REVIEW

Hormonal Etiology of Epithelial Ovarian Cancer, With a Hypothesis Concerning the Role of Androgens and Progesterone

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In the United States, ovarian cancer is the fourth most frequent cause of cancer death among women, following lung, breast, and colorectal cancers. Each year, approximately 26 000 women are diagnosed with ovarian cancer and 14 000 die of it. Germline mutations in BRCA1, BRCA2, or other genes have been implicated in a small fraction of cases. However, it has been suggested that, for the great majority of patients, the risk of epithelial ovarian cancer could be related to ''incessant ovulation'' (i.e., to the chronically repeated formation of stromal epithelial clefts and inclusion cysts following ovulation) or to some type of hormonal stimulation of ovarian epithelial cells, either on the surface of the ovary or within ovarian inclusion cysts, possibly mediated through excessive gonadotropin secretion. From the evidence to date, the relative importance of these two hypotheses—incessant ovulation and gonadotropin stimulation—cannot be distinguished. While either or both may play a role in the development of ovarian cancer, it appears that an additional major factor must also be involved. The purpose of this review is to evaluate evidence for and against the incessant ovulation and gonadotropin hypotheses, as well as to consider the possibility that risk of ovarian cancer may be increased by factors associated with excess androgenic stimulation of ovarian epithelial cells and may be decreased by factors related to greater progesterone stimulation. Many features of the evidence bearing on the pathophysiology of ovarian cancer appear to support a connection with androgens and progesterone. [J Natl Cancer Inst 1998;90:1774–86]

In the United States in 1997, it has been estimated that there were more than 26 000 new cases of ovarian cancer and that approximately 14 000 women died of it *(1)*. Close to 2% of women are affected over the lifetime. It is the fourth most frequent cause of cancer death among women, after lung, breast, and colorectal cancers. Ovarian cancer is difficult to treat because patients frequently present late in the course of the disease, which may be asymptomatic until advanced stages. The established risk factors (low parity, nonuse of oral contraceptives, and family history) account for a portion of disease incidence, but the possible mechanisms by which these factors affect risk of developing ovarian cancer are not fully understood.

In this review, the term ''ovarian cancer'' has generally been used to denote the borderline (low malignant potential) and invasive epithelial tumors of the ovary, which constitute the more than 90% of all nonbenign ovarian neoplasms of adult women. Germ cell, stromal, and other kinds of primary tumors also occur but are not the main focus here. Even among the epithelial tumor types, there may be some etiologic heterogeneity according to histologic subtype; this has been discussed by the author in detail elsewhere *(2)*.

In 1971, Fathalla *(3)* suggested that chronic repeated ovulation without pregnancy-induced rest periods contributes to neoplasia of the ovarian epithelium. Fathalla noted that the ovarian surface epithelium—a single-cell layer surrounding the ovary and derived from the same mesodermal celomic epithelium as that lining the peritoneal cavity and other Müllerian structures—undergoes rapid proliferation during 24 hours after ovulation, and that invaginations of the epithelium to form clefts and inclusion cysts within the ovarian stroma are most pronounced just after ovulation. Casagrande et al. *(4)* extended this concept to decreased cancer risk associated with anovulation resulting from oral contraceptive use. Those authors postulated that proliferation or malignant transformation of the surface epithelium occurs because of exposure to estrogen-rich follicular fluid following ovulation. In 1983, Cramer and Welch *(5)* organized what was then known about the etiology of ovarian cancer in an important paper on its pathogenesis that still merits attention. In brief, they noted that the ovarian epithelium repeatedly invaginates throughout life to form clefts and inclusion cysts and suggested that, under excessive gonadotropin (follicle-stimulating hormone [FSH] or luteinizing hormone [LH]) stimulation of the ovarian stroma and resulting stimulation by estrogen or estrogen precursors, the epithelium may undergo proliferation and malignant transformation. They concluded that factors affecting systemic estrogen regulation would therefore influence gonadotropin stimulation and indirectly the paracrine estrogen environment of the ovarian epithelial cells. The gonadotropin model is consistent with the known protective effects of parity and oral contraceptive use, inverse associations seen in the great majority of ovarian cancer studies [three studies evaluated in *(6);* 12 in *(7);* and many other studies]. Both pregnancy and oral

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contraceptive use suppress ovulation and may lower basal as well as peak gonadotropin stimulation. Postmenopausally, the Cramer–Welch model relates extraovarian estrogens—exogenous or through obesity or possibly diet—to estrogenic stimulation of the ovarian epithelium. The main point stressed by Cramer and Welch was that it is excessive gonadotropin secretion, leading ultimately to increased estrogenic stimulation of the epithelial cells, which is responsible for the increased risk of cancer.

The purpose of this review is to evaluate pathologic, endocrinologic, and epidemiologic evidence for and against the incessant ovulation and gonadotropin hypotheses. In addition, we will discuss a new hormonal hypothesis, in which risk of ovarian cancer is increased by factors associated with excess androgenic stimulation of ovarian epithelial cells and decreased by factors related to greater progesterone stimulation. Many of the findings bearing on the etiology of ovarian cancer appear to support the involvement of androgens and progesterone.

Review Methods

A MEDLINE® search from 1966 was used as a starting point to identify papers relevant to our review. Additional papers were found by examination of reference lists and by perusal of current and recent issues of appropriate journals. Our purpose was to examine suitable evidence in a number of fields, rather than to calculate quantitative estimates of effect. Thus, formal quantitative methods have generally not been used in this review. We have attempted to be comprehensive in exploring the evidence and have included for discussion all relevant papers identified. Consideration has been given to applicability of findings, but no inclusion/exclusion criteria have been employed based on quality of individual reports.

