 years of oral contraceptive use confers a 30% to 50% reduction in ovarian cancer risk (23, 31, 32). The favorable effect has been observed for at least 10 to 15 years since last use (23, 31, 33, 34), and recent studies showed that the protection may persist for even longer (>20-25 years; refs. 13, 35, 36). In contrast, use of exogenous hormones for menopause-related symptoms could be associated with an increased risk of ovarian cancer incidence or mortality (37-43). Most of the recent and well-designed studies showed that prolonged periods of hormone replacement therapy (HRT) use (≥5-10 years) conferred 1.5- to 2-fold increase in risk (39-44). Three studies (40, 42, 45) could investigate the effect of type of HRT on ovarian cancer, and in two of these (40, 42), risk was elevated specifically among those who used estrogen unopposed by progesterone (40, 42). However, the Women’s Health Initiative randomized trial showed that women assigned to combined estrogen plus progesterone HRT also experienced higher risk of ovarian cancer in comparison with women on placebo [1.58 (0.77-3.24); ref. 43]. Some data suggest that the adverse effect of HRT use may be more pronounced for endometrioid and clear cell tumors and less so for mucinous tumors (13, 46, 47). Data regarding possible modification of the effect of HRT according to previous hysterectomy are largely inconclusive (40, 42, 43, 45).

Ovarian cancer risk is possibly decreased with increasing duration of breast-feeding (13, 22) and twin pregnancies (48-50), possibly increased in women with polycystic ovary syndrome (PCOS; ref. 51) and endometriosis (52-54), but not clearly related to ages at menarche or menopause (13, 22, 55, 56) or fertility drug use (57-60). Infertility seems to increase risk of ovarian cancer among nulligravid/nulliparous women with refractory infertility but not among parous women (22, 23). A recent pooled study combining data from eight case-control studies conducted between 1989 and 1999 in the United States, Denmark, Canada, and Australia showed that among nulligravid women attempts for >5 years to become pregnant compared with attempts for <1 year increased risk of ovarian cancer close to 3-fold (57).

Excess body weight possibly confers a moderate increase of risk of developing ovarian cancer risk (on average between 20% and 40%; refs. 61-65), although in many studies the direct associations did not reach statistical significance (66-70), and no association (66, 71, 72) or even a decrease in risk has been observed (73, 74). Few studies indicated that a higher body mass index during adolescence or early adulthood may increase risk of ovarian cancer overall (71, 72, 75) or during premenopausal years specifically (66), but no association was observed in other studies (67, 76). Generally, weight gain throughout adulthood has not been related to increased risk of ovarian cancer (66, 69, 71, 75-77).

Only four studies have reported on the association of waist-to-hip ratio, as a measure of central (android) obesity, with ovarian cancer (66, 69, 71, 78). Despite the lack of association of body mass index with ovarian cancer in one cohort (71) and one large case-control study (69), high waist-to-hip ratio was associated with significantly increased risk of ovarian cancer in both of these studies as well as in a small case-control study in premenopausal women (78). No association was observed in a fourth (cohort) study (66).

Several recent studies have indicated a direct association of height with risk (61, 72, 74, 76, 77), and in some of these, the association seemed stronger in younger women (72, 74), whereas in other studies no association with height was found (67, 69, 75). The reports on the association of physical activity; with ovarian cancer have been contradictory, and no clear tendency has yet become evident (65, 68, 79-81). Several recent studies have shown increased risk of ovarian cancer with tobacco smoking overall (82, 83) and specifically for mucinous tumors (83-85).

The epidemiologic characteristics of ovarian cancer have given rise to several etiologic hypotheses. Among those that do not postulate a direct involvement of endogenous hormones are the incessant ovulation (86), the inflammation (87) and retrograde transport (88) hypotheses. The most widely cited is the incessant ovulation hypothesis, proposed by Fathalla, which states that long duration of ovulatory menstrual cycles increases risk of developing ovarian cancer. Indeed, well-established epidemiologic risk factors for ovarian cancer, such as the protective effect of oral contraceptive use, parity, and breast-feeding and the adverse effect of a high lifetime number of ovulatory cycles (89, 90), are consistent with the incessant ovulation hypothesis (Table 1; ref. 21). Fathalla’s hypothesis fails, however, to provide a rationale for other observations, such as the greater protective effect of both pregnancy and oral contraceptive use than that expected simply on the basis of the number of suppressed ovulations, the protection associated with twin pregnancies, and the lack of clear association with ages at either menarche or menopause (Table 1; refs. 4, 21, 23, 24, 48, 49).

