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Fertility Drugs and Ovarian Cancer

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INTRODUCTION

In 1992, a pooled analysis of the risk of ovarian cancer was published by Whittemore et al. (1). It received a great deal of attention because of its size and many strengths. The study confirmed most of the previously identified risk factors for ovarian cancer, but, in addition, a considerably increased risk of ovarian cancer was observed among infertile women who had used fertility drugs (1). This finding started an intense debate over the long term effects of artificial ovarian stimulation, because the use of fertility drugs has increased extensively during the past three decades, they are used on healthy women, and use of new and more potent drugs has expanded. The main concern with regard to public health has been possible carcinogenic effects, especially an effect on ovarian cancer and other hormone-associated cancers.

An association between use of fertility drugs and an increased risk of cancer was initially suggested by several case reports of ovarian cancer (2–17) and breast cancer (17–19) occurring subsequent to treatment with fertility drugs. Following this finding, six analytical studies examined ovarian, breast, and cervical cancer risk in cohorts of women who sought treatment for infertility (20–26). Results from these studies consistently indicated that there was no overall effect of infertility on the risk of premenopausal breast cancer (27). Use of fertility drugs was examined in four of these studies (20–24). There was no overall effect of fertility drug use on the risk of either cervical cancer (21, 22, 24) or breast cancer (22, 23). In contrast to this finding, research on the overall effect of infertility per se, and especially the effect of fertility drug use on the risk of ovarian cancer, has been inconsistent, which has caused debate (28–30).

It is well established that nulliparous women have an increased risk of ovarian cancer and that increasing parity protects against ovarian cancer. Thus, the fundamental issue in the debate has been whether use of fertility drugs increases a woman's risk of ovarian cancer over and above that predicted by infertility or low parity. The aim of this review is to present findings on the general biologic effects of fertility drugs in relation to possible carcinogenic effects on the target organ of infertility treatment (i.e., the ovary), and especially to review the existing epidemiologic data on the relation between infertility/fertility drug use and risk of ovarian cancer.

FERTILITY DRUGS

The first available fertility drugs, first marketed around 1955, were all preparations with follicle-stimulating hormone (FSH) activity. Approximately 10 years later, clomiphene citrate (CC) and human menopausal gonadotrophin (hMG) began to be marketed in most Western countries (31, 32). Before and during this period, treatment for infertility also included pituitary irradiation and administration of pregnant mare serum gonadotrophins, conjugated estrogen, oral contraceptives, and diethylstilbestrol (31, 33).

Currently, there are four major drugs being used for infertility treatment, all of which can induce ovulation: 1) the antiestrogen CC; 2) hMG, which contains FSH and luteinizing hormone (LH); 3) human chorionic gonadotrophin (hCG); and 4) gonadotrophin-releasing hormone agonists (GnRH(a)). These hormones are used alone or in combination depending on the cause of infertility and the protocol used (table 1). In addition, most regimens of in vitro fertilization programs and other assisted reproductive technologies include luteal phase support by exogenous administration of natural progesterone or synthetic gestagen preparations (34, 35). Ovulation induction regimens may be used to induce ovulation or superovulation. Superovulation is defined as ovulation of greater than the nor-

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Abbreviations: CC, clomiphene citrate; CI, confidence interval; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; GnRH(a), gonadotrophin-releasing hormone (agonist); hCG, human chorionic gonadotrophin; hMG, human menopausal gonadotrophin; LH, luteinizing hormone; RR, relative risk; SIR, standardized incidence ratio.

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TABLE 1. Indications for the use of selected fertility drugs*

Clomiphene citrate (CC)	Human menopausal and chorionic gonadotrophins (hMG and hCG)	Gonadotrophin-releasing hormone (agonist) (GnRH(a))
Normogonadotropic, euprolactinemic anovulation (WHO† group I)	Anovulatory infertility	Primary hypothalamic amenorrhea
Artificial insemination (male factor)	Hypogonadotropic, hypogonadal with negative progestin challenge (hypoestrogenic) (WHO group II)	Secondary hypothalamic amenorrhea
Unexplained infertility	Normogonadotropic, refractory to clomiphene therapy	In vitro fertilization
In vitro fertilization	Oligo-ovulation	Gamete intrafallopian tubal transfer
Gamete intrafallopian tubal transfer	Cervical dysmucorrhea	Pituitary down-regulation prior to gonadotrophin therapy
	Luteal phase deficiency	
	Unexplained infertility	
	In vitro fertilization	
	Gamete intrafallopian tubal transfer	

* Based on data from Blacker (34) and Derman and Adashi (35).
† WHO, World Health Organization.

mal number of ova, usually the result of menotropin or clomiphene therapy. Superovulation is used mainly for unexplained infertility or in association with in vitro fertilization, gamete intrafallopian tubal transfer, and intracytoplasmic sperm injection programs (34).

Biologic effects of fertility drugs

When assessing the biologic effects of fertility drugs on serum sex steroid levels, one must consider the fact that the effect depends on the woman's individual hormonal milieu, i.e., the specific cause of the infertility and the woman's age. Thus, it should be noted that response to administered ovarian stimulants is governed by the FSH : LH ratio *prior to* stimulation (36). It is important to understand that the administration of fertility drugs must be individualized, as sensitivity to the drug differs between patients (35). In fact, endogenous hormone levels, the rate of follicular growth, and the number of developing follicles may vary considerably from cycle to cycle in a given patient, even when she is using the same treatment regimen (35).

CC is a weak synthetic estrogen, but it acts clinically as an estrogen antagonist for ovulation induction at typical pharmacologic doses (37–39). CC acts as an anti-estrogen by interacting with estradiol receptors in the hypothalamus. "Blinded" by CC molecules, the estrogen receptor sites in the hypothalamus are unable to correctly perceive the real level of estrogen in the blood, which results in an increase in LH and, to a lesser extent, in FSH (37–40). In light of its mechanism of action, CC can only effectively be used for inducing ovulation in women with an intact hypothalamo-pituitary-ovarian axis (table 1) (35, 41). As a consequence of elevated pre-ovulatory FSH and LH levels, CC treatment generally results in a two- to threefold increase in the mean estradiol level. Among women with normal, spontaneous ovulatory menstrual cycles, this results in an increase in the number of ovulations per cycle to approximately three, the number of ovulatory follicles and serum concentrations of estradiol being correlated with the regimen of ovulation induction employed. Also, the level of progesterone is higher in stimulated cycles than after spontaneous ovulation because multiple follicles mature and are luteinized (31, 35, 42–45).

Gonadotrophins (hMG, hCG) are mainly used to treat anovulatory infertility according to the World Health Organization's classification of group I and group II ovulatory disorders (table 1) (46, 47). In regularly ovulating women, gonadotrophin therapy is usually used to induce multifollicular development, while in anovulatory infertility it is used to achieve ovulation of a few mature oocytes. All stimulation protocols designed for this purpose are aimed at augmenting the normal gonadotrophin signals. By keeping FSH levels high during the early- and mid-follicular phases, the normal selection of one follicle is usually replaced by several follicles' being rescued from atresia and reaching the preovulatory stage. Thus, FSH/hMG is given to amplify and prolong the endogenous secretion of FSH (41, 47) and to ensure

that at least two or three follicles (preferably 8–10) are developed in order to maximize pregnancy potential (35, 48). At present, three modes of gonadotrophin treatment are used: substitution therapy (applied to patients in World Health Organization group I), stimulation therapy (given to patients in World Health Organization group II), and hyperstimulation therapy (used in in vitro fertilization programs) (35, 49).

Gonadotrophin-releasing hormone (GnRH) was isolated in 1971. In that same year, the first pregnancy resulting from GnRH treatment was reported (41, 50). The primary indication for pulsatile GnRH therapy is infertility associated with hypogonadotrophic hypoestrogenic chronic anovulation (47) (table 1). Administration leads to a prompt release of LH and FSH, with the absolute amount of LH exceeding that of FSH (35, 41, 50). In GnRH or GnRH(a) regimens, the number of follicles developed and the number of oocytes obtained is greater than the number obtained from other stimulation protocols. These protocols induce low levels of LH, FSH, and estrogen for 3–4 weeks but considerably increase the number of ovulations (51, 52).

Fertility drugs and cancer

Of the fertility drugs currently being used, only CC has been evaluated for its possible carcinogenic role by the International Agency for Research on Cancer expert group. The group's report, published in 1987, concluded that the evidence for carcinogenicity of CC in humans was inadequate (53). However, following this report, two studies with relevant data on toxicity (54) and possible carcinogenic activity (55) were published. Cunha et al. (54) showed that human fetal tract tissue (grown in athymic nude mice) exposed to CC had markedly influenced stromal (müllerian tissue) differentiation. The authors concluded that CC (and also the anti-estrogen tamoxifen) is a potent estrogen in the human female genital tract and that it may be teratogenic. Furthermore, Ohnishi et al. (55) found that CC causes DNA-strand breaks in *Escherichia coli*.

Epidemiologic and experimental studies consistently suggest that endogenous as well as exogenous sex hormones play an important role in the development of female reproductive cancers (56). Since exogenously administered fertility drugs increase the woman's endogenous levels of gonadotrophins, estrogen, and progestogen, a role of fertility drugs in the development of hormone-associated cancers (e.g., cancers of the ovary, breast, endometrium, cervix, colon, and rectum, as well as melanoma) is theoretically possible. Whether fertility drugs act as direct carcinogens or by inducing or promoting tumors through interference with the endogenous hormone balance is not known.

Case reports of cancer occurring subsequent to infertility treatment have only been published on ovarian cancer (2–17) and breast cancer (17–19). In one case-control study (57) and three cohort studies (21–24, 58) of infertile women, the effect of fertility drug use on the risk of breast cancer, cervical cancer, and melanoma was analyzed. Among these studies, two found no effect for cervical cancer (21, 22) and three found no effect for breast cancer (22, 23, 57). In contrast, Rossing et al. observed that CC use was associated with a decreased risk of breast cancer (standardized incidence ratio (SIR) = 0.5, 95 percent confidence interval (CI) 0.2–1.2) (23) as well as of cervical cancer (SIR = 0.4, 95 percent CI 0.2–0.8) (24) but an increased risk of melanoma (SIR = 1.8, 95 percent CI 0.9–3.1) (58). Only the study by Venn et al. (22) also examined the risk of other hormone-associated cancers associated with fertility drug use, but the number of cases was too small to estimate an overall effect in the cohort of infertile women exposed to in vitro fertilization treatment.

INFERTILITY

The term "infertility" has not been used in a standardized manner in different studies, but it is commonly defined as the inability of a couple to conceive after 1 year of unprotected sexual intercourse. This condition may be further classified as primary infertility, in which no previous pregnancies have occurred, and secondary infertility, in which a prior pregnancy, although not necessarily a live birth, has occurred (59). Infertility may also be defined according to its specific cause: i.e., ovulatory infertility, tubal infertility, cervical or endometrial infertility, infertility due to a male factor, or unexplained infertility.

Most studies from industrially developed countries indicate that about 10–15 percent of all couples will experience either primary or secondary infertility at some time during their reproductive lives (60–62). Information on secular trends in the prevalence of infertility is sparse because of large differences in definitions and methods of measurement and a lack of good population-based studies (61). However, data from the US National Survey of Family Growth showed that the prevalence of infertility among women who had not been surgically sterilized was 13.3 percent in 1965, 13.9 percent in 1982, and 13.7 percent in 1988 (63). These data, together with similar results from two UK studies (60, 62), indicate that the prevalence of infertility has remained virtually the same over the past few decades (60, 62, 63). In surveys conducted in industrialized countries during the period 1970–1992, it was found that 4–17 percent of

the general population, 32–95 percent of primarily infertile women, and 22–79 percent of secondarily infertile women had sought medical treatment for infertility (61). Data from a 1988 US survey (64) estimated that administration of fertility drugs was the most common specialized treatment for infertility. Approximately 20 percent of infertile women had been treated with fertility drugs to stimulate or induce ovulation; approximately 5 percent had undergone artificial insemination; and only about 2 percent had undergone in vitro fertilization (64).