Incessant Ovulation

It seems evident that the proliferative behavior of the ovarian epithelium following ovulation could support a role for ovulation in the etiology of ovarian cancer. Poultry hens kept hyperovulatory under continuous, long-term photostimulation are extremely likely to develop ovarian or tubal adenocarcinomas *(8),* and repeatedly recultured rat ovary epithelial cells—forced to proliferate—spontaneously acquire features of malignant transformation and produce serous cystadenocarcinomas when injected into nude athymic mice *(9).* After ovulation, besides repairing the ovulatory wound, the epithelium has an increased tendency to form clefts extending into the cortical stroma *(3).* Clefts may occur through retraction of a corpus albicans or from collapse of a cystic follicle, by direct spread of the surface epithelium into the cavity of a corpus luteum or through other processes *(10).* The clefts frequently appear to close off, becoming ovarian inclusion cysts. The cysts may then remain in the stroma for long periods of time or for unknown reasons may regress and disappear *(10).* Prevalence of inclusion cysts in contralateral ovaries of women with unilateral ovarian cancers provides some evidence that these germinal inclusion cysts may be associated with cancer development *(11).* However, increased frequency of inclusion cysts among cancer cases was not seen in another, larger study *(12).* The latter study found that both germinal inclusion cysts and unilateral ovarian cancers occur more frequently in the right ovary than the left, and others have also reported a higher frequency of right-sided ovarian cancer *(13),* although this finding was not confirmed in the large Surveillance, Epidemiology, and End Results (SEER)[1] incidence database *(14).* Ovulation has also been reported to occur somewhat more often in the right ovary than in the left *(15),* but this finding too has not been confirmed *(16).*

p53 and Ovulation

Somatic mutation and/or inactivation of the p53 cell cycle checkpoint regulatory gene (also known as TP53) has been implicated in carcinogenesis in a number of organs. Aside from a general ability to cause increased rate of cancer progression, p53 mutations appear to be involved early in the neoplastic process of glioblastoma *(17),* esophageal cancer *(18),* and hepatocellular carcinoma *(19)* but late in the development of colon cancer *(20).* Mutation in or inactivation of p53 is found in about 46% of invasive ovarian tumors *(21–45)* but in only 8% of borderline (low malignant potential) tumors *(25,32,36,38,39,46,47)* and is virtually nonexistent in benign tumors or normal ovarian epithelium *(21,26,32,33,37,46,47).* Thus, p53 inactivation is likely to be a late event in ovarian carcinogenesis, although as evidenced by a high degree of expression concordance between primary tumors and metastases *(22,38,48),* it may frequently occur prior to metastatic spread. Nevertheless, the types of mutations seen in ovarian cancers suggest that many p53 mutations are caused by generalized genomic instability, rather than being the cause of the instability *(49).* The same appears to be true in breast cancer *(50).*

A recent report by Schildkraut et al. *(51)* asserted that exposure to a high calculated lifetime number of ovulatory cycles was associated with increased risk of p53-overexpressing ovarian cancer but not of p53-negative ovarian cancer. On the basis of the supposed specificity of the association for p53-positive tumors only, the authors concluded that both repeated ovulation and p53 inactivation were involved in the etiology of ovarian cancer. However, the analysis by Schildkraut et al. *(51)* indeed showed that the principal reproduction factors affecting the calculated lifetime number of ovulatory cycles—attained parity and duration of oral contraceptive use—were equally associated with p53-positive and p53-negative ovarian cancers *(52).* Only the term ''age at menopause/interview,'' which for the case subjects was essentially the same as age at diagnosis, differed between p53-positive and p53-negative subjects. It is also known that, except for the factors age at diagnosis and presence of a germline BRCA1 or BRCA2 mutation, borderline ovarian tumors have the same risk factor associations (including low parity and nonuse of oral contraceptives) as invasive tumors *(2,53,54),* yet the borderline tumors rarely show p53 mutations. As well, the results of Schildkraut et al. *(51)* showed p53-positive ovarian cancers to be more associated with distant rather than local/regional stage at diagnosis in comparison with p53-negative cancers. What is more, p53 positivity was strongly associated with tumor grade ($P<10^{-5}$) *(51),* and this relationship has been seen in a number of other studies *(38,41,44,45).* Thus, the data of Schildkraut et al. *(51)* provide support for the view that p53-overexpressing ovarian tumors are likely to be those diagnosed later in the neoplastic process, when more genetic errors have

accumulated, and not evidence for a role of ovulation in causing p53 damage leading to ovarian cancer.

Ovulation and Breast-feeding

Breast-feeding can suppress ovulation, particularly if the breast-fed infant is not supplemented with bottle feeding. Total duration of breast-feeding or average amount of breast-feeding per pregnancy has been observed to be associated with reduced risk of ovarian cancer in a number of studies *(7,55,56)*.

Ovulation and Menstrual Variability

Certain aspects of the menstrual cycle bear upon the probability of ovulation or anovulation. Cycles less than 25 days or more than 35 days in length or with more than 8 days of flow are appreciably more likely to be anovulatory [reviewed in *(57)*]. Moderate physical activity can induce menstrual alterations and is examined in the ''Progesterone'' section below. Aside from ages at menarche and menopause, few epidemiologic studies of ovarian cancer have considered factors related to the menstrual cycle. In a large case–control study, Parazzini et al. *(58)* observed significantly decreased risk (adjusted odds ratio [OR] = 0.45; 95% confidence interval [CI] = 0.31–0.65) associated with cycles less than 21 or more than 35 days in length. No case–control difference in menstrual cycle length was seen in studies by Wynder et al. *(59)* and McGowan et al. *(60)*, although those investigators apparently did not consider that excessively long cycles might have the same effect on risk as excessively short cycles. In two other studies, nonsignificantly reduced ORs of 0.75 (95% CI = 0.43–1.32) and 0.87 (95% CI = 0.65–1.18) were seen for irregular cycles *(61,62)*, and nonsignificantly increased risk (relative risk = 1.12) with this factor was seen in a prospective study *(63)* and in three other case–control studies (OR = 1.4 [95% CI = 0.83–2.50], 2.7 [95% CI = 0.8–9.0], and 1.3 [95% CI = 0.5–3.2]) *(64–66)*. Finally, an ovarian cancer case–control study by the author *(56)* found that menstrual cycles less than 25 days or greater than or equal to 35 days long, generally irregular, or with more than 8 days of flow all were associated with ORs less than unity (OR = 0.65 [95% CI = 0.43–0.98], 0.67 [95% CI = 0.46–0.99], and 0.39 [95% CI = 0.15–0.99], respectively) (Risch HA: unpublished data).