Thus, the involvement of additional, especially hormonal, mechanisms have been proposed.

**Epithelial Origin of Most Ovarian Tumors and Formation of Inclusion Cysts**

Epithelial ovarian tumors are thought to arise from the ovarian surface epithelium (OSE) and its inclusion cysts (8). The OSE is composed of modified peritoneal mesothelial cells, which replicate as generative stem cells (i.e., the division of a surface epithelial cell yields two daughter cells with equal potential for further cell division; ref. 91). The OSE is separated from the hormone-producing ovarian stroma by a basement membrane and, underneath, a collagenous tissue layer, the tunica albuginea (92).

OSE has the potential to differentiate to either stromal or ectopic (aberrant) epithelial phenotypes (93). Auesperg et al. (92) proposed that the capacity of OSE cells to undergo epitheliomesenchymal conversion might be a homeostatic mechanism to accommodate OSE cells that become trapped within the ovary as stromal fibroblasts. An inability to undergo...
such conversion would preserve the epithelial forms within the ovarian stroma, which could lead to OSE aggregation and inclusion cyst formation (92). The epithelial phenotypes seem to be prone to metastasis, and the dysplastic changes they might ultimately lead to tumorigenesis (92, 94). It was proposed that differentiation of OSE cells specifically toward Mullerian types of epithelia, similar to those of the fallopian tube, endometrium, or endocervix, might confer a selective growth advantage through changes in hormone/growth factor receptors and responsiveness (reviewed in detail in ref. 66). Indeed, the most frequent type of epithelial ovarian malignancies—serous tumors—resemble the epithelium of the fallopian tube, whereas endometrioid tumors resemble the epithelium of the endometrium and mucinous tumors that of the endocervix (5, 9).

Although not conclusive (95), there is evidence to suggest that OSE inclusion cysts are more prone to malignant transformation than the surface epithelium itself (reviewed in refs. 8, 92). First, most early carcinomas of the ovary seem to be confined within the organ without involvement of its surface (8). Second, tubal metaplasia has been observed more frequently in inclusion cysts than in OSE itself (96). Third, OSE metaplasia has been found twice to thrice more frequently in inclusion cysts of ovaries contralateral to ovaries containing carcinomas than in ovaries of cancer-free subjects (97). Fourth, several ovarian carcinoma tumor markers, such as CA-125 or CA19-9, were identified immunohistochemically more often in the epithelium of the inclusion cysts than in the OSE itself (98-101). Epithelial tumors are also less frequent in the related pelvic peritoneal mesothelium, which has a much larger surface area (21).

It has been suggested that ovarian inclusion cysts form as a consequence of entrapment of OSE cells in the stromal tissue during repeated damage and remodeling of the OSE induced by ovulations (102). However, this may not invariably be the case, because the majority of cortical inclusion cysts are not in fact related to the repair of the ovulatory defect (8). Inclusion cysts can be found in ovaries from women of all ages, but their frequency increases with age, and they are commonly observed at late reproductive and postmenopausal ages (103). Alternative possible origins of the inclusion cysts are inflammatory adhesions involving the ovarian surface, PCOS (104), or infoldings, which are typical of the normal surface of the ovary. The ovarian stromal-mesothelial interface is dynamic, and proliferation of either component may result in the pinching off of portions of the mesothelium to form small cysts (8, 91). Finally, the OSE cells can secrete proteolytic enzymes, which may enable them to penetrate the underlying stroma (8, 105).

The epithelium covering the ovaries is avascular; therefore, its cells thrive more likely in the absence than in the presence of growth factors through paracrine than through endocrine mechanisms (106). OSE cells in inclusion cysts; embedded in the ovarian stroma and in immediate proximity to hormone and growth factor-producing cell types may be exposed through both endocrine and paracrine mechanisms (21, 106, 107).

