Chapter 1

# Ovarian Cancer Following Use of Clomiphene and Gonadotropin in Assisted Reproduction

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## Introduction

The incidence of infertility is highest in Western countries where it affects about 10%–15% of all couples [1, 2]. On the basis of the data from the 2002 National Survey of Family Growth, 12% of the 61.6 million US women between the ages of 16 and 44 sought infertility services. The use of infertility services was more common among older women, women with higher incomes, and women who were childless [3].

The usage of fertility drugs and other infertility services is expected to continue to rise as the percentage of women who postpone pregnancy until after the age of 35 years because of economic and social reasons increases. Stephen and Chandra [4] estimated that the number of infertile women will increase to between 5.4 and 7.7 million in 2025. Despite the growing number of women seeking fertility treatment, the effects of fertility drug use on ovarian cancer risk remain uncertain.

Early studies reported an association between exposure to fertility drugs and the development of ovarian cancer, which raised concern with regard to the safety of these drugs [1, 5, 6]. Subsequent studies did not provide hard evidence of an increased risk of ovarian cancer with the use of fertility drugs [7–16]. However, concern about fertility drug use remained after some studies reported that women who had been exposed to fertility drugs for more than 12 cycles were at an increased risk of ovarian cancer [6]. In addition, nulliparous women who failed to conceive after treatment have also been reported to have an increased risk of epithelial ovarian cancer (EOC) [5, 6]. Finally, several studies have shown that fertility drug use may increase the risk of borderline ovarian tumors (BOT) [11, 15–21].

The literature data regarding a hypothetic correlation between ovarian cancer and infertility treatments remain conflicting and hard to interpret. This is due to several factors characterized by some methodological limitations. For example, many studies evaluated fertility schedules of treatment containing drugs used in the past. Furthermore, many reports did not show an optimal control on potential confounding factors, or reported on small number of patients, were frequently retrospective in nature, had short follow-up periods, or did not identify the role of other reproductive factors influencing ovarian cancer risk. There was often a lack of clear distinction between invasive epithelial tumors and their borderline counterpart [1, 20–22].

Given that the first in vitro fertilization (IVF) baby was born in 1979, there has been limited ability to assess ovarian cancer risk in a cohort of women old enough to be at risk of developing cancer. Thus, the number of epidemiologic studies with relatively long-term follow-up that have assessed effects of fertility drugs used in conjunction with IVF is relatively small and contains a limited number of ovarian tumors [1, 20–22].

## Epidemiology of Ovarian Cancer

Ovarian cancer is the leading cause of death from all pelvic gynecologic malignancies. Despite the significant advances in surgery, chemotherapy and radiotherapy, the resulting 5-year survival is about 40% [23]. The highest incidence rates are reported in North America and Western Europe, where incidence rates of 19 per 100,000 have been reported [23]. In the United States, there are approximately 22,000 new cases and 14,000 cancer-related deaths each year from ovarian cancer [24]. The average age at diagnosis of invasive ovarian cancer in the USA is 63 years [24].

The incidence by age is:

- < 20 years old: 0.2–1.4 per 100,000
- 20–29: 1.8–2.2 per 100,000
- 20–39: 3.1–5.1 per 100,000
- 40–49: 9.0–15.2 per 100,000
- 50–59: 21.8–28.3 per 100,000
- 60–69: 36.2–41.5 per 100,000
- ≥ 70: 47.6–56.7 per 100,000

*Complications and Outcomes of Assisted Reproduction*, ed. Botros Rizk and Jan Gerris. Published by Cambridge University Press. 

Ovarian cancer occurs at a younger age among women with a hereditary ovarian cancer syndrome. The risk of ovarian cancer reaches 2%–3% in women with a *BRCA1* gene mutation at age 35 and for those with a *BRCA2* mutation at age 50. The typical age at diagnosis of ovarian cancer in women with hereditary nonpolyposis colon cancer is 43–50 years [24].

The prognosis is dependent on tumor stage at diagnosis as defined by the International Federation of Gynecology and Obstetrics (FIGO). About two-thirds of patients with epithelial ovarian cancer (EOC) will have widespread tumor dissemination in the abdominal cavity at the time of diagnosis (FIGO stage III). The 5-year survival rates are 73%, 46%, 17%, and 5% for FIGO stages I, II, III, and IV, respectively [23]. Efforts at early detection and new therapeutic approaches to reduce mortality have been largely unsuccessful because the origin and pathogenesis of EOC are not completely understood [23, 25].

The histological classification of ovarian tumors by the World Health Organization (WHO) is based on histogenetic principles, and this classification categorizes ovarian tumors with regard to their derivation from coelomic surface epithelial cells, germ cells, and mesenchyme (the stroma and the sex cord). Epithelial tumors, representing more than 90% of all ovarian malignancies, are further grouped according to their patterns of differentiation into the following histological subtypes: serous, mucinous, endometrioid, clear cell, transitional cell tumors (Brenner tumors), carcinosarcoma, mixed epithelial tumor, undifferentiated carcinoma, and others [23–25]. The serous, mucinous, endometrioid, clear cell, and transitional cell tumors can present as invasive tumors but also as tumors of borderline malignancy [20, 23, 24, 26]. It is crucial to understand the major differences between the invasive epithelial tumors and their borderline counterpart while analyzing data regarding the impact of fertility drugs on the subsequent risk of ovarian cancer.