Magnitude of Effect of Ovulatory Events

While ovulation still could have some role, it is clear that it (or inclusion-cyst formation) by itself is insufficient to account for the pathogenesis of ovarian cancer. Ovulations occur over a period of at least 20 years [*see (57)*]. On average, each full-term pregnancy suppresses ovulation for perhaps a year, at most 5% of the total number of ovulations. Even including a latency effect as in the methods of Pike *(67)*, we calculate this would correspond to only a 6% risk reduction, using recent SEER data *(68)*. Epidemiologic studies demonstrate that the reduction in risk among parous women for each additional pregnancy after the first is about 14%–16% *(7,56)*, an amount statistically inconsistent with the 5% [e.g., $P<10^{-5}$ for the data of *(7)*]. As well, each year of oral contraceptive use also suppresses ovulation for a year, but the risk reduction for an additional year of use among ever users is only about 9%, and that too is inconsistent with the reduction in risk for each pregnancy [$P = .001$ for *(7)*]. Similar discrepancies have been seen for other ovarian cancer risk factors *(69)*.

Hormonal Factors in General

Even if ovulation or inclusion cysts are involved in the etiology of ovarian cancer, additional mechanisms must also be involved. These mechanisms are probably hormonal, as some recent evidence suggests. Salazar et al. *(70)* observed significantly increased frequencies of hyperplastic or metaplastic changes in the ovarian epithelium and excessive ovarian stromal activity in women with family histories of ovarian or breast cancer. Resta et al. *(71)*, examining a total of 200 oophorectomy specimens, found hyperplastic or metaplastic changes in the surface epithelium or in inclusion cysts in 92% of ovaries of women with epithelial tumors of the contralateral ovary (benign and malignant), in 76% of ovaries of women with endometrial adenocarcinomas, in 68% of women with polycystic ovary syndrome, but in only 22% of control women whose ovaries had been removed during surgery for uterovaginal prolapse or other non-neoplastic conditions of the fallopian tubes, uterus, or vagina. Since excessive ovulation is not associated with polycystic ovary syndrome [e.g., shown in *(72)*], some other factor must be responsible for the proliferative changes. It should be noted that the women with polycystic ovary syndrome in the study by Resta et al. *(71,73)* were on average 10 or more years younger than the women in the three other subject groups, making this observation somewhat uncertain. Resta et al. *(73)* also observed a loss of surface epithelium more frequently in the ovaries of the (nonpregnant) control women without neoplastic conditions than among those of the other three groups. While other authors have suggested that the surface epithelium is so fragile that it is easily destroyed by surgical handling or by delay in fixation *(74)*, this would not account for its differentially more frequent presence in the three case groups *(71)* nor would it account for increasing loss with increasing age among older ovulatory women *(73)*. The greater presence of epithelium on the ovarian surface in the three case groups seems not to be attributable to ovulation but to some other factor *(73)*. Ovaries of women with luteinized unruptured follicles typically have completely intact, highly proliferative surface epithelium *(75)*.

In addition, epithelial cancers of the ovary appear to arise most frequently within the cortical stroma, in epithelial inclusion cysts, compared with the same cells of the ovarian surface epithelium *(76)* and even less often in the related pelvic peritoneal mesothelium, which comprises a much larger surface area. In the study by Resta et al. *(71)*, hyperplastic or metaplastic changes were observed more frequently in inclusion cysts than in surface epithelium in all four subject groups. Inclusion cysts are not affected by the trauma and repair processes of ovulation *(73)*. Within the cortex of the ovary, the epithelial cells of the inclusion cysts are brought into closer proximity to the vasculature and to the steroid hormone-producing cells and activity *(76)*. Hormonal effects are similarly seen during the fourth and fifth months of fetal development, when the ovarian surface epithelium undergoes diffuse multilayered proliferation, in intimate contact with the interstitial cells, which appear to be active in steroidogenesis at the same time *(77)*. By the 24th week of gestation, the surface epithelium is reduced to a single layer and

proliferation terminates, following the formation of a tunica albuginea separating the epithelium from the underlying cortex *(77).* Thus, the evidence appreciably points to hormonal influences on the behavior of the ovarian epithelial cells.

Specific Hormones

Gonadotropins

We have noted that lower basal as well as peak gonadotropin stimulation may occur during pregnancy and oral contraceptive use *(5,78–80)* and that both of these factors reduce risk of ovarian cancer. Additional evidence bearing on gonadotropins in the pathogenesis is as follows.