### Table 1. Agreement between observed association of some epidemiologic factors with ovarian cancer in epidemiologic studies and as predicted by several etiologic hypotheses

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<thead>
<tr>
<th>Epidemiologic factor</th>
<th>Observed relationship with risk</th>
<th>Agreement between observed and predicted by etiologic hormonal hypothesis</th>
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<td>Incessant ovulation</td>
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<td>Endometriosis</td>
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<td>PCOS</td>
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<td>Tubal ligation/hysterectomy</td>
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NOTE: ↑/↓, increase/decrease/no clear or weak association with risk; +/−/0, agreement/disagreement between observed association with risk and as predicted under an etiologic hypothesis.

*Relationship established for endometrioid and clear cell tumors only.

Endogenous Hormones and Ovarian Cancer Development

Hormones and growth factors play a central role in regulating cell proliferation, differentiation, and apoptosis. Dysregulations of these processes may allow cells that have harbored mutations in proto-oncogenes and tumor suppressor genes to survive and expand clonally (108-110).

Pituitary gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)], androgens, estrogens, progesterones, and IGF-I have all been proposed to influence ovarian cancer development (21, 111). However, establishing their role in the pathogenesis of the disease is hindered by several characteristics of ovarian cancer. The traditional case-control design is of limited use, as a tumor growing in a key endocrine organ is likely to cause major alterations in the synthesis and circulating levels of sex steroids and other hormones. Consequently, the observed case-control differences in hormone levels may be a result of the presence of the tumor (inverse causation bias). Although conferring high mortality, the incidence rates of ovarian cancer are relatively low; thus far, very few prospective studies could investigate the role of prediagnostic hormone levels (112-114). Further difficulties in the design of analytic studies, especially in premenopausal women, are related to the large menstrual cycle variations in circulating estrogens, progesterone, and gonadotropins.

### Gonadotropins

The “gonadotropin hypothesis” is the first hormonal hypothesis that was developed to explain ovarian cancer pathogenesis. It postulates that ovarian cancer develops where high mortality, the incidence rates of ovarian cancer are relatively low; thus far, very few prospective studies could investigate the role of prediagnostic hormone levels (112-114). Further difficulties in the design of analytic studies, especially in premenopausal women, are related to the large menstrual cycle variations in circulating estrogens, progesterone, and gonadotropins. The effect of gonadotropins may be exerted either directly through activation of gonadotropin-responsive genes in those cells that would...
eventually undergo malignant transformation or indirectly through stimulation of ovarian production of sex steroids that could influence malignant transformation through paracrine or endocrine mechanisms (21, 107).

The existing experimental data on the direct effect of gonadotropin hormones on the proliferation of normal human OSE cells is inconclusive, the majority of studies showing either an increase in proliferation (115-117) or no effect (117, 118). The generalizability of the animal models, from which the gonadotropin hypothesis initially originated, to human epithelial ovarian cancer has been questioned, as the tumors induced in rodents are mostly of nonneoplastic origin (reviewed in refs. 21, 119).

Epidemiologic evidence indirectly supporting the gonadotropin hypothesis includes the well-documented protective effects of pregnancies and oral contraceptive use, which both suppress pituitary gonadotropin secretion (Table 1; ref. 21), and the increased ovarian cancer risk among women with PCOS [who have elevated circulating LH (51)]. However, the lack of increase in risk related to early age at menopause and with twin pregnancies [both of which are associated with increase in gonadotropin levels (120, 121)], the absence of an elevation; in the rate of increase of ovarian cancer incidence after menopause in spite of increasing LH and FSH levels (Fig. 1; refs. 1, 2), and the moderately increased risk with HRT use (38, 40) or obesity, which lower circulating FSH and/or LH (122, 123), suggest that gonadotropins themselves may not be directly responsible for alterations in ovarian cancer risk. Furthermore, three prospective studies showed either no difference or decreased blood levels of FSH and LH in ovarian cancer cases compared with controls (112, 113, 124).

In conclusion, more experimental and epidemiologic data are needed to establish a role for gonadotropins in the development of ovarian cancer and to understand the possible direct or indirect mechanisms mediating such action.