Borderline tumors of the ovary (BOT) are epithelial tumors with histopathologic features and biologic behavior intermediate between clearly benign and frankly malignant. They are considered to be tumors of a separate entity as they possess major differences in their biologic behavior and prognosis from their invasive counterparts. Over the decades, misunderstanding of these major differences has lead to an overtreatment of BOT both surgically and pharmacologically [23, 26, 27]. The histologic diagnosis of BOT is based on criteria as established by Hart and Norris and detailed by Scully: epithelial cellular proliferation (stratification of the epithelial lining of the papillae, multi-layering of the epithelium, mitotic activity, and nuclear atypia without stromal invasion. BOTs account for 10%–20% of all EOC [21, 23, 24, 26–28]. As opposed to women with invasive carcinoma, those with BOT tend to present at a younger age and mostly in early stage disease. The median age at presentation is younger than 45 years, which is 10–15 years less than the median age seen with invasive tumors. About 27% will present during the reproductive age [26]. In over 85% of cases, BOT is limited to one or both ovaries. Intra-abdominal spread occurs in < 10%. This is the opposite of the situation seen in invasive EOC, which presents with advanced stage in two-thirds of cases. Women with BOT have much better prognosis than those with invasive EOC, and the 5-year survival for stage I disease exceeds 95% [23, 24, 26]. Fertility-conserving surgery with appropriate staging performed by a gynecologic oncologist is safe in patients with BOT and carries no negative impact on survival, as has been re-ensured by a recent meta-analysis [27–29]. Adjuvant therapy is not beneficial and is only associated with morbidity. The use of fertility drugs in subfertile patients with BOT who did not spontaneously conceive following fertility-conserving surgery is permitted and does not seem to increase the risk of disease recurrence or have a negative impact on the pregnancy outcome [20, 24, 29]. The use of oral contraceptives seems to have a protective effect, as is the case of invasive EOC [20, 24].

Serous BOTs account for approximately 55% of all BOTs. They present as a cystic mass with intra- and extra-cystic vegetations (Figure 1.1). Bilaterality exists in about 40% of cases. The outcome of serous BOT correlates with the FIGO stage. Stage I has a 15-year survival of 99%. Peritoneal implants at the time of diagnosis have been reported in 30% of the cases (stages II–III). The survival rate depends on the presence of invasive peritoneal implants. Invasive implants must be differentiated from foci of endosalpingiosis and noninvasive implants that arise from benign glandular elements of the peritoneal serosa (with its potential for Müllerian differentiation) [20, 23, 24, 26, 28]. Fertility-conserving surgery in FIGO II–III disease is also permitted in the absence of invasive implants. Mucinous BOTs account for 40% of all BOTs. They present as a uni- or multilocular cystic mass with smooth capsule (Figure 1.2). Mucinous BOT present

**Table 1.1** Significant differences between invasive epithelial ovarian cancer (EOC) and borderline tumors of the ovary (BOT).

| | Invasive EOC | BOT |
|---|---|---|
| Median age at diagnosis | > 55 years | 45 years, one-third are < 40 years |
| Disease stage at presentation | 2/3 in stage III–IV | More than 85% in stage I |
| Management | Debulking surgery and postoperative chemotherapy | Staging and fertility-conserving surgery. No adjuvant chemotherapy |
| Chemo-sensitivity | Chemo-sensitive especially in high-grade, nonmucinous tumors | Chemo-resistant |
| CA 125 in follow-up | Useful, independent prognostic factor for survival | Elevated in < 20% of recurrences |
| % Mucinous tumors | < 10% | Up to 45% |
| % Serous tumors | > 80% | About 55% |
| ER and PR expression | Low | High and almost in all cases |
| Lymphatic spread | Common | Rare (with invasive implants) |
| 5-year disease recurrence | > 75% | < 10% (long-term recurrence 10–15 years) |
| 5-year survival (all stages) | < 40% | > 95% |

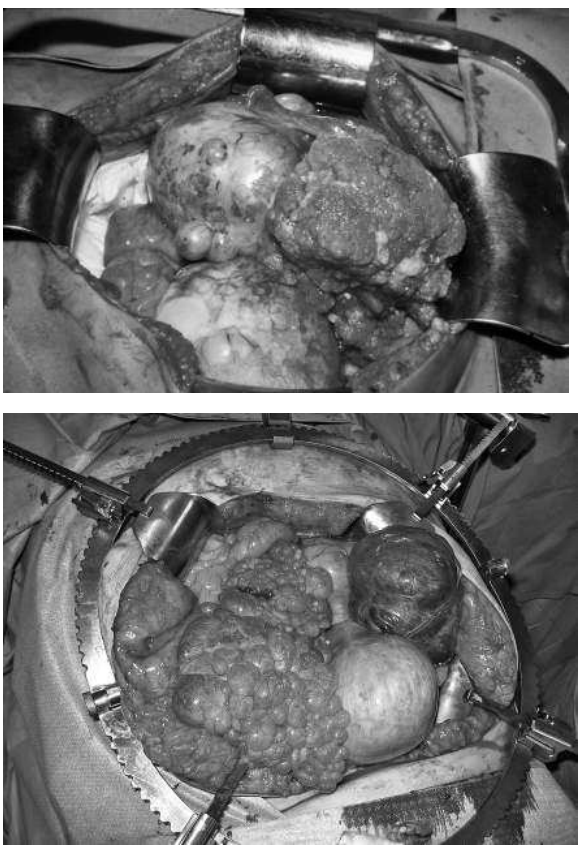

**Figure 1.1** Bilateral serous BOT with typical vegetative capsular growth. The presence of capsular vegetations in serous BOT does not imply a bad prognosis as in the case of its invasive counterpart.