Despite the relatively stable prevalence of infertility, data show that the use of fertility services has increased significantly in recent years (60, 62, 64, 65). For instance, between 1968 and 1984, the number of office visits made for infertility increased nearly threefold in the United States (65). In the United Kingdom, analyses of trends in medical services showed an increase in the use of general practitioners, as well as in subsequent referrals to infertility specialists, among successively younger age cohorts of women. This finding applied to both primary and secondary infertility (60, 62, 64). In the study by Templeton et al. (60), 95.1 percent of the younger cohort (women aged 36–40 years) had sought medical advice, compared with 72.1 percent of the older cohort (women aged 46–50 years).

TIME TRENDS IN FERTILITY DRUG USE AND INDICATIONS

Only limited data concerning the actual use of fertility agents are available. In the United States, Wysowski (32) reported an approximate doubling in the number of prescriptions written for fertility drugs between 1973 and 1991. In Denmark, during the same period (1973–1993), there was an 11-fold increase in the sale of CC, and since 1986, the sale of hMG has increased 13-fold (66). It appears, in keeping with the increasing use of fertility services, that the number of women who have been exposed to fertility drugs is growing rapidly. A simplified overview of indications and trends in fertility drug use is presented in figure 1. As can be seen, a shift in indications for the use of fertility drugs has occurred. Until in vitro fertilization procedures became widely used in the mid- and late 1980s, infertility drugs such as CC and hMG were not used on normally ovulating women. Rather, CC and hMG were used primarily to treat women with ovulatory abnormalities such as anovulation, polycystic ovaries, and luteal phase defects, as well as some women with unexplained infertility (31, 41, 44, 49, 59). Thus, a "weak" drug was given to a limited number of women, primarily those with oligomenorrhea or amenorrhea and those with low/normal

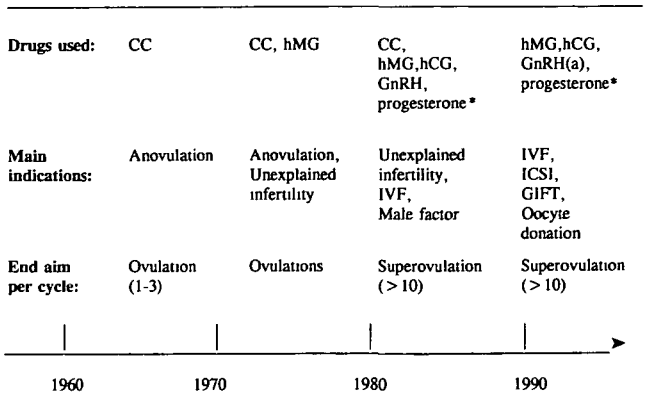

FIGURE 1. Trends in fertility drug treatment, 1960–1990. An asterisk (*) indicates that progesterone was administered for luteal phase support in most in vitro fertilization programs and other assisted reproductive technologies. CC, clomiphene citrate; hMG, human menopausal gonadotrophin; hCG, human chorionic gonadotrophin; GnRH, gonadotrophin-releasing hormone; GnRH(a), gonadotrophin-releasing hormone (agonist); IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; GIFT, gamete intrafallopian tubal transfer. Based on data from March (41), Glasier (44), Insler (49), and Healy et al. (59).

gonadotrophin and estrogen secretion who ovulated infrequently or not at all. In a natural cycle, usually only one ovum is released. Use of CC increases the number of ova released to two or three. In programs using CC plus hMG, the number of ova released is, on average, 6.5. When GnRH(a) is added, the number increases to an average of nine ova (22, 35, 48). Thus, numbers of ovulations and mean hormone levels have increased proportionally with the potency of the induction programs. In addition, indications for fertility drug use have changed to include women without prior decreased ovulations or hormone levels—i.e., women who receive in vitro fertilization, gamete intrafallopian tubal transfer, or intracytoplasmic sperm injection because of tubal damage, unexplained infertility, or partner infertility problems.

OVARIAN CANCER PATHOLOGY AND EPIDEMIOLOGY

Ovarian cancer constitutes a heterogeneous group of tumors. Overall, about 85 percent are of epithelial origin. This group comprises serous, endometrioid, and mucinous adenocarcinomas, of which serous adenocarcinoma is by far the most common type (67, 68). Ovarian cancers also include germ cell tumors (2–3 percent) and sex cord tumors (2–3 percent). Germ cell tumors are dominated by dysgerminomas and sex cord tumors by the granulosa cell tumors. The remainder are malignant neoplasms with unspecified morphology (that is, undifferentiated carcinoma) and others with specified morphology, such as sarcomas and stromal/fibroepithelial tumors (68).

Intermediate between the completely benign tumors and the malignant carcinomas are the borderline tumors or tumors with low malignant potential, including the granulosa cell tumors (69). The borderline tumors constitute 10–17 percent of all ovarian malignancies (70, 71). Borderline tumors are characterized by cellular stratification with variable nuclear atypia and mitotic activity but without evidence of stromal invasion (67). It is not known whether the natural history of ovarian cancer includes a borderline phase and, if so, what proportion of borderline tumors progress to ovarian cancer.

There is marked geographic variation in ovarian cancer incidence. In general, the incidence is highest in the United States, Canada, and Scandinavia (the world's highest incidence is in Denmark) and lowest in Japan, Italy, and developing countries (72). Ovarian cancer rates have remained almost constant in the high risk areas (European and North American countries) throughout the period 1958–1982 (72). In the same period, a rising trend was observed in low risk areas like Japan, India, and Singapore (72, 73). The age-standardized incidence rate, which is about 14 per 100,000 women in the high risk areas, corresponds to a lifetime risk of approximately 1.9 percent. Malignant ovarian tumors are most common in women over 60 years of age, with only 10 percent of ovarian cancers occurring in women under the age of 40 years (74, 75). In contrast, borderline tumors are more common in younger women, the mean age at diagnosis being approximately 40 years (71, 76).

Ovarian cancer is the most lethal gynecologic malignancy in the Western world. In high risk areas, it ranks fourth in cancer mortality after cancers of the breast, lung, and colon (77). Overall, 70 percent of women present with stage III or stage IV disease (78). Only 5 percent of patients with stage III or stage IV disease, 21 percent of patients with stage II disease, and 64 percent of patients with stage I disease are alive 5 years after diagnosis (78). The prognosis is substantially better for borderline ovarian tumors than for invasive tumors: After a mean follow-up period of 7 years, one study on borderline tumors found that 99 percent of patients with stage I disease and 92 percent with stage II and stage III disease were still alive (79).

There are several established risk factors for ovarian cancer. The strongest of these relate to reproductive events. Multiparity and oral contraceptive use have been shown to be associated with a substantial reduction in risk (1, 80–83). It is generally agreed that the protective effect increases with increasing numbers of births (1, 83, 85) and that the association with oral contraceptive use is dose-dependent, with a 50 percent decrease in risk being seen after 3 years of use (80). A decreased risk of ovarian cancer is also found among women who have had a tubal ligation or hysterectomy (86–88). No consistent trend in risk has been observed with age at menarche, age at first pregnancy, or age at menopause (1, 84, 85, 89). Similarly, most studies have not found an association between duration of hormone replacement therapy and ovarian cancer risk (90). An increased risk of ovarian cancer has been found among women with a familial aggregation of breast and ovarian cancers, and the *BRCA1* gene has been located in these families (91).

Many studies that have assessed infertility as a risk factor for ovarian cancer have been impaired by only being able to use surrogate measures of infertility. Data on length of unprotected intercourse before a pregnancy or on physician-diagnosed infertility have frequently not been obtained. Furthermore, a distinction has often not been made between involuntary and voluntary infertility or between primary and secondary infertility (20–22, 85, 88, 92). Instead, several studies have found increased ovarian cancer risk among nulliparous married women (compared with nulliparous unmarried women) or among women who reported difficulties in conceiving or with unplanned childlessness (compared with women without such problems) (85, 92–97). Other studies have evaluated contraceptive-free years of marriage and found an overall increased risk of ovarian cancer in the range of 1.5–2.0 (96, 98). However, in several recent studies that used a quantitative measure of attempting pregnancy without success (and not a surrogate measure), it was revealed that after stratification for parity, the risk was confined to nulliparous women, with relative risks in the range of 1.5–2.7 (1, 88, 99).

Infertility may also be assessed by specific cause, yet few studies have included this information. It appears, however, that ovarian cancer risk may indeed vary by type of infertility, with some studies suggesting that ovulatory dysfunction is most predictive of subsequent ovarian cancer risk (1, 21, 100). Whittemore (1) found an odds ratio of 2.1 (95 percent CI 0.9–4.7) and Rossing et al. (100) reported a relative risk of 3.7 (95 percent CI 1.4–8.1) in women with ovulatory infertility compared with women in the general population. Similarly, Brinton et al. (21) reported an almost doubling in risk among women with luteal phase defects compared with women with other causes of infertility. In contrast, two other studies that had information on type of infertility found that unexplained infertility related most strongly to ovarian cancer risk (20, 22).

In most studies, reproductive risk factors for borderline and invasive ovarian tumors have been found to be similar (73, 89, 93, 101). This was confirmed in a

recent pooled analysis of nine case-control studies, with the exception that the association with oral contraceptive use was less pronounced for borderline tumors than for invasive tumors (102). Fewer studies have assessed the association between infertility and borderline ovarian tumors, although, as with invasive ovarian cancer, infertility appears to increase risk, with risk estimates ranging from 2.3 to 4.0 (73, 101, 102).

STUDIES OF FERTILITY DRUG USE IN RELATION TO RISK FOR OVARIAN CANCER

The evidence indicating a possible association between use of fertility drugs and development of ovarian tumors has emerged from both descriptive studies (case reports and case series) (table 2) and analytical studies (cohort and case-control studies) (tables 3–5).

Descriptive studies

The first case report was published by Bamford and Steele in 1982 (2). Since then, case reports on 50 women with a malignant or borderline ovarian tumor detected during or after infertility treatment have been published (2–17) (table 2). These reports have varied widely in terms of the degree of clinical information presented. The type of infertility is frequently not specified, and only two case reports included information on potentially confounding factors such as a family history of breast cancer or ovarian cancer (15, 16). Some of the case reports lacked information on the total amount of fertility drugs received (6–8, 11, 12, 14, 16) or on the amount of time between diagnosis and the first or last treatment cycle (3, 8, 11), although all patients had received treatment before diagnosis. Cases were 22–41 years of age at diagnosis. By histology, the tumors can be divided into three groups: granulosa cell tumors (26 percent), borderline tumors (28 percent), and adenocarcinomas (42 percent). The young age of the patients in these reports probably reflects the fact that the majority (54 percent) were patients with tumors of low malignant potential. The patients had received an average of six treatment cycles for infertility. Overall, CC was the treatment in 81 percent of the patients; 72 percent received gonadotrophins; and 56 percent were treated with both CC and gonadotrophins. Of the nine patients who received treatment for 12 or more cycles (marked with a double dagger (‡) in table 2), seven developed invasive epithelial carcinoma and two developed granulosa cell tumors.

Some concern has been expressed as to whether the association between fertility drugs and ovarian cancer can be explained by detection bias. One of the case series (12) found that all of the women studied became pregnant after removal of their granulosa cell tumors; thus, there was concern that they actually had had their tumors before their infertility treatment started. Furthermore, Lais et al. (103) found an increased frequency of ovarian tumors during microsurgery for infertility evaluation. Among 571 infertile women under 40 years of age who were undergoing laparoscopy, a malignant ovarian tumor (cystadenocarcinomas) occurred in six women, as compared with only one tumor among 5,806 women with no fertility problems from the same population who underwent cholecystectomy or appendectomy. Findings from these case reports may reflect an increased likelihood of detection of ovarian tumors in women undergoing infertility surgery rather than an effect of fertility drug use on ovarian cancer risk. However, in the absence of an appropriate comparison group, descriptive studies can only be used to generate hypotheses for further research and cannot directly address the relation between infertility and ovarian cancer risk.