Androgens. In a seminal review article, Risch (21) proposed that ovarian cancer risk may be increased by excess androgenic stimulation of ovarian epithelial cells (the "androgen hypothesis").

In premenopausal women, theca-interstitial cells surrounding the developing ovarian follicles secrete ~50% of circulating androstenedione and ~25% of testosterone and dehydroepiandrosterone (DHEA; refs. 125-127). The adrenal glands contribute ~50% of circulating androstenedione and DHEA, up to 25% of testosterone, and virtually all of DHEA sulfate (125-127). The remaining 50% to 60% of circulating testosterone and 25% to 30% of DHEA are derived from peripheral conversion (e.g., in fat, liver, and kidneys) from androstenedione and DHEA sulfate (125-127). After menopause and the loss of ovarian follicles (together with the associated theca-interstitial and granulosa cells), the elevated levels of gonadotropins stimulate the ovarian synthesis of androgens by the secondary interstitial cells (direct descendants of the theca-interstitial cells of atretic follicles) and by the hilar cells (equivalent to testicular Leydig cells; refs. 127, 128). As a consequence, the ovarian contribution to circulating androstenedione decreases to ~20%, whereas testosterone production remains largely unchanged (or even increases slightly), so that its relative contribution to circulating levels is ~40% (128-130).

Several in vitro studies have shown increased cell proliferation of normal OSE cells after androgen administration (116, 131, 132). In an experimental study with guinea pigs, testosterone administration stimulated the growth of ovarian epithelial cells, resulting in formation of benign cyst and small adenomas in the ovarian parenchyma and papillomas on the ovarian surface (133).

Epidemiologic evidence in support of the androgen hypothesis includes the protective effect of oral contraceptive, which reduce androgen levels in both normoandrogenic and hyperandrogenic women (134-136) and the increased risk in women with a previous diagnosis of PCOS, a hyperandrogenic syndrome (Table 1; ref. 51). Histologic examinations of the ovaries of PCOS patients have shown an increased occurrence of epithelial inclusion cysts compared with ovaries of normoandrogenic women (104). PCOS is often associated with obesity and usually develops during puberty (137, 138), and it is tempting to relate recent findings of increased ovarian cancer risk in adolescents with high body mass index to a higher prevalence of PCOS among obese girls (71, 72, 75). Nevertheless, PCOS is observed also among lean women, in whom the androgen excess is more strongly related to elevated LH secretion, than in obese women (139).

Finally, patients with endometriosis who had been treated with danazol (which suppresses the pituitary secretion of gonadotropins but has androgenic properties) had ~3-fold higher risk of ovarian cancer in comparison with patients who took leuprolide (a gonadotropin-releasing hormone agonist) or no medication (54).

More direct epidemiologic evidence for androgen involvement in ovarian cancer pathogenesis comes from two prospective studies (112, 140). The first study was nested within a population-based serum bank in Washington County, Maryland, and included 31 case and 62 control subjects, of which 13 case and 26 control subjects were premenopausal at blood donation (112). The second study combined 132 ovarian cancer cases and 258 controls from three cohorts in New York, Umeå (northern Sweden), and Milan (Italy; 44 case and 84 control subjects were premenopausal; ref. 140). In premenopausal women, Helzlouer et al. (112) found that case subjects had 44% higher androstenedione levels than control subjects. In the pooled study, the difference was of a smaller magnitude (12%), although an evident, not statistically significant increase in risk with increasing androstenedione concentrations was observed [odds ratios (95% confidence intervals) for the second and third tertile of 1.72 (0.60-4.99) and 2.35 (0.81-6.82); P trend < 0.12; ref. 140]. Mean DHEA levels, measured in the study by Helzlouer et al., were also significantly higher in case than in control women (112), whereas no such difference was observed for DHEA sulfate in either study or for testosterone in the pooled study (140).

In postmenopausal women from the two studies, no difference in mean levels of any of the measured androgens between cases and controls was observed.