Historically, the gonadotropin hypothesis arose from observations that ovarian tumors occurred in rodents following bilateral oophorectomy and ovary transplantation under the splenic capsule *(81).* Intact ovarian function in such animals suppressed the tumor formation, apparently by reducing the gonadotropin hypersecretion *(82).* Aside from chronic photostimulation of poultry hens, ovarian tumors may also be produced in animals by treatment with chemical carcinogens or x irradiation *(83)* or by neonatal thymectomy *(84),* the three methods causing destruction of follicles and ovarian failure. In addition, ovarian tumors occur spontaneously in animal strains (e.g., W^x/W^v and Sl/Sl^t mice) that are congenitally deficient in or that rapidly lose oocytes *(85,86).* These animals are subject to excessive gonadotropin stimulation, and tumor occurrence is reduced or blocked by treatment with depot gonadotropin-releasing hormone agonists, which suppress the gonadotropins *(87,88).* The tumors seen in animals are tubular adenomas, benign epithelial neoplasms that grow within and replace the ovarian stroma, but which do not invade in the uncontrolled fashion characteristic of malignant cancers and do not metastasize. Nonepithelial tumors, particularly granulosa cell tumors, are also seen. Thus, the generalizability of the various animal models to human epithelial ovarian cancer is uncertain. Nevertheless, Cramer *(89)* has argued that the presence of epithelial inclusion cysts within the stroma of human ovaries could lead to a different spectrum of tumor types than would occur in rodents, under the same physiologic stimuli.

In North America, the mean age of incidence of ovarian cancer (borderline and invasive in total) is about 57–59 years [*(56);* also calculated from *(68,90)*], while childbirths and oral contraceptive use are frequent at ages 25–35 years, suggesting some 25–30 years of latency. Follow-up of the atomic bomb survivors cohort also provides a consistent estimate of 25 years of latency among the most heavily exposed women, particularly those less than 40 years of age at the time of the bombing *(91).* Serum FSH and LH reach maximal values in the perimenopausal and immediately postmenopausal years with the depletion of oocytes *(92)* and remain highly elevated thereafter *(93,94).* Age-specific ovarian cancer incidence rates peak in the mid- to late-70s *(68,90),* the same 25 years of latency after the menopause. This suggests that a relationship may exist between the rise in gonadotropins and the later peak in cancer incidence. However, given the mean age of ovarian cancer occurrence in the late-50s, perimenopausal and postmenopausal exposure to high gonadotropin levels would be related to incidence mostly after age 70 years, well after the majority (75%) of cases have occurred.

In addition, ovarian epithelial cells near the time of ovulation are receptive to human chorionic gonadotropin (hCG) stimulation, in that they progress with lysosomal secretory activity and ovulation *(95).* Proliferative behavior is not seen though *(95).* About 25% of benign ovarian tumors have been observed to bind hCG (and by implication, LH, which is very similar in protein sequence) *(96).* However, in that study, the epithelial binding was very weak, in all cases less than 5 fmol/mg protein homogenate, values that are routinely considered negative in studies of receptor binding. In analogy with testicular surface epithelium development, Gondos *(77)* concludes that it is something other than hCG that stimulates the ovarian epithelial cell proliferation during weeks 12–20 of fetal gestation. Also, in *in vitro* cell culture, high concentration of hCG has been seen to stimulate the proliferation of rabbit ovarian epithelial cells, but FSH and LH applied together did not result in growth stimulation *(97).* The applicability of this finding to human epithelial cells *in vivo* is uncertain however (*more discussion below*).

Finally, a recent prospective study provides some direct evidence bearing on the effects of gonadotropins. Among participants to a specimen bank who were followed for more than 15 years after providing blood samples, 31 cases of ovarian cancer occurred *(98).* These case subjects were compared with 62 matched noncancer cohort control subjects on baseline (prediagnosis) serum hormone levels. Case subjects were found to have significantly lower FSH levels than control subjects ($P = .04$), but significant differences were not observed for serum LH levels. Altogether, the evidence thus seems to suggest that the gonadotropins, while involved in the feedback regulation of ovarian steroid hormones, may not in themselves be responsible for alterations in ovarian cancer risk but could reflect certain hormonal circumstances that are related to risk.

Estrogens

In general, much of the evidence in support of hormonal mechanisms is indirect. The ovarian surface epithelium is avascular, suggesting a largely paracrine rather than endocrine influence of hormonal factors *(74).* With regard to estrogens, the ovarian epithelium is not itself normally (i.e., in the nonmalignant state) hormonally active *(99),* save for the presence of 17β-hydroxysteroid dehydrogenase (which reversibly converts estradiol to estrone and testosterone to androstenedione) *(100)* and possibly Δ^5–3β-hydroxysteroid dehydrogenase (which, e.g., converts pregnenolone to progesterone) *(101).* Neither of these enzymes converts androgens to estrogens. Most of ovarian steroidogenesis occurs under the control of the gonadotropins FSH and LH in the granulosa and theca interna cells of developing and mature follicles *(74),* under LH stimulation within secondary interstitial stromal cells, which are derived from the theca cells following follicular atresia *(92),* and within the granulosa- and theca-lutein cells of the corpus luteum under trophoblast hCG stimulation during the first 8–9 weeks of pregnancy *(102).* Estrogen biosynthesis peaks sharply in the granulosa cells prior to ovulation *(92),* again somewhat in the midluteal phase, and declines after cycle day 22 *(74).* As follicular growth distends the surface of the ovary, the epithelial cells multiply and flatten in shape *(103)* until ovulation, when epithelial cell proteases dissolve the follicle apex and rupture it *(104).* The epithelium, up to this point possibly exposed to more indirect paracrine influences

of the granulosa and theca cells [perhaps through intercellular gap junctions that allow small molecules to pass *(75)* or through diffusion *(105)*], is now bathed in follicular fluid that may contain estradiol in concentrations some 10 000 times higher than circulating levels *(74).* After ovulation, epithelial cells proliferate at the edges of the ovulatory wound, migrate over it, and contribute to wound repair *(104).* Estrogen receptors frequently appear to be present in the cytosol of normal ovaries and ovaries with benign lesions *(106–111).* Whether this finding applies specifically to the epithelium is uncertain, although it is possible, since receptor presence seems similar in normal ovaries and in benign epithelial tumors *(106,108,109).* As a whole, during menstrual cycles, the epithelium proliferates at times when estrogenic influences are relatively greater, and the increased mitotic activity is likely to enhance the risk of mutations occurring, which could then propagate clonally with additional epithelial cell divisions in further cycles. Ovarian surface epithelial cells appear not to differentiate into end-stage cells but remain developmentally relatively uncommitted *(104);* cell division results in daughter cells with the same potential for further growth *(76).*