Analytical studies

At present, the results of eight epidemiologic studies on the relation between fertility drug use and risk of ovarian and borderline tumors have been published (tables 3–5). These studies included four cohort studies of infertile women (20–22, 100) (one study also included an analysis of a nested case-cohort study (100)), one hospital-based case-control study (104), two population-based case-control studies (99, 105), and a pooled analysis using original data from 12 case-control studies. The results of the pooled analysis were published as three separate articles on risk factors for invasive epithelial (1), borderline (102), and nonepithelial (106) ovarian tumors, respectively.

Cohort studies. A cohort study by Ron et al. (20) consisted of 2,575 women evaluated for primary or secondary infertility (table 3). Infertility was diagnosed by a physician, and from the clinical files the type of infertility was also defined. A total of four ovarian cancer cases were observed among all infertile women as compared with 1.9 expected, yielding a nonsignificantly elevated SIR of 2.1. The cancer risk associated with use of CC or hMG was similar to the risk observed in infertile women receiving other hormonal treatment or no hormones (risk estimates not provided). One strength of this study was the nearly complete follow-up, as the authors succeeded in matching 96 percent of the identified patients from the gynecologic outpatient clinic records with the population registry. Also, this study included information on important risk factors for ovarian cancer, such as parity and use of oral contraceptives. However, since the cohort was young (mean age at the end of follow-up

was 41.0 years), the study was limited by small numbers of observed events and by not presenting detailed information on the different hormonal treatments or their risk estimates.

Brinton et al. (21) studied 2,335 women evaluated for infertility (table 3). Infertility was defined as the inability to conceive in spite of attempts to become pregnant for at least 1 year. The observed number of ovarian cancer cases did not differ from that expected. In comparison with the general population, ovarian cancer risk was higher in women with progesterone deficiencies (SIR = 1.6) than in women with other causes of infertility (SIR = 1.1) (data not shown). There were no differences in ovarian cancer risk between women treated for infertility and those not treated (risk estimates not provided). A limitation of the study, apart from its small size, is that it covered a period during which primarily estrogens and progesterone were being used. In addition, the study lacked detailed information on these infertility treatments; furthermore, only 67 percent of the women in the cohort could actually be traced.

Rossing et al. (100) examined a cohort of 3,837 women evaluated for infertility at several infertility clinics (table 3). All of the women had attempted conception for at least 1 year and had made at least two visits to an infertility clinic. Cases were compared with the general female population in the study area, as well as with a control group of 135 women selected at random from the cohort. In comparison with the general population, the SIR for invasive epithelial ovarian cancer was 1.5 (95 percent CI 0.4–3.7), and it was 3.3 (95 percent CI 1.1–7.8) for borderline tumors (data not shown). Women who had used hCG, hMG/FSH, or CC were at increased risk for developing an ovarian tumor: The SIRs were 2.8 (not significant), 5.6 (not significant), and 3.1 (95 percent CI 1.4–5.9), respectively (data not shown). Infertile women with ovulatory abnormalities had an approximate doubling of risk for an ovarian tumor compared with infertile women with other abnormalities. Ever use of CC was associated with a relative risk of 2.3 (95 percent CI 0.5–11.4) compared with infertile women with no CC use. The risk was mostly pronounced in women with long term use (≥12 cycles), and it was observed in both nulligravid and gravid women (table 4). Data on the use of hMG/FSH were not presented, but there was no increase in risk of ovarian tumors associated with the use of hCG when cases were compared with the subcohort.

In this carefully conducted investigation, specific information on type of infertility, type of infertility drugs, and number of cycles of use was retrieved directly from the clinical records, and blindly with regard to cancer diagnosis. Nevertheless, some limitations of the study should be noted. The results were based on a small number of tumors, of which nearly half were reported to be borderline. In the within-cohort analysis, all types of ovarian tumors were grouped together, and risks associated with CC use were not provided separately for invasive and borderline tumors. Ideally, borderline and invasive ovarian tumors should be analyzed separately, since they differ in terms of behavior, prognosis, and age distribution (70, 71). Furthermore, despite having similar risk factors (107), it is still not known whether there are etiologic differences between ovarian tumors of low malignant potential and invasive tumors. More importantly, the inclusion of granulosa cell tumors in the group of epithelial tumors has been criticized because of their different embryologic, pathologic, and epidemiologic characteristics (33). Rossing et al. (29) have recently responded to this critique by eliminating women with granulosa cell tumors from the analysis, revealing an attenuated but still elevated risk associated with exposure to 12 or more cycles of CC (relative risk (RR) = 6.7, 95 percent CI 0.8–58.8). Additionally, it has been suggested that the high number of borderline tumors in this cohort and the high ratio of borderline tumors to invasive tumors may be the result of detection bias due to intensive ultrasound surveillance in this population (108). This explanation seems unlikely, however, since the majority of tumors (9 of 11) were diagnosed *after* infertility treatment had stopped. Furthermore, the increased risk associated with long term CC use was observed in both women with ovulatory abnormalities (RR = 7.4, 95 percent CI 1.0–53.1) and women without them (RR = 9.1, 95 percent CI 1.0–86.5) (data not shown), which suggests that the association was not due simply to an ovarian abnormality leading to both infertility and CC use.

The most recent and, to date, the largest cohort study carried out among infertile women was conducted by Venn et al. (22), with specific emphasis on in vitro fertilization treatment (table 3). A total of 5,564 women were treated with ovarian stimulation, while 4,794 women had no ovarian stimulation. Three malignant ovarian tumors were observed in each group. Relative to the general population, this yielded an SIR of 1.7 (not significant) for the exposed women and an SIR of 1.6 (not significant) for the unexposed women. When only women with 5 or more years of follow-up were included in the analysis, risk estimates increased slightly. Finally, infertile women treated with ovarian stimulation had a nonsignificant increased risk of ovarian cancer compared with infertile women without this treatment.

Although this is the largest cohort study yet con-

TABLE 2. Case reports on fertility drug use and ovarian cancer, 1982–1997

Study (reference no.) and year	Country	Histology	Age (years) at diagnosis	Preparations*	Durations*,† (cycles)	Months since last/first treatment
Bamford and Steele (2), 1982	United Kingdom	Endometrioid carcinoma	32‡	CC, hCG FSH, LH, hCG	24 for hMG 24 for hCG	4/40
Atlas and Menczer (3), 1982	Israel	Borderline	26	CC (hCG)	Several years (1)	Several years
Ben-Hur et al. (4), 1986	Israel	Serous adenocarcinoma	27‡	Bromocriptine, CC, hCG	12, 12, 2	1/
		Serous adenocarcinoma	22	CC	3	2/
Carter and Joyce (5), 1987	United Kingdom	Serous adenocarcinoma	25	CC, LH, FSH, hCG	3	0/
Kulkarni and McGarry (6), 1989	United Kingdom	Papallary serous adeno-carcinoma	35‡	CC, cyclofenil	Several months, 18	60/
Lappöhn et al. (7), 1989	The Netherlands	Granulosa cell tumor	29	CC	1	1/
Dietl (8), 1991	Germany	Papillary serous adenocarcinoma	34‡	CC, FSH, LH, GnRH	>12 for CC; 1 for FSH and LH; 4 for GnRH	?
				Buserelin	6 for buserelin	
Goldberg and Runowicz (9), 1992	United States	Borderline	32	hMG	2	9/11
		Borderline	25	hMG	3	5/8
		Borderline	28	hMG	2	5/7
Nijman et al. (10), 1992	The Netherlands	Borderline	38	LHRH-a, hMG, hCG	2	4/5
		Borderline	36	hMG, LHRH-a	4	1/15
Lopes and Mensier (11), 1993	France	Borderline	39	hMg, hCG	4	?
		Borderline	36	CC, hMG, hCG	8	?
		Borderline	37	CC, hMG, hCG	?	?
		Borderline	41	CC, hMG, hCG	4	?
		Borderline	34	hMG, hCG	4	?
		Borderline	34	Ovulation induction	6	?
		Endometrioid adenocarcinoma	37	hMG, hCG	3	?
		Endometrioid adenocarcinoma	35	CC	1	?
		Endometrioid adenocarcinoma	39	CC, hMG, hCG	8	?
		Serous adenocarcinoma	36‡	CC, hMG, GnRH(a), hCG	>20	?
		Clear cell adenocarcinoma	34‡	CC, hMG, GnRH(a), hCG	>20	?
		Clear cell adenocarcinoma	36	CC, hMG	6	?
		Undifferentiated carcinoma	36	CC, hMG, GnRH(a), hMG	2, 5	?
Willemsen et al. (12), 1993	The Netherlands	Granulosa cell tumor	37	CC, bromocriptine, hMG	?, ?, 4	24/
		Granulosa cell tumor	27	CC, hMG	?, 3	0
		Granulosa cell tumor	30	CC, GnRH, hMG	?	?
		Granulosa cell tumor	35	CC, hMG	?, 4	?
		Granulosa cell tumor	30	CC, bromocriptine	?	?
		Granulosa cell tumor	25	CC	?	?
		Granulosa cell tumor	31‡	CC, hMG, GnRH	12, 8, 2	2/
		Granulosa cell tumor	27	CC	6	6/

		Granulosa cell tumor	38	hMG, CC	5, ?	60/
		Granulosa cell tumor	33‡	CC, tamoxifen	36	?
		Granulosa cell tumor	32	CC, hMG	?, 2	?
		Granulosa cell tumor	31	CC	?	?
Balasch and Barri (13), 1993	Spain	Borderline	35	CC	6	26/32
Karlan et al. (14), 1994	United States	Papillary adenocarcinoma	39	CC	5	0/5
Komatsu et al. (15), 1995	Japan	Papillary serous carcinoma	34‡	CC, hMG, hCG, bromocriptine	15, 3, 8, ?	2/52
Grimbizis et al. (16), 1995	Greece	Borderline	29	hMG, CC, buserelin	5, 1, 3	8/14
		Benign, mucinous cystadenoma	34	CC, hMG, buserelin	7, 2, 1	9/14
Unkila-Kallio et al. (17), 1997	Finland	Granulosa cell tumor	29	CC	?	?
		Granulosa cell tumor	32	CC	2	0/3
		Papillary serous carcinoma	28	CC	24	9/34
		Papillary serous carcinoma	31	CC, FSH	36, 3	0/72
		Papillary serous carcinoma	33	CC	24	36/60
		Papillary serous carcinoma	47	CC	12	84/108
		Mucinous carcinoma	38	CC, FSH	3, 3	36/60
		Malignant teratoma	43	FSH	26	0/168

* Abbreviations: CC, clomiphene citrate; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; GnRH(a), gonadotrophin-releasing hormone (agonist); hCG, human chorionic gonadotrophin; hMG, human menopausal gonadotrophin; LH, luteinizing hormone; LHRH-a, luteinizing hormone-releasing hormone agonist.
† Different durations of treatment are separated by commas and correspond to the individual preparations, respectively.
‡ Indicates patient received 12 or more treatment cycles.

ducted, it has some limitations: 1) exposure to specific fertility drugs was not analyzed—instead, exposure was defined only as "in vitro fertilization" treatment (table 3); 2) the median length of follow-up was short, and only 51 percent of women in the exposed group were followed for at least 5 years; 3) women who had started but not completed a stimulated in vitro fertilization cycle were included in the exposed group; 4) there was no information on parity or other potential confounders; and 5) the number of in vitro fertilization treatment cycles was generally low (mean = 2; range = 1–22), with 77 percent of the women having had three or fewer stimulation cycles, thus limiting the investigators' ability to study a potential dose-response relationship with increasing numbers of in vitro fertilization cycles. In addition, Rossing and Weiss (109) subsequently pointed out that an inconsistency in the analysis of included person-time between registration and the first stimulated cycle could have contributed to an underestimation of the relative rates in the women who received in vitro fertilization. This, together with limitations 2 and 3 above, may have caused an underestimation of the true risk of ovarian cancer.