The lack of association of circulating androgens with ovarian cancer in postmenopausal women and the increase in risk observed with androstenedione and DHEA, but not with testosterone, in PCOS patients suggests that among premenopausal women suggest that ovarian cancer risk may be more strongly related to the intraovarian environment during a woman’s reproductive years rather than to the circulating levels of these hormones. Androstenedione is considered a weak androgen (or proandrogen; refs. 125, 138), but OSE cells possess the enzymes necessary for its conversion to the 10 times more potent testosterone (21, 141) and theca and granulosa cells can convert it to dihydrotestosterone (142) or estrogens (143).

Insights about the possible role of ovarian versus circulating hormone levels come also from the observation that during the fourth to fifth month of fetal development there is a parallel increase in the steroidogenic activity of ovarian interstitial cells and in the proliferative processes of OSE, which undergoes diffuse multilayered proliferation (144). These proliferative processes seem to end when the tunica albuginea is formed and separates the OSE cells from the hormonally active stroma (144). In contrast, in the testis, there is an early separation of the surface epithelium from the underlying sex cords by a much denser tunica albuginea, a difference that may account for the divergence in the growth patterns of the surface epithelium of the ovary and testis during fetal life (144, 145) and in the types of malignancies that develop during adult life.
Endogenous Hormones and Ovarian Cancer

(∼95% of testicular tumors are of germ cell origin and the remaining part are mostly sex cord-stromal tumors (146)). The potential importance of paracrine and intracrine exposures has been discussed in relation to other malignancies, including those of the prostate (47-149), and they may be of particular importance in ovarian pathogenesis.

In conclusion, preliminary data for androgen involvement in ovarian cancer development are accumulating, but more studies are necessary to establish the role and contribution of circulating and intraovarian androgens.

**Estrogens.** Granulosa cells of the premenopausal ovary secrete both estradiol and estrone and, at ovulation, OSE cells are bathed in the estrogen-rich follicular fluid, which may have concentrations of magnitude higher than circulating levels (21). In vitro experiments have shown the potential of estrogens to stimulate the proliferation of OSE cells (116), and treatment of guinea pigs with estradiol resulted in development of serous ovarian cysts (150).

Epidemiologic evidence about the role of estrogens in ovarian cancer is conflicting. Long-term use of any type of HRT seems to increase risk of ovarian cancer (39-42, 44) and points to a direct etiologic importance of persistently elevated estrogen concentrations. The weaker effect of combined estrogen and progestin than estrogen-only formulations observed in some studies might be due to the beneficial role of the progestin component. However, given the long latency of ovarian cancer (21) and that in some studies the effect of HRT on risk was confined to recent users support a role of estrogens as a stimulus of the growth of preexisting, undiagnosed cancers.

In postmenopausal women, obesity is associated with increased levels of circulating estrogens (151), but obesity seems to have a relatively weak adverse effect on risk, in contrast to the more pronounced relationships observed for cancers of the endometrium and postmenopausal breast (65). The lack of association between prediagnostic estrogen concentrations and risk of ovarian cancer in the two prospective studies also does not support a major etiologic role of elevated estrogens in postmenopausal women (112, 140).

The protective effect of oral contraceptives could be interpreted as supportive of the estrogen hypothesis as oral contraceptive use decreases ovarian estrogen production, and early to midfollicular phase circulating estrogens are maintained during use (21). At a first glance, the well-established protective effect of pregnancy seems to sharply contradict the estrogen hypothesis, as pregnancy is associated with ~100-fold increase in maternal circulating estrogen concentrations. However, along with other proposed beneficial effects of pregnancy in terms of ovarian cancer (e.g., increased progestogen levels and suppression of ovulation), after the first 4 to 5 weeks of pregnancy, nearly all estrogens are synthesized in the trophoblasts (i.e., the placenta and the ovarian contribution is minimal; refs. 152-154).

Estrogens could play an etiologic role in the development of endometrioid and clear cell tumors of the ovary as supported by observations that patients with endometriosis are at increased risk to develop these tumors (52, 54, 155). Endometriotic lesions have been shown to harbor molecular aberrations that favor increased local production of estradiol, including higher expression of aromatase and deficiency of 17β-hydroxysteroid dehydrogenase type 2 (that converts estradiol to estrone; ref. 156). Along the same lines, ~15% to 20% of endometrioid carcinomas of the ovary, for which estrogens are an established etiologic risk factor (157, 158), are associated with carcinoma of the endometrium (159), and in some studies, the adverse effect of HRT use was stronger for endometrioid and clear cell tumors (13, 46, 47). Finally, the plateau in the incidence of endometrioid and clear cell tumors after menopause (Fig. 1) also supports for a possible etiologic role of ovarian estrogens in the development of these histologic subtypes of epithelial ovarian cancer.