in 85% of the cases as stage I that have an excellent 15-year survival (97%). In stage III, the reported mortality rate has been 64%. Extra-ovarian spread at the time of diagnosis is rare. Only 10%–15% of the cases are associated with pseudomyxoma peritonei. Mucinous BOT can be separated into endocervical (Müllerian) and intestinal subtypes and pseudomyxoma is only present in the intestinal subtype. Mucinous tumors of the appendix coexist in 8% of these cases. It is controversial whether a patient with a mucinous BOT in the ovary and in the appendix has two primaries or has a primary appendiceal lesion and a metastatic ovarian lesion. About 5% of the BOT cases are nonserous, nonmucinous tumors (endometrioid 2%, mixed 2%, clear cell < 1%, and Brenner < 1%). The clear-cell type has the worst prognosis [20, 24, 26, 28]. It is of significance to the current topic to mention that unlike invasive EOC, the majority of BOTs possess high concentrations of hormonal receptors both for estrogen and for progesterone [20, 28]. Hormonal receptor expression is even higher in serous than in mucinous BOT [20, 28]. The major differences between invasive EOC and BOT of the ovary are summarized in Table 1.1.

## Pathogenesis of Ovarian Cancer

Traditionally, two main hypotheses have been suggested.

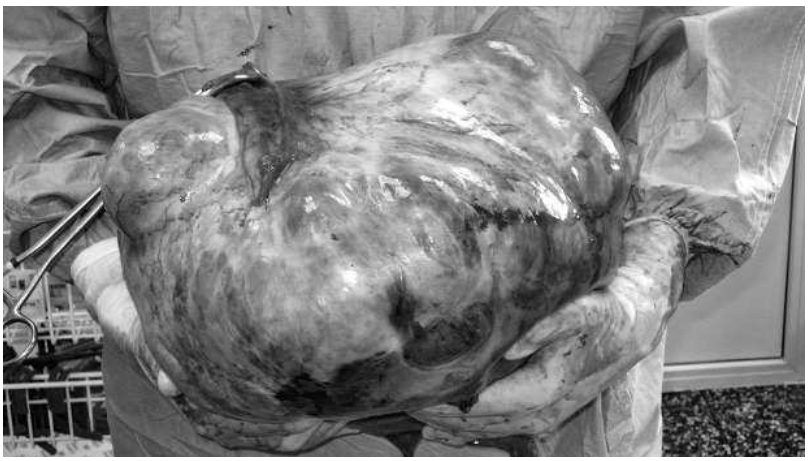

**Figure 1.2** Voluminous mucinous BOT. Note the smooth lobular capsule without vegetations.

## Incessant Ovulation Theory

This theory holds that repeated ovulation results in minor trauma to the ovarian epithelium. To repair the disruption of the surface epithelium, an increased cellular proliferation and consequently a higher rate of DNA synthesis are necessary. This predisposes the cells to a higher chance of mutations, which in turn can lead to malignant transformation [20, 21, 24, 30]. The incessant ovulation theory has been supported by the results of different epidemiological studies showing that any factor which suppresses ovulation, such as pregnancy, oral contraception, lactation, and an early menopause, clearly reduce the risk of ovarian cancer [20, 21, 24]. Thus, according to the "incessant ovulation" theory, an association between ovulation-inducing drugs and ovarian cancer risk is biologically plausible. Clomiphene citrate (CC) and gonadotropins are the most commonly used medications in ovulation induction and ovarian hyperstimulation. The use of both medications has been steadily increasing since the 1960s. CC induces ovulation indirectly. It is considered as first-line treatment for women with anovulatory infertility, and is also widely used for ovulatory women with unexplained infertility. CC is a selective estrogen-receptor modulator (SERM). Chemically, CC is a nonsteroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties [1, 30]. As an anti-estrogen, it competes with estradiol for binding sites at the hypothalamic level, leading to an increased secretion of GnRH and hence of FSH and LH from the pituitary, resulting in ovarian follicular maturation. This is followed by the preovulatory LH rise, ovulation and the subsequent development of the corpus luteum [31]. Use of CC is thus associated with an increase in estradiol and progesterone serum levels. In-vitro studies showed that CC is able to increase cell proliferation and to exert genotoxic effects [32]. CC is structurally similar to tamoxifen, which has been used rarely as an ovulation induction agent but has been widely used in the treatment of breast cancer. The use of tamoxifen among breast cancer patients has been associated with an increased risk of uterine cancer [1, 21, 30]. Gonadotrophins are also used for ovulation induction and in so-called controlled ovarian hyperstimulation. These include human menopausal gonadotrophins (HMG) obtained from the urine of menopausal women and their purified derivatives as well as the more recent recombinant FSH preparations obtained by recombinant technology [30]. It has been estimated that a single cycle of ovulation induction preparing for IVF can be equivalent to two years of menstrual cycles in terms of the number of follicles produced and estrogen concentrations achieved [21, 33]. It is also impressive how a hyperstimulated ovary could macroscopically look like a real ovarian malignancy. Ovarian hyperstimulation is usually associated with elevated CA125 levels and levels up to 1500 U/ml have been reported [34]. Hatzipetros *et al.* [35] recently reported the presence of atypical cells suggestive for ovarian malignancy in the ascitic fluid of nine women with severe ovarian hyperstimulation syndrome [35]. The significance of these atypical cells was not clear, as subsequent cytological and histological examinations following regression of the ovarian volume failed to find evidence of any malignant tumors in these patients [35].