Case-control studies. Whittemore et al. (1, 110) performed a collaborative pooled analysis using original data collected from 12 case-control studies of ovarian cancer diagnosed between 1956 and 1986. Only three (95, 98, 111) of the 12 studies included had data on infertility, use of fertility drugs, and epithelial ovarian cancer (table 5). An increased risk of ovarian cancer was observed in women who had used fertility drugs as compared with women without a history of infertility. However, the risk associated with the use of fertility drugs was much higher among nulligravid women than among gravid women (table 4). This is in contrast to the study by Rossing et al. (100), in which the risk associated with fertility drug use was increased in both nulligravid women and gravid women (table 4). This inconsistency might result from the nulligravid women in Whittemore et al.'s pooled analysis using fertility drugs longer than the women who subsequently conceived. However, it has been argued that the difference in the magnitude of risk between nulligravidae and gravidae cannot be explained by the protective effect of pregnancy or by duration of fertility drug use, but is more likely due to differences in other patient characteristics (unknown confounding factors) (112). Caro et al. (113) suggested that this difference could be caused by selection bias, if women who were treated for infertility and did not conceive were less likely to participate in the control group, or by recall bias, if these women were reluctant to reveal fertility drug use.

TABLE 3. Cohort studies of infertility drug use and risk of ovarian cancer

Study (reference no.) and year	Country	Type of cohort	Median age (years) at entry	End of follow-up	Composition of cohort*	Mean length of follow-up (person-years in parentheses)	Infertility treatment classification†	No. of cases and type of infertility	Risk of ovarian cancer
Ron et al. (20), 1987	Israel	Women referred for infertility, 1964–1974	28.7	1981	n_t = 1,343‡ n_u = 1,232 T = 2,575	12.3 years (31,622)	CC, hMG, "other hormonal treatments," no hormonal treatment	4 ovarian cancers: 2 unclassified infertility; 1 male partner infertility; 1 mechanical infertility	Compared with general population: SIR† = 2.1 (NS†) for infertility SIR = 6.1 (NS) for unclassified infertility Compared with infertile women with no use of fertility drugs: No excess risk among infertile women‡ treated with CC or hMG
Brinton et al. (21), 1989	United States	Women evaluated for infertility, 1935–1964	28.6	1981	n_t = 530 n_u = 1,805 T = 2,335	19.4 years (45,408)	Primarily "estrogens or progesterones"	11 ovarian cancers: 5 progesterone deficiencies; 2 structural defects; 2 male factors; 2 other or unknown	Compared with general population: SIR = 1.3 (NS) for infertility Compared with infertile women: SIR = 1.8 for hormonal infertility compared with other types of infertility
Rossing et al. (100), 1994	United States	Women evaluated for infertility, 1974–1985	29.7	1992	In a subcohort of 135 patients: n_t = 64% with CC; 25.9% with hCG; 4.4% with hMG T = 3,837	12.3 years (43,438)	CC hCG hMG	11 ovarian tumors: 4 epithelial invasive, 2 non-epithelial, and 5 borderline; 5 with non-ovulatory infertility and 6 with ovulatory infertility	Compared with general population: SIR = 3.7 (95% CI† 1.4–8.1) for ovulatory infertility Compared with infertile women: Never CC use (2/48§): adjusted RR† = 1.0 Ever CC use (9/87): adjusted RR = 2.3 (95% CI 0.5–11.4) 1–11 months of use (3/64): adj. RR = 0.8 (95% CI 0.1–5.7) 12 months of use (5/18): adj. RR = 11.1 (95% CI 1.5–82)
Venn et al. (22), 1995	Australia	Women referred for IVF† treatment, 1978–1992	32	1988–1993	n_t = 5,564 n_u = 4,794 T = 10,358	5.2 years for treated group; 7.6 years for untreated group (66,927)	IVF treatment: Until 1987, CC plus hMG or hCG 1987–1992, GnRH(a) plus hMG or hCG 1990–1992, GnRH plus hMG/FSH	3 serous or mucinous adenocarcinomas in *treated* group and 3 *serous or* mucinous adenocarcinomas in *untreated* group; 5 with unexplained infertility and 1 unknown	Compared with general population: Treated with IVF: SIR = 1.7 (NS); SIR = 2.1 (NS) including only women with ≥5 years of follow-up No IVF: SIR = 1.6 (NS); SIR = 1.8 (NS) including only women with ≥5 years of follow-up Compared with infertile women: Infertile women *with* IVF treatment had an RR = 1.5 (95% CI 0.3–7.6) compared with women *without* IVF treatment RR for unexplained infertility = 19.1 (95% CI 2.2–165)

* n_t = treated with fertility drugs; n_u = untreated; T = total.

† Abbreviations: CC, clomiphene citrate; CI, confidence interval; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; GnRH(a), gonadotrophin-releasing hormone (agonist); hCG, human chorionic gonadotrophin; hMG, human menopausal gonadotrophin; IVF, in vitro fertilization; NS, not significant; RR, relative risk; SIR, standardized incidence ratio.

‡ Treated for hormonal infertility.

§ Number of cases/number of controls.

TABLE 4. Results from selected studies of the risk for ovarian cancer associated with infertility and use of fertility drugs in never and ever pregnant women

Study (reference no.) and year	Variable	Never pregnant women						Ever pregnant women					
		No. of cases	No. of controls	Crude RR*	95% CI*	Adjusted RR	95% CI	No. of cases	No. of controls	Crude RR	95% CI	Adjusted RR	95% CI
Whittemore et al. (1), 1992	History of infertility												
	No	58	78	1.0		1.0		472	888	1.0		1.0	
	Yes	34	23	2.1†	1.1–4.0†	2.1	1.0–4.2	62	112	1.0†	0.7–1.4†	1.0	0.6–1.2
	Fertility drug use												
	No infertility	54	78	1.0		1.0		472	888	1.0		1.0	
	Never use	22	22	1.4†	0.7–2.9†	1.6	0.7–313	54	102	1.0†	0.7–1.4†	0.8	0.6–1.2
	Ever use	12	1	17.3†	3.6–84†	27.0	2.3–315‡	7	10	1.5†	0.6–3.8†	1.4	0.5–3.6§
	Fertility drug use among infertile women												
	Never use	22	22	1.0				54	102	1.0			
	Ever use	12	1	12.0†	2.0–71†			8	10	1.5†	0.6–4.0†		
Rossing et al. (100), 1994	Fertility drug use (CC*) among infertile women												
	0–11 cycles	No data				1.0		No data				1.0	
	≥12 cycles	No data				10.8	1.5–78	No data				17.0	1.2–243
Mosgaard et al. (99), 1997	History of infertility												
	No	20	53	1.0		1.0		458	1,286	1.0		1.0	
	Yes	64	58	2.9†	1.6–5.4†	2.7	1.3–5.5	71	187	1.1†	0.8–1.4†	1.1	0.6–2.2
	Fertility drug use												
	No infertility	20	53	1.0		1.0		458	1,286	0.9		1.0	0.6–1.8
	Never use	46	39	3.1		2.7	1.3–5.5	61	148	1.0		1.1	0.6–2.2
	Ever use	18	19	2.5		2.3	0.9–5.6	10	39	0.6		0.7	0.3–1.8
	Fertility drug use among infertile women												
	Never use	46	39	1.0		1.0		61	148	1.0		1.0	
	Ever use	18	19	0.8†	0.4–1.7†	0.8	0.4–1.7	10	39	0.6†	0.3–1.3†	0.6	0.2–1.3

* RR, relative risk; CC, clomiphene citrate; CI, confidence interval.
† Estimated from data provided in the paper.
‡ Adjusted for age, study, and oral contraceptive use.
§ Adjusted for age, study, parity, breastfeeding, and oral contraceptive use.

TABLE 5. Case-control studies of infertility, use of fertility drugs, and risk of borderline tumors and ovarian cancer

Study (reference no.) and year	Country	Type of case-control study	Type and no. of cases and controls	Data collection period	Age (years) of cases at diagnosis	Data collection method	Response rates	Type of infertility drug	Prevalence of infertility among controls (%)	Variable	No. of cases	No. of controls	Crude RR*	95% CI*	Adjusted RR	95% CI
Whittemore et al. (1), 1992	United States	Pooled analysis with data† from three case-control studies (95, 98, 111)	622 epithelial ovarian cancers; 1,101 hospital and population controls	1977–1981	18–80	Personal interview	Cases: 5–24% died before contact 70–90% included among the remaining Controls: 60–78%	Limited information	12	History of infertility						
										No	526	966	1.0		1.0	
										Yes	96	135	1.3	1.0–1.7‡	1.0	0.8–1.4
										Fertility drug use						
										No infertility	526	966	1.0		1.0	
										Never use	76	124	1.1	0.8–1.5‡	0.9	0.7–1.3
										Ever use	20	11	3.3	1.7–6.7‡	2.8	1.3–6.1§
										Fertility drug use among infertile women						
										Never use	76	124	1.0			
										Ever use	20	11	2.9	1.4–6.4‡		
Harris et al. (102), 1992	United States	Pooled analysis with data† from three case-control studies (95, 98, 111)	88 borderline ovarian tumors; 752 population controls	1977–1981	18–80	Identical to Whittemore et al. (1) above	NI*	NI	12	History of infertility						
										No	180	2,779	1.0		1.0	
										Yes	43	370	1.8	1.3–2.5‡	1.9	1.3–2.7
										Fertility drug use						
										No infertility	69	657	1.0		1.0	
										Never use	15	86	1.7	0.9–3.0‡	1.6	0.8–3.1
										Ever use	4	9	4.2	1.4–12.8‡	4.0	1.1–13.9¶
										Fertility drug use among infertile women						
										Never use	15	86	1.0			
										Ever use	4	9	2.5	0.7–9.0‡		
Horn-Ross et al. (106), 1992	United States	Pooled analysis with data† from two case-control studies (98, 111)	18 germ cell tumors; 1,142 population controls 45 stromal tumors; 2,617 population controls	1977–1981	18–80	Identical to Whittemore et al. (1) above	Cases: 5–24% died before contact; 70–90% included among the remaining Controls: 60–90%	NI	NI	Fertility drug use						
										Never use	44	2,615 #	NI		1.0**	
										Ever use	1	2			13.4	0.9–191

Franceschi et al. (104), 1994	Italy	Hospital-based case-control study	195 epithelial ovarian cancers; 1,339 controls	1992–1993	NI	Personal interview	NI	NI	2.5	History of infertility						
										No	191	1,305	1.0			
										Yes	4	34	0.8	0.3–2.3‡		
										Fertility drug use						
										No infertility	191	1,305	1.0		1.0'	
										Never use	2	19	0.7	0.1–3.8‡		
										Ever use	2	15	0.9	0.2–4.0‡	0.7	3.3††
										Fertility drug use in infertile women						
										Never use	2	19	1.0			
										Ever use	2	15	1.3	0.7–2.4‡		
Shushan et al. (105), 1996	Israel	Population-based case-control study	164 invasive epithelial tumors‡‡ and 36 borderline tumors‡‡; 408 controls	1990–1993	36–64	Telephone interview	Cases: 25% of eligible died before contact; 70% included among the remaining	CC* hMG*	11.3	History of infertility						
										No	166	362	1.0			
										Yes	34	46	1.6	1.0–2.6‡		
										Fertility drug use						
										No infertility	166	362	1.0			
										Never use	10	17	1.3	0.6–2.9‡		
										Ever use	24	29	1.8	1.0–3.3	1.3	2.7§§
							Controls: ~63%			CC alone	11	18	1.0	0.9–3.1‡		
										hMG alone	11	6	3.2	0.9–10.8‡		
										CC +/– hMG	22	24	1.6	0.6–4.1		
Mosgaard et al. (99), 1997	Denmark	Population-based case-control study	684 epithelial (87%) and *non*-epithelial (7%) cases; 1,721 controls	1989–1994	18–59	Posted questionnaire	Cases: 37% of eligible died *before* contact; 81% included among the remaining	Tablets only—CC	15.5	History of infertility						
										No	613	1,484	1.0			
								Tablets and 1 injection—CC and hCG*		Yes	135	245¶¶	1.5	1.2–2.0‡		
										Fertility drug use						
										No infertility	613	1,484	1.0			
										Never use	107	187	1.4	1.1–1.8‡		
										Ever use	28	58	1.2	0.7–1.8‡		
							Controls: ~78%	Several injections—hMG or hCG		Fertility drug use among infertile women						
										Never use	107	187	1.0			
										Ever use	28	58	0.8	0.5–1.4‡		
										CC alone	15	27	1.0	0.5–1.9‡		
										CC and hCG	6	13	0.8	0.3–2.1‡		
										hMG and hCG	7	13	0.9	0.4–2.4‡		

* RR, relative risk; CI, confidence interval; NI, no information; CC, clomiphene citrate; hMG, human menopausal gonadotrophin; hCG, human chorionic gonadotrophin.
† Studies in which data on fertility drugs were available.
‡ Estimated from data provided in the paper.
§ Adjusted for age, study, parity, breastfeeding, and oral contraceptive use.
¶ Adjusted for age, study, and parity.
# Stromal tumors.
** Both infertile and nonfertile.
†† Adjusted for age, area of residence, education, use of oral contraceptives, and number of pregnancies.
‡‡ Results shown are totals of all ovarian tumors, i.e., invasive and borderline.
§§ Adjusted for age, parity, body mass index, region of birth, education, family history, and interviewer.