More epidemiologic data are necessary to establish the role of elevated estrogens in the development and progression of ovarian cancer by histologic subtype. Of particular interest would be studies addressing the effect of ovarian estrogen synthesis.

**Progesterone.** Several observations led Risch to suggest that progesterone might protect against ovarian tumor development (Table 1; ref. 21). He proposed that the protective effect of pregnancy, which exceeds that due to simple suppression of ovulation, may be due to the elevated ovarian production and blood levels of progesterone (21). Moreover, such protective effect of progesterone could account for the beneficial effect of twin pregnancies, which is in sharp contradiction with both the incessant ovulation and the gonadotropin hypotheses. Progesterone levels during multiple pregnancies are higher in comparison with singleton pregnancies (160-163), and there is some evidence that mothers of dizygotic twins may also have higher follicular phase serum progesterone than women with single pregnancies (164). A 3-year study in primates showed that the progestin component of the oral contraceptive Triphasil (levonorgestrel) has a potent apoptotic effect on ovarian epithelium (165), which could be mediated by transforming-growth factor-β (166) and up-regulation of p53 expression (167). These observations, together with the observed protective effect of later age at first birth and last birth in some studies are in line with the suggestion of Adami et al. (27) that pregnancy might clear the ovaries from cells that have already undergone malignant transformation.

Some of the protection conferred by oral contraceptive use might be due to the strong potency of the synthetic progestins, which may more than compensate for the decrease in endogenous progesterone synthesis in pill users (21, 168). The indication of a protective effect of progestin-only formulations is also intriguing (169, 170), because they suppress ovulation in ~40% of the users and their contraceptive effect is exerted through thickening of the cervical mucus and reduction of the endometrial receptivity to implantation (21, 31). Additionally, one study showed that combination oral contraceptive formulations with high progestin potency confer greater protection than those with low-progestin potency (171) and two other studies found that risk of ovarian cancer is higher in users of estrogen-only HRT than in combined estrogen-progestin HRT users (40, 42). A summary, evidence is accumulating that progesterone levels may be inversely related to risk of ovarian cancer but await confirmation from epidemiologic studies.

**Insulin.** Insulin has been shown to exert mitogenic and ant apoptotic properties (172, 173) and has been directly implicated in the development of several cancer types (174-178). At high concentrations, insulin might bind to the IGF-I receptor and activate intracellular signaling pathways under its control (172). Insulin might also enhance ovarian cancer development through its effects on the synthesis and metabolism of other hormones. It can stimulate LH-induced synthesis of androgens and is a powerful down-regulator of the synthesis of sex hormone binding globulin and IGF-I binding protein-1 and hence is a determinant of the free, biologically active fraction of sex steroid hormones and; IGF-I (65, 178).

Despite the above-mentioned characteristics of insulin, which might link it to ovarian cancer pathogenesis, the available epidemiologic evidence thus far does not support its involvement in ovarian cancer development (Table 1). Obesity, which is a widespread hyperinsulinemic condition in affluent societies, seems to be only weakly associated with ovarian cancer. Moreover, weight gain throughout adulthood does not seem to influence ovarian cancer risk. Regular physical activity improves insulin sensitivity, but the data...
relating physical activity to ovarian cancer are conflicting and do not show a clear-cut protective effect (65). Type 2 diabetes, which is generally associated with a long history of insulin resistance and hyperinsulinemia before diagnosis and also for several years after diagnosis, also is not associated with an increased risk of ovarian cancer (179-181). Only one prospective cohort study has investigated prediagnostic levels of C-peptide (a marker of pancreatic insulin secretion) in relation to ovarian cancer, and no significant association was found (Table 1; ref. 182). A limitation of the study was that about half of the available blood samples were collected from nonfasting women, although attempts were made to account for the effect of fasting in the analyses.

In conclusion, insulin is unlikely to be a risk factor for ovarian cancer.