## Exposure to Gonadotropins Theory

This theory holds that persistent ovarian exposure to gonadotropins and elevated estradiol concentrations may be carcinogenic. The close temporal

relation between the rise in gonadotropin levels and the increased ovarian cancer incidence during menopause supports the gonadotropin theory [1, 20, 21, 24, 30]. This hypothesis is supported by animal models and by the observation that experimentally induced ovarian tumors contain gonadotropin receptors [1, 20, 21, 24]. The review of Schüler *et al.* [20] showed that FSH and LH regulate ovarian steroid biosynthesis via their receptors (FSH-R and LH-R) on the membranes of granulosa and theca cells, respectively. FSH-R and LH-R are also located on ovarian surface epithelium (OSE) cells and partly on benign and malignant epithelial ovarian tumor cells. Receptor expression seems to decrease with tumor dedifferentiation. While the gonadotropins receptor expression was found to be 80% in benign cystadenomas, it decreased to 71% and 40% in BOT and invasive EOC, respectively. Overexpression of FSH-R in OSE cells was followed by an increased expression of EGFR, c-myc and Her2/neu-receptor and a higher rate of proliferation [20].

### Role of Hormonal Factors

Ovarian stimulation is associated with increased serum levels for estrogen and progesterone. The possible role of estrogen and progesterone in ovarian carcinogenesis has also been reviewed by Schüler *et al.* [20].

Elevated estradiol (E2)-serum levels are detected in patients with EOC and estrogen receptor a (ERa) as well as estrogen receptor b (ERb) are expressed in most ovarian malignancies, especially in BOT. Estrogen regulates a number of proteins influencing the rate of mobility and invasion and interacts with growth factors like EGF, TGF-alpha, IGF and IL-6. Cells overexpressing ERb showed notably slower cell growth, exhibited an increased apoptosis rate and a significantly decreased motility. Estrogen can abolish the antiproliferative effect of progesterone and GnRH on EOC [20]. However, the clinical use of SERM or GnRH antagonists in the treatment of patients with advanced EOC has been associated with marginal benefit [36].

In fertility treatment, progesterone is used routinely in most IVF protocols to support the luteal phase. The rationale for using progesterone during the luteal phase in IVF treatment cycles is due to the GnRH agonist dysregulation of luteal pituitary LH pulses, which normally support the corpus luteum. Progesterone receptor (PGR)-A and PGR-B are expressed in some invasive EOC but in most BOT. In-vitro studies show that high doses of progesterone are associated with an apoptotic effect in EOC cells. Loss of heterozygosity and/or polymorphisms of PGR is associated with increased EOC risk, which implies that PGR activation might protect against the development of ovarian cancer. About 75% of all EOC exhibit a loss of heterozygosity in gene locus 11q23.3–24.3, the region where the progesterone receptor gene is located. Although a meta-analysis showed that elevated levels of PR predicted favorable survival in EOC, the results of clinical trials evaluating the therapeutic benefit of high dose of progesterone in EOC are disappointing [20].

Finally, the review of Schüler *et al.* [20] also showed that the androgen receptor is expressed by more than 80% of EOC cells. Tubal fimbriae and OSE cells, both putative precursors of OEC, also express the androgen receptor and it has been shown that androgens stimulate the growth of OSE [20].

## New Concepts on the Origin of Epithelial Ovarian Cancer (EOC): The Fallopian Tube Theory

Recent molecular studies showed that invasive EOC is a heterogenic disease that can be divided into two types according to the molecular origin, biological behavior, and prognosis. Both types develop independently along different molecular pathways. Both types develop outside the ovary and involve it secondarily.

Type 1 EOC is generally indolent, presents in stage I (tumor confined to the ovary) and develops from well-established precursors, so-called BOT. These tumors are characterized by specific mutations including *KRAS*, *BRAF*, *ERBB2*, *HNF1*, *CTNNB1*, *PTEN* and *PIK3CA*, but rarely *TP53.* They are relatively genetically stable.

Type II EOC is composed of tumors that are aggressive, present in advanced stage, and develop from intraepithelial carcinomas in the fallopian tube. They have a very high frequency of *TP53* mutations but rarely harbor the mutations detected in type I tumors. They are genetically highly unstable [25].