Additional epidemiologic evidence bears on the role of estrogens in the pathophysiology of ovarian cancer. Breastfeeding, which appears protective in a number of studies [e.g., *(7,55,56)*], is associated with reduced serum concentrations of estradiol (and also LH, but elevated levels of FSH) *(112).* Menopausal conjugated estrogen therapy raises serum estradiol and estrone levels somewhat and lowers levels of the gonadotropins *(113).* While increased risk of ovarian cancer with menopausal estrogen replacement was not generally seen in older studies [10 studies in *(7); (114–116)*], a number of recent large studies *(117–120)* do suggest increased risk with usage, and one older study *(121)* found significantly increased risk with use of premarin and especially with diethylstilbestrol. In addition, the established protective effect of oral contraceptive use on risk of ovarian cancer, if greater than that attributable to suppression of ovulation, may be due in part to reduction in endogenous estradiol production *(122),* through suppression of the mid-cycle LH peak *(80),* and possibly by some lowering of basal LH and FSH levels *(78,79).* It seems unlikely that reduction of risk associated with combined oral contraceptive use would be directly due to the estrogen absorbed from the pill (i.e., that estrogen could be protective), since low serum ethinyl estradiol (or equivalent) levels (24-hour average) comparable to the low estradiol levels of the early- through mid-follicular phases of the menstrual cycle are maintained during usage *(78,123–126).* Due to the progestin as much as the estrogen component of the pill, the low estrogen levels are lower than late follicular- or luteal-phase estradiol levels. If estrogens are indeed related to increased ovarian cancer risk, an overall lower estrogen climate could mediate some of the protective effect of oral contraceptive use.

However, other evidence suggests that estrogens may not be the most relevant etiologic factor. Pregnancy raises serum estrogen levels about 100-fold *(102),* yet is protective; thus, some other hormone must be involved. If ovarian epithelial cells were responsive to estrogenic stimulation, then estrogen receptors should be present in them. As we have noted, early studies *(108,109)* did suggest that estrogen receptors are detectable in cytosols of normal human ovaries. However, using monoclonal antibodies directed against human estrogen and other hormone receptors, a study of 35 normal ovaries showed no estrogen receptors in surface epithelial cells or in inclusion cysts, but more than 85% of the sections of surface epithelium and 100% of the epithelial inclusion cysts contained progesterone receptors *(127)* (relevant for the progesterone hypothesis below). Another study found exceedingly low levels of estrogen-receptor messenger RNA in the ovarian epithelial cell line IOSE-Van *(128).* Finally, with respect to serum hormone levels, the cohort study of Helzlsouer et al. *(98)* (*mentioned above*) observed at baseline slightly lower estradiol levels for case subjects compared with matched control subjects.

Androgens

Appreciable evidence implicates androgens in the pathogenesis of ovarian cancer. To start with, in normal nonpregnant women of reproductive age, the overwhelming majority of plasma estradiol comes from direct ovarian secretion, whereas plasma estrone is produced by extraglandular (adipose) aromatization of androstenedione, about half of the latter ovarian and half adrenal *(92).* Androstenedione is a relatively weak androgen *(92),* but ovarian epithelial cells express the enzyme 17β-hydroxysteroid dehydrogenase *(100),* which converts it to testosterone. Plasma concentrations of androgens—even during the late follicular phase of the menstrual cycle when estrogens are at their peak—are greater than estrogens. For example, typical concentrations of androstenedione, testosterone, and dehydroepiandrosterone (in nmol/L) are 5.6, 1.3, and 17, respectively, and those of estradiol and estrone, 1.2–2.6 and 0.5–1.1, respectively *(92).* Circulating estrogens and androgens are mostly bound to plasma albumin and sex hormone-binding globulin, leaving some 2%–3% free *(92).* The free hormones are presumed to be completely active, but the bound hormones also have some ability to enter target tissues *(92,129).* Within the ovaries, the secretion rate of androgens is higher than that of estrogens. The two ovaries produce about 0.8–2.8 mg/day of androstenedione, 0.3–3.0 mg/day of dehydroepiandrosterone, and 0.06–0.10 mg/day of testosterone compared with late-follicular (peak) 0.4–0.8 mg/day of estradiol and 0.25–0.50 mg/day of estrone *(92,130).* The more steady-state early follicular- and luteal-phase estradiol and estrone production rates are one quarter to one half these amounts *(92).* Thus, the epithelial cells, particularly those within inclusion cysts, appear to be appreciably exposed to paracrine ovarian androgens, if not circulating androgens. Postmenopausally, the ovary is also relatively androgenic, as evidenced by 15-fold higher testosterone concentrations seen in ovarian vein compared with peripheral vein serum *(131).*

Androgens are also present in follicular fluid and are the principal sex steroid of fluid in growing follicles *(132).* The follicular fluid concentration of androstenedione in follicles less than 10 mm in diameter is more than 10 times greater than that of estradiol *(133,134).* Appreciable amounts of testosterone are also found in follicular fluid *(133).* Toward ovulation, follicular fluid in the principal large follicle that will ovulate becomes estrogenic through FSH stimulation of granulosa cell aromatase *(135).* However, the smaller follicles that undergo atresia continue to synthesize androgens *(135).* Since even small (<5 mm in diameter) preovulatory growing follicles have huge androstenedione concentrations *(134),* we infer that steroid production in much of the ovary during the follicular part of the menstrual

cycle is relatively androgenic and that epithelial cells within stromal inclusion cysts located near developing follicles may be particularly exposed to high levels of androgens.