**Insulin-Like Growth Factor-I.** IGF-I has well-documented mitogenic and antiapoptotic properties shown on many cell types (183). Elevated concentrations of IGF-I and decreased concentrations of some of its binding proteins have been directly related to several common cancers, including cancers of the breast and prostate (178). IGF-I and IGFBPs play a role in the regulation of ovarian follicle development, steroidogenesis, and cellular mitosis and apoptosis in ovarian tissue (184, 185). Experimental studies have shown that overexpression of the IGF-I receptor can induce malignant transformation of ovarian epithelial cells (186). The mitogenic and antiapoptotic effects of IGF-I might be particularly relevant during ovulation-related tissue remodeling of the surface epithelium (187).

In the only prospective epidemiologic study on ovarian cancer and prediagnostic IGF-I reported thus far, globally there was no association between IGF-I and cancer risk (114). In analyses restricted to subjects who developed ovarian cancer at a relatively early age, women in the top tertile of IGF-I concentrations had an ~5-fold increase in risk in comparison with those from the bottom tertile [odds ratio (95% confidence interval), 4.73 (1.31-17.1); ref. 114]. The observed effect of IGF-I only in younger women parallels the observed effect of IGF-I in younger women in the relationship of IGF-I with breast density (188) and breast cancer (189, 190). More epidemiologic data are necessary to establish the role of elevated IGF-I levels in ovarian pathogenesis, as IGF-I alone cannot account for the overall pattern of the disease occurrence (Table 1), and also to understand the possible interactions of IGF-I with sex steroid hormones, which may be of special relevance before menopause.

**Summary and Discussion**

We have reviewed the major hypotheses implicating endogenous hormones in the etiology of ovarian cancer, and the epidemiologic evidence that may be interpreted as being in support or against them. A summary of the association of epidemiologic evidence that may be interpreted as being in favor or against one hormone (or group of hormones) as a major determinant of risk. Perhaps, this is most striking for the reduction in risk among oral contraceptive users, and observation that is consistent with hypotheses that implicate gonadotropins, androgens, progesterone, estrogens, and even IGF-I (the oral intake of estrogens reduces circulating IGF-I levels (191, 192)). Third, only very few studies could account for the diverse histologic subtypes of ovarian cancer separately with reasonable statistical power. However, differences in the effects of established risk factors have been reported and age-specific incidence rates do vary according to histologic subtype: the incidence of endometrioid, clear cell, and mucinous tumors plateau after menopause, whereas the incidence of serous tumors continues to increase until much greater age (Fig. 1). The distinct mutational spectra that characterize the specific subtypes of epithelial ovarian cancer and the putative involvement of diverse mechanisms (and possibly different hormonal factors) in tumor development and progression to clinically overt disease (91, 193). Finally, the presence of sex steroid (111, 131, 194, 195) and IGF-I (196) receptors in malignant ovarian tissues and differences in the type of estrogen and progesterone receptors expressed by normal and malignant tissues (197-201) together with the potential of these hormones to stimulate malignant cell proliferation support an important role of these hormones in ovarian tumor progression.

In conclusion, the current epidemiologic evidence suggests possible etiologic roles for elevated androgens and estrogens and decreased progesterone in the pathogenesis of ovarian cancer. A challenging scientific task would be to determine the relative contribution of ovarian synthesis and circulating levels of sex steroid hormones in both disease development and progression. Stimulation of ovarian steroidogenesis appears as the major mechanism of gonadotropin involvement in ovarian cancer, although direct effects of elevated LH or FSH cannot be entirely ruled out. Hyperinsulinemia seems to be unlikely risk factor for ovarian cancer. IGF-I emerges as a hormone that may also be directly involved in the pathogenesis of the disease, but future studies will be essential to elucidate the role of the IGF system, including IGF-I, IGF-II, IGFBPs, and growth hormone. The role of other hormones and growth factors (e.g., hepatocyte growth factor and inhibins) as well as the possible roles of inflammation and retrograde menstrual flow through the Fallopian tubes present intriguing opportunities for future research that will ultimately form the basis for the design of preventive strategies and treatment schemes to help combat this devastating disease.

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**References**