The "Fallopian tube theory," hypothesized by Kurman and Shih [25], suggests that serous carcinomas developed from normal residual fimbrial epithelium localized on the ovarian surface after ovulation. The authors suppose that, following implantation of tubal epithelium in the ovary, the adjacent stromal cells are activated and secrete steroid hormones that can stimulate malignant transformation. The fallopian tube theory was based on pathological evidence of a putative precursor lesion in the fallopian tube that

**Table 1.2** New concepts on the of ovarian adenocarcinomas (AC). From J. Prat, *Annals of Oncology* 2012; 23:S111–S117.

| | Type 2 OC | Type 1 OC | | | |
|---|---|---|---|---|---|
| | **High-grade serous AC** | **Low-grade serous AC** | **Mucinous AC** | **Endometrioid AC** | **Clear cell AC** |
| Risk factors | *BRCA1/2* | Unknown | Unknown | HNPCC | Unknown |
| Precursor | Tubal Intraepithelial carcinoma | Serous borderline tumor | Cystadenoma/ borderline tumor | Atypical endometriosis | Atypical endometriosis |
| Spread | Very early transcoelamic | Transcoelamic | Confined to ovary (usually) | Confined to pelvis (usually) | Confined to pelvis (usually) |
| Molecular abnormality | *BRCA, p53* | *BRAF, KRAS* | *KRAS, HER2* | *PTEN, ARIDIA* | *HNF1, ARIDIA* |
| Chemo-sensitivity | High | Intermediate | Low | High | Low |
| Prognosis | Poor | Intermediate | Favorable | Favorable | Intermediate |

OC, ovarian cancer; HNPCC, hereditary nonpolyposis colorectal carcinoma.

morphologically and molecularly resembles high-grade ovarian serous carcinoma and that has been designated "serous intraepithelial tubal carcinoma" (STIC) [25]. Thus, rather than developing *de novo* from the ovary, as previously thought, the majority of type II tumors appear to arise from a STIC in the fimbriated end of the fallopian tube that spreads to the ovary. Another possible mechanism for the development of "ovarian" carcinoma is dislodgement of normal tubal epithelium from the fimbria, which implants on the site of rupture where ovulation occurred, resulting in the formation of an inclusion cyst that may then undergo malignant transformation. Thus, serous tumors may develop from inclusion cysts, as has been thought, but by a process of implantation of tubal (Müllerian-type) tissue rather than by a process of metaplasia from ovarian surface epithelium (mesothelial) [25]. Endometrioid and clear-cell carcinomas may also originate from nonovarian, Müllerian-type tissue, as it is widely accepted that these tumors develop from endometriosis. The origin of mucinous and transitional cell (Brenner) tumors is still not well established, although recent data suggest a possible origin from transitional epithelial nests located in para-ovarian locations [25].

Table 1.2 summarizes molecular aspects and heterogeneity of EOC.

## Ovarian Cancer and Protective Factors

### Oral Contraceptives

Studies have consistently shown that prolonged use of oral contraceptives (OCs) reduces the risk of ovarian cancer. An analysis of 45 epidemiological studies found that, compared with women who had never used OCs, any use of OCs was associated with a statistically significant reduction in risk of developing ovarian cancer (relative risk [RR] 0.73, 95% CI 0.70–0.76) [24, 37]. Larger reductions in ovarian cancer risk occurred with increasing duration of OCs use (RR decreased by approximately 20% for each 5 years of use; by 15 years, the risk of ovarian cancer was reduced by 50%). Importantly, the protective effect persisted for 30 years after cessation of OCs, although the effect attenuated over time [24, 37]. The use of OCs offers also protective effect against BOT, with the least protective effect in the case of mucinous tumors. OCs with the current standard or low dose (≤ 35 μg ethinyl estradiol) were associated with a similar or lower likelihood of EOC compared with the higher-dose OCs previously used, based upon a case-control study ($n = 745$ women with ovarian cancer) [24, 38]. The use of OCs is also associated with a decrease in mortality rate of ovarian cancer. In women who never used OCs, 1.2% are diagnosed with ovarian cancer and 0.7% die as a result of this disease before the age of 75 years. In women who used OCs for 10 years, the estimated cumulative incidence is only 0.8% and only 0.5% die as a result of this disease before the age of 75 years [37]. The literature contains several meta-analyses evaluating the impact of OC use in *BRCA* mutation carriers. The published data also confirm significant risk reduction in this population. No difference in the risk reduction was notable between *BRCA1* and *BRCA2* mutation carriers [39].

### Breastfeeding

A meta-analysis of six studies found that breastfeeding for a cumulative duration of > 12 months compared with never breastfeeding was associated with a statistically significant decrease in the risk of EOC (OR 0.72, 95% CI 0.54–0.97) [24].

### Nonhormonal protective factors

These include surgical resection of the ovaries and tubal ligation [24].