Androgen receptors are also frequently seen in normal ovaries and have been directly identified within ovarian epithelial cells *(109).* As we have noted, androstenedione does not bind with high affinity to the androgen receptor *(92),* but the epithelial cells are able to convert it to testosterone, which does bind. The exact function of androgens within ovarian epithelial cells is currently unknown, but the presence of receptors suggests at least that the epithelial cells are exposed to and respond to androgens *(136).*

Do androgens (or other sex steroids) directly stimulate epithelial cell proliferation? One recent study by Karlan et al. *(137)* examined *in vitro* proliferation of human epithelial cells treated with estradiol, progesterone, or dihydrotestosterone. In the assay, estrogen receptor-positive, progesterone receptor-positive cells were thinly plated on plastic substrate, and all of the treatment groups showed exponential growth curves for 10 days in culture *(137).* The authors stated that compared with controls without hormone treatment, none of the hormones significantly increased the rate of cell growth, and they concluded that the sex hormones do not affect epithelial cell proliferation *(137).* However, by a week in culture, all of the three hormone-treatment groups showed sustained greater cell numbers than the controls, about 25% more, and the combined result was significant. In addition, it has been pointed out that ovarian epithelial cells proliferate on plastic much more rapidly than on collagen gel, fibrin clot, or Matrigel (basement-membrane components) *(104).* Even on plastic, the epithelial cells grow until forming a confluent monolayer, at which time proliferation ceases *(138).* Thus, normal ovarian epithelial cells appear subject to contact inhibition, and the conditions employed for the experiment of Karlan et al. *(137)* do not seem relevant for evaluating hormone stimulation of growth *in vivo.* Indeed, animal models do indicate that testosterone stimulates the growth *in vivo* of ovarian surface papillomas and cystadenomas *(139)* and that dysgenetic ovaries of neonatally thymectomized mice (which develop tubular adenomas) produce abnormally large amounts of androstenedione and testosterone, but not estrogens, prior to tumor occurrence *(140).*

Available epidemiologic evidence also generally supports a relationship between androgens and risk of ovarian cancer. First, in the prospective cohort study of Helzlsouer et al. *(98)* described above, in addition to case subjects having approximately normal prediagnostic serum LH levels and lower FSH levels than control subjects, they were found to have significant elevations (about 50% higher levels) of androstenedione and dehydroepiandrosterone ($P = .03$ and .02, respectively). This pattern appeared for both premenopausal and postmenopausal subjects in the study. It is uncertain whether the excess androgens were due to an ovarian or adrenal source; the lower FSH may be a feedback response required to maintain the relatively normal estrogen levels as were seen. A second piece of evidence concerns the association observed between history of polycystic ovary syndrome and risk of epithelial ovarian cancer. In the Cancer and Steroid Hormone case–control study, case subjects were significantly more likely than control subjects to report a history of physician-diagnosed polycystic ovary syndrome (OR = 2.4; 95% CI = 1.0–5.9) *(141).* Polycystic ovary syndrome may have more than one causal defect, but in general, it results in elevated serum LH, normal or low FSH, and elevated androstenedione and testosterone levels *(142–145).* Third, in the only study that appears to have considered the following possibly androgen-related factors, both a history of acne (OR = 1.6; 95% CI = 0.7–3.3) and having a diagnosis of hirsutism (OR = 2.0; 95% CI = 0.4–10.) have been somewhat associated with increased risk of ovarian cancer *(59).* Fourth, oral contraceptive use (which is protective) is known to suppress ovarian testosterone production 35%–70% *(79,146–149).*

Finally, in a prospective cohort study of 31 000 Iowa women followed more than 7 years, a significantly increasing trend in risk of epithelial ovarian cancer was seen with increasing waist-to-hip ratio ($P = .03$) *(150).* Waist-to-hip ratio and other measures of truncal adiposity or central obesity have been significantly associated with serum levels of both ovarian and adrenal androgens among both premenopausal and postmenopausal women *(151–159).* Only two other studies appear to have examined central obesity and ovarian cancer. A very small cohort study *(160)* did not observe an association; however, a case–control study *(161)* in which subjects were matched on age and body mass index showed significantly ($P = .005$) increasing risk with increasing waist-to-hip ratio among premenopausal women.

Progesterone

Evidence for a possible protective role of progesterone in the etiology of ovarian cancer starts with consideration of the increased sex hormone activity during pregnancy. Over the first month of pregnancy, maternal LH and FSH decline strongly with the increase in trophoblast hCG *(162).* The hCG also stimulates the corpus luteum to continue producing progesterone and not regress *(102).* After the seventh week, the luteal-placental shift occurs in which the functional capacity of the corpus luteum of pregnancy drops, while the massive placental production of progesterone during pregnancy begins *(102).* In addition, the placenta extracts maternal (and later, fetal) adrenal androgens, which remain at stable maternal serum concentrations while both production and utilization rates increase; maternal serum estrone and estradiol are made from the adrenal androgens *(102).* During pregnancy, the placental synthesis thus causes 10-fold increases in maternal circulating progesterone levels *(102).* Maternal testosterone and androstenedione levels increase some twofold to threefold, although most of the testosterone is bound to the pregnancy-induced higher levels of sex hormone-binding globulin, preventing virilization of female fetuses *(102).* These maternal ovarian androgens are in any case dwarfed by the huge estrogen and progesterone concentrations. In terms of the pathogenesis of ovarian cancer, we suggest that the additional protective aspect of pregnancy not mediated through suppression of ovulation may be due to the 8–9 months of elevated progesterone. As we have noted, it seems unlikely to be due to the pregnancy estrogens, since most of the evidence relating estrogens to risk of ovarian cancer (as well as to endometrial cancer and perhaps breast cancer) points either to no effect or to increase in risk.