In the pooled analysis of Whittemore et al. (1), the comparison of fertility drug use to nonuse was not restricted to women diagnosed as infertile, and it may, therefore, reflect the risk due to infertility itself as well as that due to the use of drugs. It may be more appropriate to assess risk associated with use of fertility drugs *within* the group of infertile women, as these women are more likely to be comparable with respect to other potentially confounding factors such as parity and use of oral contraceptives. We calculated new risk estimates, using infertile women who had not used fertility drugs as the reference group, for all of the case-control analyses (tables 4 and 5). In the pooled analysis of epithelial ovarian cancer by Whittemore et al., the crude risk related to fertility drug use, when the analysis was restricted to infertile women, was increased in ever users (table 5). An even higher risk for fertility drug use was noted among nulligravid women (table 4).

Harris et al. (102) published results from the pooled analysis of Whittemore et al. concerning risk factors for borderline ovarian tumors (table 5). In three of the studies included in the pooled analysis, information on physician-diagnosed infertility among ever-married women and use of fertility drugs had been collected (95, 98, 111). Relative to women who did not report infertility problems, the risk associated with use of fertility drugs was 4.0 (95 percent CI 1.1–13.9). Restricting the analysis to women with a history of infertility, the crude risk estimated from data provided in the paper was 2.5 (95 percent CI 0.7–9.0) for fertility drug use compared with never use (table 5). This is similar to the crude estimate of 2.9 in infertile women with invasive ovarian cancer (1). Data that would allow calculation of risk estimates among nulliparous and parous women separately were not provided.

Finally, Horn-Ross et al. (106) published results on nonepithelial ovarian cancer, as part of the pooled analysis of Whittemore et al. (table 5). However, information on use of fertility drugs was available only for two of the studies (98, 111). One case (nulligravid) with a stromal tumor and two controls (one nulligravid, the other multiparous) reported use of fertility medications, yielding a nonsignificant elevated risk.

The results emerging from the pooled analysis of the 12 US case-control studies of ovarian cancer have received international attention and have been much debated. Authors have expressed their concern with regard to several methodological issues (28, 33, 112–114). One concern relates to the fact that results on fertility drugs and epithelial ovarian cancer were based on only three of the 12 original studies; consequently, the numbers of exposed cases and controls were small. In some of the original studies, up to 24 percent of cases had died before enrollment (table 5). Furthermore, a high proportion of the cases were diagnosed before the most currently used fertility drugs were available (33). Other major limitations include the lack of information on cause of infertility and type of fertility drug used (33). Additionally, it has been suggested (113) that the prevalence of fertility drug use among infertile control subjects is much lower (4 percent) than expected (20–30 percent) from surveys conducted in the United States (64, 113). Thus, the inability to assess fertility drug type, dose, or time of drug administration in those patients taking fertility drugs makes the results difficult to interpret. Finally, the methodological problems inherent in combined data sets and pooled analyses merit attention.

In the case-control study by Franceschi et al. (104), a medical diagnosis of infertility was not associated with an increased risk of ovarian cancer (table 5). No difference in the risk of ovarian cancer was found between women with and without fertility drug use. Confining the analysis to infertile women, the crude relative risk for use of infertility drugs was 1.3 (95 percent CI 0.7–2.4). Additional results from this study (104), based on 208 cases and 873 controls, have been published in a letter (115). Fertility drugs were used by 1.9 percent of cases and 1.5 percent of the controls, yielding a relative risk of 1.1 (95 percent CI 0.4–3.6) (data not shown). This study was not initially designed to evaluate infertility, but instead had an emphasis on dietary habits and hormone levels in relation to ovarian cancer. As a result, there was no information on cause of infertility or specific types of fertility drugs used. The results were based on very small numbers of fertility drug users, and the prevalence of infertility among controls appears low compared with that reported in similar studies (1, 105).

In the case-control study by Shushan et al. (105), analyses were performed on a combined case group of invasive and borderline epithelial ovarian tumors (table 5) and on a case group including only women with borderline tumors (data not shown). From data presented in the paper, it was estimated that, compared with women without a history of medically diagnosed infertility, infertile women who had never used fertility drugs had a crude relative risk of 1.3 (95 percent CI 0.6–2.9) for an ovarian tumor, while the relative risk for infertile women who had ever used fertility drugs was 1.8 (95 percent CI 1.0–3.3). When adjustment was made for confounding factors, the risk associated with fertility drug use became nonsignificant. Analyses were not performed separately for nulliparous and parous women. When the analysis was restricted to borderline tumors, the risk remained elevated (RR = 3.5, 95 percent CI 1.2–10.1) (data not shown). The use

of CC was not associated with an increase in risk: The adjusted relative risk was 0.9 (not significant) in the combined case group (data not shown) and 1.3 (not significant) among women with borderline tumors (data not shown). When the analysis was confined to women reporting fertility problems (combined case group), the use of fertility drugs was associated with a slightly increased crude risk (RR = 1.4, 95 percent CI 0.5–3.6). However, this effect was not apparent when infertile women who had only used CC were compared with infertile women without such use (table 5). A total of 11 women from the combined case group and six women from the control group reported that they had used hMG, yielding an adjusted relative risk of 3.2 (95 percent CI 0.9–11.8) in comparison with women with no hMG use (data not shown). The risk was especially pronounced among women with borderline tumors (adjusted RR = 9.4, 95 percent CI 1.7–52.1) (data not shown).

In this study, data were collected by telephone interview, with no verification of the information on fertility drug use. Furthermore, there was no information included on type of infertility, as most subjects could not recall the particular cause of their infertility. It may also be of methodological concern that 25 percent of the cases, defined as women diagnosed with cancer during the period 1990–1993, had died before contact was established; that a relatively low percentage of eligible controls were included in the study; and that oral contraceptive use, usually a strong risk determinant for ovarian cancer, was not associated with ovarian cancer in this study. In addition, as was noted above, combining invasive and borderline tumors may not be appropriate. Finally, it would have been of interest to see the estimates calculated separately for nulliparous and parous women, given previous findings by Whittemore et al. (1).

Findings from the largest case-control study have been published by Mosgaard et al. (99) (table 5). Cases were ascertained through the Danish Cancer Registry and the Danish National Patient Registry. Controls were selected randomly from the National Person Registry. Infertility was defined as having attempted pregnancy for more than 12 months without success. The risk of ovarian cancer was not increased among treated infertile women versus nontreated infertile women, and the risks were similar among nulliparous and parous women (table 4). Exclusion of non-epithelial tumors did not change these odds. No data on the specific types of drugs used were collected; this information had to be extrapolated from the mode of administration (tablets, injections, or both). Neither use of tablets (CC) nor use of combination treatment (CC plus hCG or hMG) increased the risk of ovarian cancer (table 5).

The study had several strengths. Cases were included in the study through two national registries, with histologic verification in all cases. A high proportion (81 percent) of the selected controls were included. However, 36 percent of the cases had died before contact was established, which could have caused selection bias if risk determinants differed between the deceased patients and the patients included in the study. Data were collected by means of a mailed questionnaire, and there was no information on the specific cause of infertility, the woman's age at the time she was receiving treatment, calendar time, or the specific fertility drug used. Given the fact that information on the specific types of fertility drugs used had to be extrapolated from their modes of action, and given the variety of former infertility treatment regimens (such as use of estrogens, oral contraceptives, steroids, etc.) (31, 33), the accuracy of the results concerning specific drug types is not known.

HYPOTHESES ON OVARIAN CANCER IN RELATION TO FERTILITY DRUG USE

The etiology and pathogenesis of ovarian cancer is still largely unknown, but epidemiologic evidence indicates that hormone-mediated carcinogenesis is thought to result from increased cell proliferation (116, 117). Proliferation of epithelial ovarian tissue is a result of cyclical gonadotrophin secretion and subsequent ovulation. With this increased cell division, the risk of errors of various kinds (i.e., amplifications, deletions, and mutations) also increases (116, 118, 119). It remains unclear whether CC and/or exogenous gonadotrophins are capable of inducing or promoting malignant transformation through increased cell division.

Two main hypotheses concerning ovarian cancer development have been proposed. In 1971, Fathalla (120) suggested that repeated minor trauma to the epithelial surface of the ovary caused by ovulations increases the risk of ovarian cancer. This "incessant ovulation" hypothesis has gained much support, as it is in accordance with several known risk factors and epidemiologic features of ovarian cancer. It agrees with the observed protective effects of multigravidity, oral contraceptive use, and breastfeeding, all conditions associated with anovulation or a decreased number of ovulations. The strong protective effect of anovulation was especially evident in the pooled analysis of Whittemore et al. (1), where the relative risk of epithelial ovarian cancer increased significantly with increasing estimated years of ovulation (121). The Fathalla hypothesis is also in agreement with the fact that 80–90 percent of ovarian cancers originate from

ovarian surface epithelial cells (67). Since ovarian epithelial cells proliferate after ovulation to cover the exposed surface of the ovary, it has also been proposed that this process may lead to entrapment of epithelium below the healing surface of the ovary, forming a germinal inclusion cyst. The ruptures are repaired by cell division, and growth ceases when repair is complete (122, 123). Thus, another mechanism by which frequent ovulation might lead to ovarian cancer is the formation of more germinal inclusion cysts, which are then stimulated by growth factors such as estrogens, gonadotrophins in high concentrations, and growth peptides within the ovary (124). If the risk of ovarian cancer is associated with the formation of germinal inclusion cysts, women with ovarian cancer would be expected to have more inclusion cysts than healthy women. However, in a case-control study, the mean numbers of germinal inclusion cysts were similar among 37 women with unilateral ovarian cancer and contralateral normal ovaries and 148 control women who underwent incidental oophorectomy (125).

A genetic basis for Fathalla's incessant ovulation theory has been suggested (126)—namely, that ovulations, with their repeated episodes of rupture and proliferation of ovary surface epithelial cells, allow tumor promotion among cells already bearing allelic loss (126). Allelic loss has consistently been shown to represent loss of tumor-suppressor genes, and this may lead to uncontrolled cell division and malignant transformation (118, 126, 127). It has been shown that a relatively high frequency of allelic loss (loss of heterozygosity) on chromosomes 6q, 17p, and 17q appears to be specific to ovarian cancer (128, 129), and some studies report a high rate of loss of the tumor suppressor-gene *p53* or overexpression of the mutated *p53* gene (118, 130, 131).