## Ovarian Cancer and Risk Factors

### Infertility

Infertility as an independent factor is associated with increased risk for cancers of the uterus and ovary. It is not clearly obvious whether this association is due to the lack of protective effects caused by pregnancy or due to pathologic conditions associated with infertility itself. Mosgaard *et al.* [31] conducted a case-control study of all Danish women (below the age of 60 years of age) diagnosed with ovarian cancer during the period from 1989 to 1994. They reported that infertility per se implied an increase in the crude risk of ovarian cancer (OR = 1.54; 95% CI = 1.22–1.95). Infertile nulliparous women without treatment had an even higher risk compared with nulliparous women without infertility (OR = 3.13; 95% CI = 1.60–6.08). The results of Modan *et al.* [7] were also in accordance. Jensen *et al.* [9] showed that infertile women are more at risk of ovarian cancer than women in the general Danish population, even after adjustment for parity. Brinton *et al.* [22], in a large, retrospective cohort study involving 12,193 infertile women, found that 581 of them developed cancer (standardized incidence ratio (SIR) = 1.23; 95% CI = 1.1–1.3). Patients with primary infertility were at an even higher risk (SIR = 1.43; 95% CI = 1.3–1.6). Particularly elevated risks among primary infertility patients were observed for cancers of the uterus (SIR = 1.93) and ovaries (SIR = 2.73). Further analysis revealed that patients with primary infertility due to anovulation were particularly predisposed to uterine cancer (SIR = 2.42; 95% CI = 1.0–5.8), while those with tubal disorders were more predisposed to ovarian cancer (SIR = 1.61; 95% CI = 0.7–3.8). Primary infertility associated with male-factor problems was associated with increases in colon (SIR = 2.85; 95% CI = 0.9–9.5) and uterine (SIR = 3.15; 95% CI = 1.0–9.5) cancers. On the other hand, Liat *et al.* [40] studied a cohort of 2431 Israeli women (more than 84,000 women-years) who were treated for infertility during the period 1964–1974. They concluded that infertility is associated with a significantly increased risk for endometrial cancer and a borderline increased risk for breast cancer, while ovarian cancer risk was not found to be elevated.

### Early Menarche or Late Menopause

In correlation with the incessant ovulation theory, both early menarche and late menopause increase the total number of ovulations in a woman's lifetime [20, 21, 24], and thereby could increase the risk of developing EOC.

### Parity and Other Obstetric Factors

Nulliparous women appear to have an increased risk of ovarian cancer. A retrospective cohort study of over 20,000 women found that parous women had a significantly decreased risk of EOC (hazard ratio 0.49, 95% CI 0.25–0.95) [41]. In addition, a history of a full-term pregnancy is associated with a decreased risk of EOC [21, 24, 42, 43]. Studies suggesting increased risk of ovarian cancer following fertility treatment showed no higher risk among those subfertile women who succeeded in becoming pregnant [5, 21, 43].

### Endometriosis

A meta-analysis of 13 case-control studies that included almost 8000 women with EOC found a statistically significant association between a self-reported history of endometriosis and an increased risk of clear cell (OR 3.05, 95% CI 2.43–3.84), endometrioid (OR 2.04, 95% CI 1.67–2.48), and low-grade serous (OR 2.11, 95% CI 1.39–3.20) EOC, but not high-grade serous (OR 1.13, 95% CI 0.97–1.32) or mucinous (OR 1.02, 95% CI 0.69–1.50) EOC [44]. The risk of malignant transformation of ovarian endometriosis has been estimated to be 2.5% [24]. Endometriosis-associated EOC appears to develop in younger women and have a better prognosis than most cases of EOC [21, 24, 25]. These epidemiologic findings are in correlation with recent pathological concepts on origin of EOC based on molecular studies (as discussed above).

### Polycystic Ovarian Syndrome (PCOS)

PCOS increases the risk of ovarian cancer (OR 2.52, 95% CI 1.08–5.89) according to a meta-analysis of eight case-control studies [21, 24].

## Postmenopausal Hormone Therapy

The absolute risk of ovarian cancer with postmenopausal hormone therapy appears to be small. The data of the Women's Health Initiative randomized trial found an increase (although not significant) in the risk of ovarian cancer with combined estrogen–progestin therapy compared with placebo (42 versus 27 per 100,000 person-years; HR 1.6, 95% CI 0.8–3.2) [45]. A recent meta-analysis showed increased risk only for serous (RR 1.53, 95% CI 1.40–1.66; $p < 0.0001$) and endometrioid subtypes (1.42, 1.20–1.67; $p < 0.0001$) [45]. The risk declined the longer ago use had ceased, although about 10 years after stopping long-duration hormone therapy use there was still an excess of serous or endometrioid tumors (RR 1.25, 95% CI 1.07–1.46, $p = 0.005$) [46].

## Genetic Factors

Several ovarian cancer susceptibility genes have been identified, primarily *BRCA1* and *2* and the mismatch repair genes (associated with Lynch syndrome). Other genes include *RAD51C*, *RAD51D*, and *BRIP1* [24]. It is estimated that *BRCA* gene mutations and Lynch syndrome account for 10%–15% of ovarian cancer cases [24].

# Ovulation-inducing Drugs – Data of Epidemiologic Studies

Concerns about whether the use of fertility drugs increases women's risk of developing ovarian cancer was elicited by two studies. Whittemore *et al.* [5] were the first to examine the possible relationship between fertility drugs and cancer. They showed in their meta-analysis of 12 case-control studies related to ovarian cancer that self-reported prior use of fertility drugs was associated with an odds ratio of 2.8 (95% CI 1.3–6.1) for developing ovarian cancer as compared to no use. In that study, infertile women who took fertility medications without ever being pregnant had a much higher risk of developing cancer (OR 27.0, 95% CI 2.3–315.6) [5]. In contrast, infertile women who had been treated for their problem and managed to get pregnant had no increased risk of ovarian cancer (OR 1.4, 95% CI 0.52–3.6) [5]. However, the notion of an increased risk limited to the subgroup of nulligravid women was disputed because the risk estimate was based on a mere 12 exposed cases and one exposed control. The study also had a number of limitations, including lack of information about type of drugs or duration of use [1, 21].