With respect to oral contraceptive usage, it is uncertain whether the synthetic progestational agents in these preparations

directly convey the decreased risk consistently seen according to duration of use; the magnitude of risk decrease is consistent with protection due to ovulation suppression or to androgen reduction (*see above*). The contraceptive progestins vary somewhat in their androgenic and estrogenic properties *(147,148).* Those progestins considered to be relatively androgenic in terms of their clinical side effects (e.g., norgestrel and levonorgestrel) *(163)* also appear to lower total and free serum testosterone the most *(148).* Epidemiologic studies *(164,165)* have shown no difference in the reduction of ovarian cancer risk between norgestrel-type contraceptives and other combined agents. More than 75% of oral contraceptive usage during the 1960s–1970s was of progestins with ''low androgenicity'' *(163,164).* During oral contraceptive use, endogenous progesterone synthesis seems to stay as low as that during the early follicular phase of the menstrual cycle, without follicle maturation or corpus luteum function *(166).* However, given that the progestational potency of the synthetic 19-nortestosterone progestins is more than 100 times that of progesterone *(167,168)* and that serum levels of progestins absorbed from oral contraceptives are comparable to luteal-phase progesterone levels [e.g., 5 ng/mL *(78)*], the net progestational environment within the ovary is likely to be quite high *(169).* Thus, the decreased risk of ovarian cancer with oral contraceptive use could also be due to the cyclic progestational climate.

Another piece of evidence suggests that combined oral contraceptives do offer ovarian cancer protection beyond that potentially from suppression of ovulation. A case–control study was large enough to have identified sufficient numbers of subjects who had used progestin-only types of oral contraceptives *(165).* These progestin-only formulations do not totally suppress ovulation and some ovulatory cycles typically occur *(169);* up to 40% of women using this method can have regular ovarian function, with normal estrogen and luteal-phase progesterone synthesis *(166).* In the case–control study, relative to never use of progestin-only contraceptives, the risks were 0.39 for use less than 3 years' duration and 0.21 for use 3 years or longer, with trend $P = .009$. These reduced risks appear comparable to those of the combined oral contraceptives or perhaps a little more protective *(165).* Thus, these progestin-only contraceptives create a progestational hormonal environment with a reduced risk that cannot in total be attributed to ovulation suppression. Given that combined oral contraceptives convey a similar degree of protection but with less ovulation, we infer that risk reduction associated with ovulation suppression cannot comprise the total protection given by the combined preparations and that the net benefit is probably due to the progestational component.

Nevertheless, a similar degree of protection is not yet clearly seen for usage of depot medroxyprogesterone acetate (DMPA). DMPA is a long-acting 17-acetoxy progesterone compound that suppresses endogenous progesterone synthesis and ovulation; estradiol levels remain in the early- to mid-follicular-phase range (<100 pg/mL) *(170).* Serum levels of DMPA stay about 1 ng/mL for 3 months after injection; these levels inhibit the mid-cycle peak in gonadotropins but do not seem to change basal LH and FSH levels *(170).* Only three studies have examined usage of DMPA and risk of ovarian cancer. A small case–control study in Shanghai *(65)* found an elevated OR of 2.8 (95% CI = 0.9–8.5), although few subjects had ever been exposed, and even use of combined oral contraceptives was not found to be protective in that study. In a follow-up study of 5000 black American women, an OR of 0.8 (95% CI = 0.1–4.6) was seen for ever use of DMPA *(171).* Last, the large and more definitive World Health Organization international collaborative case–control study observed for nonmucinous ovarian cancer a significantly decreased risk of 0.42 (95% CI = 0.15–0.96) with ever usage *(172).* DMPA use may thus protect against the development of ovarian cancer, although further studies are needed to confirm this fact.

The effects of physical exercise may also bear on the hypothesis of progesterone activity and ovarian cancer. We return to the prospective study of 31 000 Iowa women followed for 7 years *(150).* In addition to the association observed with waist-to-hip ratio, a significant increasing trend in risk of ovarian cancer was seen according to increasing value of an index of usual physical activity. Other studies have also suggested increased risk with employment in jobs categorized as having moderate (compared with low) physical activity levels: manual workers *(173),* physical education teachers *(174),* and jobs with little sitting time *(175).* Physical activity may not be related to serum androgens (or progesterone) postmenopausally *(176,177)* but premenopausally is associated with a shortened luteal phase *(178–182),* resulting in lower luteal progesterone levels *(183,184).* This finding applies both to female nonathletes as well as athletes. Even moderate recreational physical activity without amenorrhea or other menstrual disturbances is associated with decreased progesterone levels *(185).* Women with menstrual cycles shortened by decrease in length of the luteal phase would also spend relatively greater proportions of time in the follicular phase and therefore may have somewhat more ovarian exposure to androgen production *(186).* Intense physical activity also produces transient elevations of serum testosterone and other androgens *(187).* However, if ovulation is indeed involved in the etiology of ovarian cancer, women whose regular physical activity is intense or frequent enough to cause amenorrhea may be at decreased risk due to the suppression of ovulation.

As we have noted above, a study using monoclonal antibodies methods showed that virtually all specimens of normal ovarian epithelium contained progesterone receptors *(127).* Defects in the progesterone receptor could lead to reduced effectiveness of available progesterone and thus to increased risk of ovarian cancer according to our hypothesis. This finding has apparently been seen: A relatively common germline polymorphism variant in the hormone-binding domain of the progesterone receptor was associated with twofold increased risk ($P<.025$) *(188).* While this finding relating to the Alu insertion was not subsequently confirmed by a second group of investigators *(189),* preliminary analysis of sister-matched ovarian cancer case–control study data of the author also shows a 60% increased risk for women with this variant (Risch HA: unpublished data).