The second major ovarian cancer hypothesis is the gonadotrophin theory proposed by Stadel (132) and discussed by Cramer and Welch (133). This theory predicts that persistent stimulation of the ovary by gonadotrophins may have a direct carcinogenic effect or may act in association with high concentrations of estrogens. The gonadotrophin theory is based on the animal studies of Biskind and Biskind carried out in 1944 (134). In these studies, it was found that rats developed ovarian tumors of stromal origin (no epithelial tumors occurred) when they were manipulated to produce high concentrations of gonadotrophins. Similarly, succeeding studies found that tumor induction can be prevented in mice by inhibiting gonadotrophin production through administration of a GnRH(a) (135). Furthermore, it has been found that FSH binds almost exclusively to membrane receptors on the granulosa cells and induces their multiplication. FSH also stimulates biochemical processes such as steroidogenesis, aromatase activity, and cyclic adenosine monophosphate production (136–138), and LH stimulates theca lutein cell development and androgen production (136). Thus, FSH and LH are capable of regulating cellular processes in the ovary. However, it still remains controversial whether various types of human ovarian tumors are target tissues for, and can be modulated by, gonadotrophins. GnRH receptors (136, 139) and LH/(hCG) and FSH receptors with high affinity have been located in benign ovarian tissue (140), as well as in ovarian cancer tissue of both epithelial and stromal origin (15, 137, 138, 141–143), indicating that gonadotrophic hormones may play a role in the growth and differentiation of ovarian neoplasms. In addition, it has been suggested that growth of well differentiated tumor cells is stimulated by gonadotrophins, whereas poorly differentiated carcinomas seem to be nonresponsive to gonadotrophins (i.e., loss of the specific receptors) (144), as is the case with breast tumors. Thus, a direct biologic effect of gonadotrophins on the development of ovarian tumors is possible, but few biologic studies exist to convincingly substantiate the hypothesis.

However, in agreement with the gonadotrophin hypothesis are epidemiologic data showing that pregnancy and use of oral contraceptives, which lower serum concentrations of gonadotrophins (145), reduce the risk of ovarian cancer. During menopause, a period accompanied by high gonadotrophin levels, the incidence of ovarian cancer increases. However, gonadotrophin levels are also lowered by the use of estrogen replacement therapy; effects of this usage on ovarian cancer risk are not consistent (1, 146). Furthermore, a large prospective nested case-control study by Helzlsouer et al. (147) that assessed prediagnostic serum gonadotrophin levels in relation to subsequent development of ovarian cancer found *lower* gonadotrophin levels (but higher androgen levels) among cases than among controls. This association was most pronounced for FSH. The relative risk for the highest tertile of FSH concentration compared with the lowest tertile was 0.1 (95 percent CI 0.0–1.0). Blaakaer et al. (148, 149) also found significantly lower preoperative serum FSH levels in postmenopausal women with malignant ovarian tumors compared with healthy postmenopausal age-matched controls. No significant associations between levels of estradiol, LH, or progesterone and FSH and ovarian cancer risk were found. Inhibin, an ovarian regulatory peptide, acts to suppress synthesis and secretion of FSH and is considered undetectable in serum from healthy postmenopausal women (150, 151). Interestingly, in some studies it has been found that postmenopausal women with malig-

nant epithelial ovarian tumors have significantly elevated levels of immunoreactive inhibin compared with healthy controls (150, 151). Preoperatively, women with a malignant ovarian tumor had significantly low FSH levels, which increased 8 months after the operation (110). This was also demonstrated among women with granulosa cell tumors (151) and could indicate ovarian suppression, most probably by inhibin. These findings, together with the findings of Helzlsouer et al. (147), do not necessarily disprove the "gonadotrophin theory," but they do raise new questions. It is unclear whether gonadotrophin levels per se or gonadotrophin intrinsic activity can induce or promote the development of ovarian tumors in humans; this must be clarified.

In addition to the two main hypotheses, some less established hypotheses on the processing of chemical carcinogenesis in the local ovarian environment have evolved (89, 93, 152, 153), but none of these can be directly or indirectly related to fertility drug use.

DISCUSSION

Infertility per se—that is, its effect separate of parity—has been found to be a risk factor in some (96, 98) but not all (1, 88, 99) ovarian cancer studies. In general, the number of cases has been too small to estimate risk by specific cause of infertility, and in studies where such data were available, no consistent pattern by cause of infertility was seen (1, 20–22, 100).

Studies that have had limited (1, 20, 21, 99, 102) or no (104) information on the specific types of infertility drugs used are difficult to interpret, and when evaluating the possible role of fertility drug use in risk of cancer, probably less emphasis should be placed on them. Instead, attention may be focused on the three studies in which such information was available (22, 100, 105). These studies all evaluated currently used fertility drugs, with two having collected information on specific drugs (100, 105) and one on specific treatment regimens (22). Furthermore, two of the studies were able to analyze effects by specific cause of infertility (22, 100). In these three studies, increased *crude* risks in the range of 1.4–2.5 were found among infertile women who had ever used fertility drugs when compared with infertile women with no drug use. Higher risk estimates were found when the comparison group was women without infertility. Higher risks were also found with the use of specific types of fertility drugs. In the study by Rossing et al. (100), the risk of CC use was dose-dependent, with risk increasing with years of use of CC. This effect was seen among both gravid and nulligravid women, as well as among women with and without ovulatory abnormalities. These findings argue against the notion that the excess risk is explained by preexisting ovarian pathology or by nulligravidity itself. However, no such effect of use of fertility drugs administered as tablets (and extrapolated to CC use) was found in the study by Mosgaard et al. (99). Unfortunately, the limited use of fertility drugs, other than CC in the cohort studied by Rossing et al. (100), prevents an assessment of risk associated with other infertility medications (hMG, hCG). However, it is of note that Shushan et al. (105) found no effect of CC use alone but an increased risk with combined use of CC and hMG, especially for borderline tumors.

Only two studies have been able to analyze separately the effect of fertility drug use on the risk of borderline ovarian tumors (102, 105). In these studies, significantly increased adjusted relative risks in the range of 3.5–4.0 were found. However, when analyses were restricted to infertile women, the risks became comparable to those observed for invasive tumors.

In general, epidemiologic studies on fertility drug use and risk of ovarian cancer are hampered by methodological problems, such as small study size, short follow-up time, and low prevalences of infertility and fertility drug use, and hence low study power. This, together with the other methodological problems described above, makes it possible that the effect of fertility drug use on ovarian cancer risk has been underestimated.

The ovarian cancer hypotheses, especially the hypothesis of high gonadotrophin levels inducing/promoting epithelial ovarian cancer in humans, need to be substantiated by more epidemiologic and biologic data. However, both hypotheses could explain the role of fertility drugs in the development of ovarian cancer. CC may act as a promoter, as it increases the number of ovulations *and* increases endogenous levels of gonadotrophins, as well as estrogens and progestogens. High levels of exogenous gonadotrophins (hMG, hCG) may themselves be carcinogenic, especially to the granulosa cells, or they may promote the carcinogenic process by significantly increasing the number of ovulations. In the past 10 years, stronger ovulation stimulants, inducing higher hormone levels and more ovulations, have been used on an increasing number of women (figure 1) (30). Infertile women with normal ovulatory function, who have been exposed to excessive CC and/or gonadotrophin administration, constantly raised estrogen and progestogen concentrations, and multiple ovulatory cycles, may be more prone to changes in the ovarian tissue than those with ovulatory disorders. Thus, although the action of fertility drugs is the same, studies that have not taken into account either pretreatment gonadotrophin/ovulation

levels or cause of infertility may actually have underestimated the risk associated with fertility drugs.

If there is a real association, the population attributable risk percentage (154), or the proportion of ovarian cancer due to fertility drug use, can be determined to quantify the effect on public health. Using data from the population-based case-control study by Shushan et al. (105), the population attributable risk percentage is 9.5, given an exposure to fertility drugs of 7 percent in the population and a relative risk of 2.5. For an individual woman, this means that her lifetime risk of developing ovarian cancer increases from approximately 1.9 percent to 4.6 percent if she uses fertility drugs. In a pilot study of risk perception among 52 women at two fertility clinics, 10 percent of the women stated that they would not accept an increased risk of ovarian cancer subsequent to fertility drug therapy (155). In contrast, 50 percent said they would accept a maximum lifetime risk of 2–4 percent, and the rest (40 percent) said they would accept a lifetime risk of >4 percent. However, further research is needed to determine the size of the problem in relevant birth cohorts.

The main methodological problems of the studies reviewed here include limited study size and limited follow-up time. Also, there has been a lack of, or questionable validity of, information on exposure and potentially confounding variables. Furthermore, analyses have not always been performed separately for different types of ovarian tumors. Invasive ovarian tumors should be analyzed separately from borderline tumors, and epithelial tumors should be analyzed separately from non-epithelial tumors.

In future studies, it will also be necessary to separate the effects of low parity, voluntary nulliparity, and infertility on the risk of ovarian cancer. Specifically, it is important to use a standardized quantitative measure of infertility and to separate infertility into its various causes and, most ideally, into different pretreatment hormonal levels. As to the extent that these various conditions or their therapy are differentially related to the incidence of ovarian cancer, adjustment simply for a history of "infertility" may be inadequate (156). Large (prospective) cohort studies of infertile women could address the problem adequately. Such studies have been started in at least three countries: the Netherlands (157), the United States (L. A. Brinton, US National Cancer Institute, Bethesda, Maryland, personal communication, 1997), and Denmark (S. K. Kjær, Danish Cancer Society, Copenhagen, Denmark, personal communication, 1997). These studies each contain cohorts of 10,000–20,000 women diagnosed as infertile. The cohorts will be linked to relevant cancer registries, or a questionnaire will be administered to the women. Risks for all female cancers will be evaluated in these studies.

In conclusion, a disturbing and important question has been raised. However, the currently available data are not adequate for drawing a solid and final conclusion with regard to the possible association between use of fertility drugs and risk of ovarian cancer. Thus, it is still important to address this issue in new studies which have a sufficient size and adequate data on both exposure and outcome to provide more precise estimates of risk. Finally, it is important to determine whether an increased risk applies to all groups of infertile women treated with fertility drugs or whether it only applies to selected subgroups, e.g., infertile women who do not subsequently get pregnant. Answers to these questions are necessary in our counseling of infertile patients.

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REFERENCES

1. Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Am J Epidemiol 1992;136:1184–203.
2. Bamford PN, Steele SJ. Uterine and ovarian carcinoma in a patient receiving gonadotrophin therapy: case report. Br J Obstet Gynaecol 1982;89:962–4.
3. Atlas M, Menczer J. Massive hyperstimulation and borderline carcinoma of the ovary: a possible association. Acta Obstet Gynecol Scand 1982;61:261–3.
4. Ben-Hur H, Dgani R, Lancet M, et al. Ovarian carcinoma masquerading as ovarian hyperstimulation syndrome. Acta Obstet Gynecol Scand 1986;65:813–14.
5. Carter ME, Joyce DN. Ovarian carcinoma in a patient hyperstimulated by gonadotrophin therapy for in vitro fertilization: a case report. J In Vitro Fert Embryo Transf 1987;4:126–8.
6. Kulkarni R, McGarry JM. Follicular stimulation and ovarian cancer. (Letter). BMJ 1989;299:740.
7. Lappöhn RE, Burger HG, Bouma J, et al. Inhibin as a marker for granulosa-cell tumors. N Engl J Med 1989;321:790–3.
8. Dietl J. Ovulation and ovarian cancer. (Letter). Lancet 1991; 338:445.
9. Goldberg GL, Runowicz CD. Ovarian carcinoma of low malignant potential, infertility, and induction of ovulation—is there a link? Am J Obstet Gynecol 1992;166:853–4.
10. Nijman HW, Burger CW, Baak JP, et al. Borderline malignancy of the ovary and controlled hyperstimulation, a report of 2 cases. Eur J Cancer 1992;28A:1971–3.
11. Lopes P, Mensier A. Ovarian cancer and assisted reproductive technology. Eur J Obstet Gynecol Reprod Biol 1993;51: 171–3.
12. Willemsen W, Kruitwagen R, Bastiaans B, et al. Ovarian stimulation and granulosa-cell tumour. Lancet 1993;341: 986–8.
13. Balasch J, Barri PN. Follicular stimulation and ovarian cancer? Hum Reprod 1993;8:990–6.