Subsequently, a large cohort study also suggested an increased risk of ovarian tumors among women using CC for 12 months or more [6]. Rossing *et al.* [6] evaluated the development of ovarian cancer in a larger cohort study of 3837 women. There were 11 invasive or borderline ovarian tumors, as compared with an expected number of 4.4 (SIR 2.5; 95% CI: 1.3–4.5). Nine of the women in whom ovarian cancer developed were treated with CC; the adjusted relative risk (RR) among these women, as compared with infertile women who had not treated with this drug, was 2.3 (95% CI: 0.5–11.4). Five of the nine women had taken CC during 12 or more menstrual cycles. This period of treatment was associated with an increased risk of ovarian tumors (RR 11.1; 95% CI: 1.5–82.3), whereas treatment with the drug for less than one year was not associated with an increased risk [6]. This was observed in subfertile women who conceived following treatment as well as in subfertile women who were refractory to therapy. The same was not shown with the use of HCG in the same cohort of patients. This study was limited by the small number of tumors, with almost half of them being BOT (5 of 11 neoplasms). The study of Rossing *et al.*, despite its limitations, raised attention regarding the use of CC for more than 12 cycles.

Some other reports have suggested that fertility drugs might even increase the risk of nonepithelial cancers [21]. In contrast, several subsequent epidemiological studies failed to show any association between women exposed to treatment with ovulation-inducing drugs and untreated infertile woman [7–14, 47]. However, most of the case-control studies contained only small numbers of ovarian tumors and were consequently limited by imprecise risk estimates, especially in subgroups of fertility drug users. Some studies used SIRs to compare the risk of ovarian cancer in cohorts of infertile women with that of the general population [47]. This comparison controls for the potential confounders of age and calendar time, but not for parity, causes of infertility, and use of oral contraceptives [9]. Jensen *et al.* [9] published a study in 2000 based on a cohort of 54,362 Danish women that comprised the largest number of reported cases of invasive ovarian cancers (156) in any cohort of women with infertility problems. The study also contained detailed histological information obtained from the Danish Cancer Registry and Danish Registry of Pathology. This enabled the authors to analyze potential differences in risk

according to histological subtype. Furthermore, they obtained detailed information on the various types of fertility drugs used. The authors evaluated the effect of four groups of fertility drugs (gonadotrophins, clomifene citrate, human chorionic gonadotrophin, and gonadotrophin-releasing hormone) on overall risk of ovarian cancer after adjustment for potential confounding factors. Analyses within cohort showed no overall increased risk of ovarian cancer after use of any of the four fertility drugs [9]. Furthermore, no associations were found between all four groups of fertility drugs and number of cycles of use, length of follow-up, or parity. Their data therefore suggested that factors related to the diagnosis of infertility (for example, genetic or biological), and not the use of fertility drugs, increase the overall risk of ovarian cancer [9]. Interestingly and in contrast to the analyses of overall risk of ovarian cancer, they found a significant increase (67%) in risk of serous tumors after the use of clomiphene, primarily when follow-up was for more than 15 years since first use. The risks for mucinous, endometrioid, and clear-cell tumors were not significantly affected by the use of any of the four types of fertility drugs. This is in accordance with several studies suggesting that mucinous tumors are less affected by hormonal (oral contraceptives) and parity-related factors than are nonmucinous tumors. One of the drawbacks of the study has been the number of women included in the cohort that have not yet reached the usual peak age for ovarian cancer [9].

Three large meta-analyses [48–50] were published. One included seven case-control and three cohort studies [48], one included only six cohort studies [49], and the third included only nine cohort studies calculating the risk of ovarian cancer in infertile women treated with fertility drugs. The authors in two of these meta-analyses [48, 49] reported a significant elevated risk of ovarian cancer in treated subfertile patients when compared to the general population. However, data from cohort studies that compared treated with untreated subfertile patients suggests that treated patients may tend to have a lower incidence of ovarian cancer [21]. The third meta-analysis reported that fertility treatment is not associated with an elevated risk of ovarian cancer [50].

A literature review has been recently published by Tomao *et al.* [1]. They included 127 studies. Of these, 97 were literature reviews or meta-analysis reports, 11 were case-control studies, and 19 were cohort studies. This report also focused on the impact of IVF on ovarian cancer risk (Table 1.3). The majority of studies (19 studies) included in their review showed that fertilization therapy did not contribute significantly to the risk of ovarian cancer. A higher risk of ovarian cancer after fertility therapy was shown in 11 studies, but in six of these the increased risk was specific to BOT. The authors stated that when considering all the studies included in their review, the most recent works appear reassuring regarding the potential risk of invasive ovarian cancer, and more accurate compared to the past, because they are conceived in order to avoid the interrelationships and potential bias derived from the different risk factors. Regarding IVF in particular, Tomao *et al.* [1] concluded that the issue of IVF must be considered as still under investigation. They explained that most studies that showed no increased risk following IVF contained few cases of EOC in this cohort of patients. In addition, some studies suggested increased risk following IVF [56, 57]. In one study, an increased risk was noticed in cohorts of women using > 4 IVF cycles [56]. In another study, the overall SIR for invasive EOC was not significantly elevated, but increased with longer follow-up after first IVF with SIR = 3.54 (95% CI: 1.62–6.72) after 15 years [57]. The same study [57] also showed that the risks of BOT and of all ovarian malignancies combined in the IVF group were significantly increased compared with risks in the subfertile comparison group (hazard ratios 1/4 4.23; 95% CI 1/4 1.25–14.33 and 2.14; 95% CI 1/4 1.07–4.25, respectively, adjusted for age, parity, and subfertility cause).