Finally, it is interesting to consider the effects of multiple gestation. Women who have delivered (naturally occurring) dizygotic twins appear to have higher gonadotropin levels during their reproductive years *(190–194)* and in general may be more likely to double ovulate *(195)* compared with women who have had singleton pregnancies only. Thus, they should be at increased risk of ovarian cancer according to either the incessant

ovulation or gonadotropin hypotheses. Such does not appear to be the case. In a record-linkage study of mothers of dizygotic twins, no excess of ovarian cancer cases appeared *(196)*. In fact, a case–control study that examined history of twin pregnancy found somewhat decreased risk with this factor (total parity-adjusted OR = 0.68; 95% CI = 0.33–1.38) *(62)*, and another by the author *(56)* also suggests decreased risk for nonmucinous ovarian cancer (parity-adjusted OR = 0.42; 95% CI = 0.14–1.26) (Risch HA: unpublished data). Some evidence exists that premenopausal women with a history of twinning may have greater follicular-phase serum progesterone levels *(195)*, and serum progesterone appears to be higher after double compared with single ovulations *(197)*. Twin pregnancies also involve greater daily production and serum levels of progesterone *(198–199)*. Thus, reduced risk for women who have had twins could be conveyed through greater ovarian progesterone exposures.

Insulin, Insulin-Like Growth Factors (IGFs), Diabetes Mellitus, and Obesity

As we have described above, the gonadotropins LH and FSH are involved in the feedback regulation of ovarian steroidogenesis. Work over the last decade has suggested that insulin and IGFs may modulate the effects of the gonadotropins *(200)*. IGFs are found at relatively high levels in serum and are produced in the ovaries as well as in the liver and elsewhere *(200)*. *In vitro*, both insulin and IGF-I enhance normal ovarian progesterone production and stimulate granulosa and granulosa-lutein cell aromatase to increase the conversion of androgens to estrogen *(200–207)*. *In vivo*, however, the relationships are less clear. Insulin-dependent diabetes mellitus is characterized by hypoinsulinemia and normal insulin sensitivity of target tissues. Among insulin-treated postmenopausal diabetic women, increased serum total estrone and estradiol are seen, but because sex hormone-binding globulin also appears to be significantly elevated, the free fractions of these hormones (as well as of testosterone, androstenedione, and dehydroepiandrosterone sulfate) remain in the normal range *(208)*. Nonobese women with noninsulin-dependent diabetes mellitus (NIDDM) tend to have slight insulin resistance, with inadequate insulin secretion as the principal abnormality, whereas obese women with NIDDM have severe insulin resistance in the liver, skeletal muscles, etc., and hyperinsulinemia *(209)*. Insulin resistance and chronic hyperinsulinemia are related to increased ovarian testosterone production *(210)*, although there is some contention about this [compare *(211)* versus *(212)*]. Obesity itself is associated with reduced glucose oxidation, increased lipid oxidation, hyperinsulinemia, and potentially the development of insulin resistance and clinical diabetes *(213)*. Body mass index (one measure of obesity) has been observed in one study to be associated with increased fasting plasma glucose, decreased sex hormone-binding globulin capacity, and increased percent free serum testosterone *(214)*. Moderate obesity, without hirsutism or menstrual abnormalities, is also associated with increased serum total- and free-testosterone concentrations *(215)* and with decreased luteal progesterone levels *(216)*. Central obesity (high waist-to-hip ratio) is perhaps even more associated with decreased sex hormone-binding globulin capacity and increased serum androgens (*see above*). Because of the apparent lack of relevant studies, the above discussion has glossed over potential differences between premenopausal and postmenopausal women. However, the various findings in both of these two groups *(151,214,217)* as well as other considerations *(159,214,218)* suggest that the androgen alterations may stem from effects on both adrenal and ovarian hormone production.

Thus, it is unclear whether (or which forms of) diabetes mellitus, as opposed to obesity, should be related to the hormonal climate of the ovary. We have omitted discussion of NIDDM in polycystic ovary syndrome, since hyperandrogenism in this disorder is established *(219–221)*. A number of epidemiologic studies *(222–224)* have shown significant positive associations between history of diabetes mellitus and risk of endometrial cancer, a disease considered to be related to unopposed estrogen stimulation. However, the same and other studies have in general failed to show positive associations between history of diabetes and risk of ovarian cancer. One study *(173)* did find a significant positive relationship (OR = 3.4; 95% CI = 1.08–10.7), a second study *(225)* a nonsignificant positive relationship (OR 1.2; 95% CI = 0.2–3.4), but all of the remaining 12 studies *(59,69,222–224,226–232)* considering this association found ORs slightly below unity. Few of the 14 studies apparently controlled for parity or obesity, etc. Whether a negative association exists, and what the hormonal implications of this would be, remain to be seen; stratification by obesity in assessing this association is likely to be important. Obesity itself, if anything, appears to increase the risk of ovarian cancer. A few studies have shown little difference in this regard between case subjects and control subjects *(65,233,234)* or only slight associations *(66,235)*, but a number of large population-based studies have observed significant positive risk associations with body mass index or with obesity *(4,62,69,164,173,236,237)*.

Conclusion

Overall, it appears that incessant ovulation could be involved in the pathogenesis of ovarian cancer, but that an additional factor, probably hormonal, must play a role. Independent evidence for the gonadotropins as that factor is not apparent. Some evidence supports estrogen, although stronger evidence implicates factors related to androgens and progesterone in the etiology. A number of interesting aspects of lifelong exposures to and manifestations of these hormonal factors remain to be observed in future studies.

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NOTES

[1]*Editor's note:* SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

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