14. Karlan BY, Marrs R, Lagasse LD. Advanced-stage ovarian carcinoma presenting during infertility evaluation. Am J Obstet Gynecol 1994;171:1377–8.
15. Komatsu T, Konishi I, Mandai M, et al. Peritoneal papillary serous carcinoma arising in an infertile woman during ovulation-induction therapy: immunohistochemical expression of LH/hCG receptors. Gynecol Oncol 1995;56:470–4.
16. Grimbizis G, Tarlatzis BC, Bontis J, et al. Two cases of ovarian tumours in women who had undergone multiple ovarian stimulation attempts. Hum Reprod 1995;10:520–3.
17. Unkila-Kallio L, Leminen A, Tiitinen A, et al. Malignant tumors of the ovary or the breast in association with infertility: a report of thirteen cases. Acta Obstet Gynecol Scand 1997;76:177–81.
18. Jourdain O, Avril A, Mauriac L, et al. Breast cancer and in vitro fertilization: about 32 cases. Eur J Obstet Gynecol Reprod Biol 1996;67:47–52.
19. Brzezinski A, Peretz T, Mor-Yosef S, et al. Ovarian stimulation and breast cancer: is there a link? Gynecol Oncol 1994;52:292–95.
20. Ron E, Lunenfeld B, Menczer J, et al. Cancer incidence in a cohort of infertile women. Am J Epidemiol 1987;125:780–90.
21. Brinton LA, Melton LJ III, Malkasian GD Jr, et al. Cancer risk after evaluation for infertility. Am J Epidemiol 1989; 129:712–22.
22. Venn A, Watson L, Lumley J, et al. Breast and ovarian cancer incidence after infertility and in vitro fertilisation. Lancet 1995;346:995–1000.
23. Rossing MA, Daling JR, Weiss N, et al. Risk of breast cancer in a cohort of infertile women. Gynecol Oncol 1996;60:3–7.
24. Rossing MA, Daling JR, Weiss NS, et al. In situ and invasive cervical carcinoma in a cohort of infertile women. Fertil Steril 1996;65:19–22.
25. Cowan LD, Gordis L, Tonascia JA, et al. Breast cancer incidence in women with a history of progesterone deficiency. Am J Epidemiol 1981;114:209–17.
26. Coulam C, Annegers JF, Kranz JS. Chronic anovulation syndrome and associated neoplasia. Obstet Gynecol 1983; 61:403–7.
27. Westhoff C. Infertility and breast cancer risk. (Editorial). Gynecol Oncol 1996;60:1–2.
28. Spirtas R, Kaufman SC, Alexander NJ. Fertility drugs and ovarian cancer: red alert or red herring? Fertil Steril 1993; 59:291–3.
29. Rossing MA, Daling JR, Weiss NS. Risk of ovarian cancer after treatment for infertility. (Letter). N Engl J Med 1995; 332:1302.
30. Shoham Z. Epidemiology, etiology, and fertility drugs in ovarian epithelial carcinoma: where are we today? Fertil Steril 1994;62:433–48.
31. Lunenfeld B, Insler V. Induction of ovulation: historical aspects. Baillieres Clin Obstet Gynaecol 1990;4:473–89.
32. Wysowski DK. Use of fertility drugs in the United States, 1973 through 1991. Fertil Steril 1993;60:1096–8.
33. Shapiro S. Risk of ovarian cancer after treatment for infertility. (Letter). N Engl J Med 1995;332:1301.
34. Blacker CM. Ovulation stimulation and induction. Endocrinol Metab Clin North Am 1992;21:57–84.
35. Derman SG, Adashi EY. Adverse effects of fertility drugs. Drug Saf 1994;11:408–21.
36. Forman RG, Demouzon J, Feinstein MC, et al. Studies on the influence of gonadotrophin levels in the early follicular phase on the ovarian response to stimulation. Hum Reprod 1991; 6:113–17.
37. Clark JH, Markaverich BM. The agonistic-antagonistic properties of clomiphene: a review. Pharmacol Ther 1981;15: 467–519.
38. Adashi EY. Clomiphene citrate: mechanism(s) and site(s) of action—hypothesis revisited. Fertil Steril 1984;42:331–44.
39. Kerin JF, Liu JH, Phillipou G, et al. Evidence for a hypothalamic site of action of clomiphene citrate in women. J Clin Endocrinol Metab 1985;61:265–8.
40. Roy S. Early developmental studies with clomiphene citrate: their subsequent validation and extension. Indian J Exp Biol 1992;30:931–46.
41. March CM. Ovulation induction. J Reprod Med 1993;38: 335–46.
42. Quigley MM, Berkowitz AS, Gilbert SA, et al. Clomiphene citrate in an in vitro fertilization program: hormonal comparisons between 50- and 150-mg daily dosages. Fertil Steril 1984;41:809–15.
43. Randall JM, Templeton A. The effects of clomiphene citrate upon ovulation and endocrinology when administered to patients with unexplained infertility. Hum Reprod 1991;6: 659–64.
44. Glasier AF. Clomiphene citrate. Baillieres Clin Obstet Gynaecol 1990;4:491–501.
45. MacDougall MJ, Tam SL, Hall V, et al. Comparison of natural with clomiphene citrate-stimulated cycles in in vitro fertilization: a prospective, randomized trial. Fertil Steril 1994;61:1052–7.
46. Fluker MR, Urman B, Mackinnon M, et al. Exogenous gonadotropin therapy in World Health Organization groups I and II ovulatory disorders. Obstet Gynecol 1994;83:189–96.
47. Nilsson L, Hamberger L. Human gonadotrophins. Ballieres Clin Obstet Gynaecol 1990;4:503–18.
48. Fishel S, Jackson P. Follicular stimulation for high tech pregnancies: are we playing it safe? BMJ 1989;299:309–11.
49. Insler V. Gonadotropin therapy: new trends and insights. Int J Fertil 1988;33:85–6, 89–97.
50. Shoham Z, Homburg R, Jacobs HS. Induction of ovulation with pulsatile GnRH. Baillieres Clin Obstet Gynaecol 1990; 4:589–608.
51. Hedon B, Bringer J, Arnal F, et al. The use of GnRH agonists with hMG for induction or stimulation of ovulation. Baillieres Clin Obstet Gynaecol 1990;4:575–87.
52. Pellicer A, Tarin JJ, Miró F, et al. The use of gonadotrophin releasing-hormone analogues (GnRHa) in in-vitro fertilization: some clinical and experimental investigations of a direct effect on the human ovary. Hum Reprod 1992;7:39–47.
53. International Agency for Research on Cancer. Clomiphene citrate (group 3). A. Evidence for carcinogenicity to humans. In: IARC monographs on the evaluation of carcinogenic risks to humans, supplement 7. Lyon, France: International Agency for Research on Cancer, 1987:172–73.
54. Cunha GR, Taguchi O, Namikawa R, et al. Teratogenic effects of clomiphene, tamoxifen, and diethylstilbestrol on the developing human female genital tract. Hum Pathol 1987;18:1132–43.
55. Ohnishi T, Ohashi Y, Amano I, et al. An ovulation inducing agent containing clomiphene citrate causes DNA-strand breaks without SOS responses in *Escherichia coli*. Mutat Res 1986;165:57–61.
56. Brinton LA, Hoover RN. Epidemiology of gynecologic cancers. In: Hoskins WJ, Perez CA, Young RC, eds. Principles and practice of gynecologic oncology. Philadelphia, PA: Lippincott, 1992:3–26.
57. Braga C, Negri E, La Vecchia C, et al. Fertility treatment and risk of breast cancer. Hum Reprod 1996;11:300–3.
58. Rossing MA, Daling JR, Weiss NS, et al. Risk of cutaneous melanoma in a cohort of infertile women. Melanoma Res 1995;5:123–7.
59. Healy DL, Trounson AO, Andersen AN. Female infertility: causes and treatment. Lancet 1994;343:1539–44.
60. Templeton A, Fraser C, Thompson B. Infertility—epidemiology and referral practice. Hum Reprod 1991;6: 1391–4.
61. Schmidt L, Münster K. Infertility, involuntary infecundity, and the seeking of medical advice in industrialized countries 1970–1992: a review of concepts, measurements and results. Hum Reprod 1995;10:1407–18.
62. Gunnell DJ, Ewings P. Infertility prevalence, needs assessment and purchasing. J Public Health Med 1994;16:29–35.
63. Mosher WD, Pratt WF. The demography of infertility in the

United States. In: Asch RH, Stubb JW, eds. Annual progress in reproductive medicine. Park Ridge, NJ: Parthenon Publishing Group, 1993:37–43.
64. Wilcox LS, Mosher WD. Use of infertility services in the United States. Obstet Gynecol 1993;82:122–7.
65. US Congress, Office of Technology Assessment. Infertility: medical and social choices. Washington, DC: US GPO, 1988. (Publication no. OTA-BA-358).
66. Mosgaard B, Lidegaard Ø, Andersen AN. Use of fertility drugs in Denmark 1973–1993: an analysis based on sale statistics. Acta Obstet Gynecol Scand 1995;74:614–18.
67. Gore H. Histopathology of ovarian cancer. Semin Surg Oncol 1994;10:255–60.
68. Krigman H, Bentley R, Robboy SJ. Pathology of epithelial ovarian tumors. Clin Obstet Gynecol 1994;37:475–91.
69. Evans AT III, Gaffey T, Malkasian GD Jr, et al. Clinicopathologic review of 118 granulosa and 82 theca cell tumors. Obstet Gynecol 1980;55:231–8.
70. Merino MJ, Jaffe G. Age contrast in ovarian pathology. Cancer 1993;71:537–44.
71. Trimble CL, Trimble EL. Management of epithelial ovarian tumors of low malignant potential. Gynecol Oncol 1994; 55(suppl):S52–61.
72. Coleman MP, Esteve J, Damiecki P, et al. Trends in cancer incidence and mortality. Lyon, France: International Agency for Research on Cancer, 1993:1–806. (IARC scientific publication no. 121).
73. Parazzini F, Restelli C, La Vecchia C, et al. Risk factors for epithelial ovarian tumours of borderline malignancy. Int J Epidemiol 1991;20:871–7.
74. Yancik R. Ovarian cancer: age contrast in incidence, histology, disease stage at diagnosis, and mortality. Cancer 1993; 71:517–23.
75. Mant JW, Vessey MP. Ovarian and endometrial cancers. Cancer Surv 1994;19–20:287–307.
76. Kaern J, Tropé CG, Abeler VM. A retrospective study of 370 borderline tumors of the ovary treated at the Norwegian Radium Hospital from 1970 to 1982: a review of clinicopathologic features and treatment modalities. Cancer 1993; 71:1810–20.
77. Ewertz M, Kjaer SK. Ovarian cancer incidence and mortality in Denmark, 1943–1982. Int J Cancer 1988;42:690–6.
78. Kjaer SK, Storm HH. Survival of Danish cancer patients 1943–1987: female genital organs. APMIS Suppl 1993;33: 107–21.
79. Kurman RJ, Trimble CL. The behavior of serous tumors of low malignant potential: are they ever malignant? Int J Gynecol Pathol 1993;12:120–7.
80. Rosenberg L, Palmer JR, Zauber AG, et al. A case-control study of oral contraceptive use and invasive epithelial ovarian cancer. Am J Epidemiol 1994;139:654–61.
81. The reduction in risk of ovarian cancer associated with oral-contraceptive use. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. N Engl J Med 1987;316:650–5.
82. Gwinn ML, Lee NC, Rhodes PH, et al. Pregnancy, breast feeding, and oral contraceptives and the risk of epithelial ovarian cancer. J Clin Epidemiol 1990;43:559–68.
83. Negri E, Franceschi S, Tzonou A, et al. Pooled analysis of 3 European case-control studies. I. Reproductive factors and risk of epithelial ovarian cancer. Int J Cancer 1991;49:50–6.
84. Franceschi S, La Vecchia C, Booth M, et al. Pooled analysis of 3 European case-control studies of ovarian cancer. II. Age at menarche and at menopause. Int J Cancer 1991;49:57–60.
85. Purdie D, Green A, Bain C, et al. Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Survey of Women's Health Study Group. Int J Cancer 1995;62:78–84.
86. Kreiger N, Sloan M, Cotterchio M, et al. Surgical procedures associated with risk of ovarian cancer. Int J Epidemiol 1997; 26:710–15.
87. Hankinson SE, Hunter DJ, Colditz GA, et al. Tubal ligation, hysterectomy, and risk of ovarian cancer: a prospective study. JAMA 1993;270:2813–18.
88. Risch HA, Marrett LD, Howe GR. Parity, contraception, infertility and the risk of epithelial ovarian cancer. Am J Epidemiol 1994;140:585–97.
89. Adami HO, Hsieh CC, Lambe M, et al. Parity, age at first childbirth, and risk of ovarian cancer. Lancet 1994;344: 1250–4.
90. Bernstein L, Ross RK, Henderson BE. Relationship of hormone use to cancer risk. J Natl Cancer Inst Monogr 1992; 12:137–47.
91. Gallion HH, Smith SA. Hereditary ovarian carcinoma. Semin Surg Oncol 1994;10:249–54.
92. Booth M, Beral V, Smith P. Risk factors for ovarian cancer: a case-control study. Br J Cancer 1989;60:592–8.
93. McGowan L, Parent L, Lednar W, et al. The woman at risk for developing ovarian cancer. Gynecol Oncol 1979;7:325–44.
94. Joly DJ, Lilienfeld AM, Diamond EL, et al. An epidemiologic study of the relationship of reproductive experience to cancer of the ovary. Am J Epidemiol 1974;99:190–209.
95. Hartge P, Schiffman MH, Hoover R, et al. A case-control study of epithelial ovarian cancer. Am J Obstet Gynecol 1989;161:10–16.
96. Whittemore AS, Wu ML, Paffenbarger PS Jr, et al. Epithelial ovarian cancer and ability to conceive. Cancer Res 1989;49: 4047–52.
97. Chen Y, Wu PC, Lang JH, et al. Risk factors for epithelial ovarian cancer in Beijing, China. Int J Epidemiol 1992;21: 23–9.
98. Nasca PC, Greenwald P, Chorost S, et al. An epidemiologic case-control study of ovarian cancer and reproductive factors. Am J Epidemiol 1984;119:705–13.
99. Mosgaard B, Lidegaard Ø, Kjaer SK, et al. Infertility, fertility drugs and invasive ovarian cancer: a case-control study. Fertil Steril 1997;67:1005–12.
100. Rossing MA, Daling JR, Weiss NS, et al. Ovarian tumors in a cohort of infertile women. N Engl J Med 1994;331:771–6.
101. Harlow BL, Weiss NS, Roth GJ, et al. Case-control study of borderline ovarian tumors: reproductive history and exposure to exogenous female hormones. Cancer Res 1988;48: 5849–52.
102. Harris R, Whittemore AS, Itnyre J, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. III. Epithelial tumors of low malignant potential in white women. Am J Epidemiol 1992;136: 1204–11.
103. Lais CW, Williams TJ, Gaffey TA. Prevalence of ovarian cancer found at the time of infertility microsurgery. Fertil Steril 1988;49:551–3.
104. Franceschi S, La Vecchia C, Negri E, et al. Fertility drugs and risk of epithelial ovarian cancer in Italy. Hum Reprod 1994;9:1673–5.
105. Shushan A, Paltiel O, Iscovich J, et al. Human menopausal gonadotropin and the risk of epithelial ovarian cancer. Fertil Steril 1996;65:13–18.
106. Horn-Ross PL, Whittemore AS, Harris R, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. VI. Nonepithelial cancers among adults. Collaborative Ovarian Cancer Group. Epidemiology 1992;3:490–5.
107. Harlow BL, Weiss NS, Lofton S. Epidemiology of borderline ovarian tumors. J Natl Cancer Inst 1987;78:71–4.
108. Del Priore G, Robischon K, Phipps WR. Risk of ovarian cancer after treatment for infertility. (Letter). N Engl J Med 1995;332:1300.
109. Rossing MA, Weiss NS. Fertility drugs and breast and ovarian cancer. (Letter). Lancet 1995;346:1627–8.
110. Whittemore AS, Harris R, Itnyre J, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. I. Methods. Am J Epidemiol 1992; 136:1175–83.