A recent Cochrane review published by Ruizzuto *et al.* [21] reviewed 11 case-control studies and 14 cohort studies, which included a total of 182,972 women. In their excellent report, the authors analyzed the impact of fertility drugs on the incidence of invasive ovarian cancer and BOT separately. Fertility drugs were categorized as follows: any fertility drug, clomiphene, clomiphene in combination with gonadotrophines, and gonadotrophines. Overall, they found no convincing evidence of an increase in the risk of invasive ovarian tumors with fertility drug treatment. Ruizzuto *et al.* [21] explained that studies showing an increase in the risk of ovarian cancer had a high overall risk of bias due to a retrospective study design, lack of accounting for potential confounding variables, and lack of details about fertility drug treatments given; estimates were based on a small number of cases, giving rise to wide confidence intervals. They added that studies with more robust estimates based on a larger number of

**Table 1.3** IVF and ovarian cancer (Cohort studies). Modified from Tomao *et al*. 2014 [1]

| Study | Population | No. of ovarian cancers | Results |
|---|---|---|---|
| Venn *et al*. [51], 1995 | 29,666 women | 3 cancers in exposed, 3 cancers in unexposed | SIR in exposed = 1.7 (CI 95%: 0.55–5.27); SIR in unexposed = 1.62 (95% CI: 0.52–5.02); RR exposed vs. unexposed = 1,45 (95% CI: 0.28–7.55) |
| Venn *et al*. [14], 1999 | 29,700 women | 7 ovarian cancers in exposed, 6 in unexposed | SIR in exposed = 0.88 (95% CI: 0.42–1.84); SIR in unexposed = 1.16 (95% CI: 0.52–2.59) |
| Dor *et al*. [52], 2002 | Retrospective cohort of 5026 women | 1 ovarian cancer case | SIR in exposed = 0.57 (95% CI: 0.01–3.20) |
| Klip *et al*. [53], 2002 | 23592 women | 17 ovarian cancers | No differences in risk exposed vs. unexposed<br>Detailed information obtained through questionnaires and from medical records |
| Lerner Geva *et al*. [47], 2003 | 1082 women | 3 ovarian cancers | SIR in exposed = 5.0 (95% CI: 1.02–14.6). SIR = 1.67 (0.02–9.27) when cancers developing within 1 year were excluded.<br>No untreated group Registry match |
| Källen *et al*. [54], 2011 | 24,058 women | 26 ovarian cancers | RR exposed vs. unexposed = 2.09 (95% CI: 1.39–3.12) |
| van Leeuwen *et al*. [57], 2011 | 19,146 IVF women, 6006 subfertile, not treated with IVF | 77 ovarian cancers (42 invasive and 35 BOT)<br>64 cancers in the IVF group and 16 in the non-IVF group | Risk of BOT increased in the IVF group compared with the general population. SIR = 1.76 (95% CI: 1.16–2.56).<br>The overall SIR for invasive ovarian cancer was not significantly elevated, but increased with longer follow-up after first IVF. SIR = 3.54 (95% CI: 1.62–6.72) after 15 years |
| Yli-kuha *et al*. [55], 2013 | 9175 women | 9 invasive ovarian cancers, 4 BOT | OR for invasive cancers = 2.57 (95% CI: 0.69–9.23) OR for BOT = 1.68 (95% CI: 0.31–9.27) |
| Brinton *et al*. [56], 2013 | 87,403 women | 45 ovarian cancers | Global HR = 1.58 (95% CI: 0.75–3.29), HR among women receiving ≥ 4 IVF cycles =1.78 (95% CI: 0.76–4.13) |

cases did not detect differences between exposed and unexposed women. However, the Cochrane review did suggest an increased risk of BOT in subfertile women treated with IVF [21].

Thus, in contrast to the reassuring results of recent epidemiological studies and meta-analyses/reviews concerning fertility drugs use on the risk of invasive EOC, there is some degree of evidence that use of fertility drugs might increase the risk of BOT [15, 17, 19, 40, 56, 57].

The apparent increase in the risk of BOT secondary to the use of fertility drugs might be biased to some extent by the epidemiology of BOT itself. The median age at diagnosis of BOT is < 45 years, with almost one-third of these patients presenting during their reproductive age [26, 28]. BOT may therefore be frequently encountered among patients seeking/under fertility treatment because the use of infertility services is more common among older nulligravida [2]. Thus, the possible correlation between fertility drugs and BOT might be secondary to the fact that infertility patients are examined more thoroughly than is the general population, leading to a higher detection rate of asymptomatic tumors [20, 21].