111. Cramer DW, Hutchison GB, Welch WR, et al. Determinants of ovarian cancer risk. I. Reproductive experiences and family history. J Natl Cancer Inst 1983;71:711–16.
112. Cohen J, Forman R, Harlap S, et al. IFFS expert group report on the Whittemore study related to the risk of ovarian cancer associated with the use of infertility agents. Hum Reprod 1993;8:996–9.
113. Caro JJ, Johannes CB, Hartz SC, et al. Re: "Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women." (Letter). Am J Epidemiol 1993;137:928.
114. International Federation of Fertility Societies. Fertility drugs and ovarian cancer. Fertil Steril 1993;60:406–8.
115. La Vecchia C, Negri E, Parazzini F, et al. Fertility drugs and breast and ovarian cancer. (Letter). Lancet 1995;346:1628.
116. Preston-Martin S, Pike MC, Ross RK, et al. Epidemiologic evidence for the increased cell proliferation model of carcinogenesis. In: Butterworth BE, Slaga TJ, Farland W, et al., eds. Chemically induced cell proliferation: implications for risk assessment. Proceedings of the Chemically Induced Cell Proliferation Conference, Austin, Texas, November 29–December 2, 1989. New York, NY: Wiley-Liss, 1991:21–34.
117. Watanabe S, Kobayashi Y. Exogenous hormones and human cancer. Jpn J Clin Oncol 1993;23:1–13.
118. Bast RC Jr, Boyer CM, Jacobs I, et al. Cell growth regulation in epithelial ovarian cancer. Cancer 1993;71:1597–601.
119. Kodama M, Kodama T. An essay on the nature of hormonal codes involved in the genesis of human neoplasias (review). Anticancer Res 1994;14:2653–65.
120. Fathalla MF. Incessant ovulation—a factor in ovarian neoplasia? Lancet 1971;2:163.
121. Whittemore AS, Harris R, Itnyre J, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. IV. The pathogenesis of epithelial ovarian cancer. Am J Epidemiol 1992;136:1212–20.
122. Radisavljevic SV. The pathogenesis of ovarian inclusion cysts and cystomas. Obstet Gynecol 1977;49:424–9.
123. Dietl J, Marzusch K. Ovarian surface epithelium and human ovarian cancer. Gynecol Obstet Invest 1993;35:129–35.
124. Godwin AK, Perez RP, Johnson SW, et al. Growth regulation of ovarian cancer. Hematol Oncol Clin North Am 1992;6:829–41.
125. Westhoff C, Murphy P, Heller D, et al. Is ovarian cancer associated with an increased frequency of germinal inclusion cysts? Am J Epidemiol 1993;138:90–3.
126. Lowry S, Russell H, Hickey I, et al. Incessant ovulation and ovarian cancer. Lancet 1991;337:1544–5.
127. Russell SE, Hickey GI, Lowry WS, et al. Allele loss from chromosome 17 in ovarian cancer. Oncogene 1990;5:1581–3.
128. Børresen AL. Oncogenesis in ovarian cancer. Acta Obstet Gynecol Scand Suppl 1992;155:25–30.
129. Amos CI, Struewing JP. Genetic epidemiology of epithelial ovarian cancer. Cancer 1993;71:566–72.
130. Marks JR, Davidoff AM, Kerns BJ, et al. Overexpression and mutation of p53 in epithelial ovarian cancer. Cancer Res 1991;51:2979–84.
131. Boente MP, Hurteau J, Rodriquez GR, et al. The biology of ovarian cancer. Curr Opin Oncol 1993;5:900–7.
132. Stadel BV. The etiology and prevention of ovarian cancer. (Letter). Am J Obstet Gynecol 1975;123:772–4.
133. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst 1983;71:717–21.
134. Biskind MS, Biskind GS. Development of tumors in the rat ovary after transplantation into the spleen. Proc Soc Exp Biol Med 1944;55:176–9.
135. Blaakaer J, Baeksted M, Micic S, et al. Gonadotropin-releasing hormone agonist suppression of ovarian tumorigenesis in mice of the Wx/Wv genotype. Biol Reprod 1995;53:775–9.
136. Qayum A, Gullick WJ, Waxman J. Gonadotrophin-releasing hormone: physiological significance and relevance to cancer. Prog Growth Factor Res 1991;3:115–30.
137. Graves PE, Surwit EA, Davis JR, et al. Adenylate cyclase in human ovarian cancers: sensitivity to gonadotropins and non-hormonal activators. Am J Obstet Gynecol 1985;153:877–82.
138. Kammerman S, Demopoulos RI, Raphael C, et al. Gonadotropic hormone binding to human ovarian tumors. Hum Pathol 1981;12:886–90.
139. Peng C, Fan NC, Ligier M, et al. Expression and regulation of gonadotropin-releasing hormone (GnRH) and GnRH receptor messenger ribonucleic acids in human granulosa-luteal cells. Endocrinology 1994;135:1740–6.
140. Nakano R, Kitayama S, Yamoto M, et al. Localization of gonadotropin binding sites in human ovarian neoplasms. Am J Obstet Gynecol 1989;161:905–10.
141. Davy M, Torjesen PA, Aakavaag A. Demonstration of an FSH receptor in a functioning granulosa cell tumour: the effect of gonadotrophin treatment on its viability following transplantation to nude mice. Acta Endocrinol (Copenh) 1977;85:615–23.
142. Rajaniemi H, Kauppila A, Rönnberg L, et al. LH(hCG) receptor in benign and malignant tumors of human ovary. Acta Obstet Gynecol Scand Suppl 1981;101:83–6.
143. Hahlin M, Crona N, Knutsson F, et al. Human granulosa cell tumor: stimulation of steroidogenesis by gonadotropins in vitro. Gynecol Oncol 1991;40:201–6.
144. Simon WE, Albrecht M, Hänsel M, et al. Cell lines derived from human ovarian carcinomas: growth stimulation by gonadotropic and steroid hormones. J Natl Cancer Inst 1983;70:839–45.
145. Dericks-Tan JS, Krög W, Aktories K, et al. Dose-dependent inhibition by oral contraceptives of the pituitary to release LH and FSH in response to stimulation with LH-RH$^+$. Contraception 1976;14:171–81.
146. Rodriguez C, Calle EE, Coates JR, et al. Estrogen replacement therapy and fatal ovarian cancer. Am J Epidemiol 1995;141:828–35.
147. Helzlsouer KJ, Alberg AJ, Gordon GB, et al. Serum gonadotropins and steroid hormones and the development of ovarian cancer. JAMA 1995;274:1926–30.
148. Blaakaer J, Djursing H, Hørding U, et al. The pituitary-gonadal axis in women with benign or malignant ovarian tumors. Acta Endocrinol (Copenh) 1992;127:127–30.
149. Blaakaer J, Bennett P, Micic S, et al. The post-operative gonadotrophin level in post-menopausal women with epithelial ovarian cancer. Eur J Obstet Gynecol Reprod Biol 1993;52:111–16.
150. Blaakaer J, Micic S, Morris ID, et al. Immunoreactive inhibin-production in post-menopausal women with malignant epithelial ovarian tumors. Eur J Obstet Gynecol Reprod Biol 1993;52:105–10.
151. Healy DL, Burger HG, Mamers P, et al. Elevated serum inhibin concentrations in postmenopausal women with ovarian tumors. N Engl J Med 1993;329:1539–42.
152. Harlow BL, Hartge PA. A review of perineal talc exposure and risk of ovarian cancer. Regul Toxicol Pharmacol 1995;21:254–60.
153. Cramer DW, Harlow BL, Willett WC, et al. Galactose consumption and metabolism in relation to the risk of ovarian cancer. Lancet 1989;2:66–71.
154. Coughlin SS, Benichou J, Weed DL. Attributable risk estimation in case-control studies. Epidemiol Rev 1994;16:51–64.
155. Rosen B, Irvine J, Ritvo P, et al. The feasibility of assessing women's perceptions of the risks and benefits of fertility drug therapy in relation to ovarian cancer risk. Fertil Steril 1997;68:90–4.
156. Weiss NS. Measuring the separate effects of low parity and its antecedents on the incidence of ovarian cancer. Am J Epidemiol 1988;128:451–5.
157. Burger CW, Klip WA, Schippers MC, et al. Ovarian stimulation and gynecologic abnormalities: a Dutch study for potential diseases in the female after in vitro fertilization (IVF). (Abstract). J Assist Repro Genet 1997;14(suppl):160S.