

## Review Article

# Menopause and ovarian cancer risk: mechanisms and experimental support

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**Abstract:** Epidemiological evidence has consistently supported a link between ovarian cancer risk and reproductive history. Pregnancies and the use of oral contraceptives reduce the risk of ovarian cancer. Moreover, the incidence of ovarian cancer rises significantly during the peri-menopausal period and is highest post-menopause. Several plausible mechanisms have been proposed to explain at the cellular level the association. Among these, the incessant ovulation hypothesis and the gonadotropin stimulation theory are best known. In this review, we consider experimental support for each mechanism, and also suggest that another idea, the follicle depletion hypothesis, may provide a unified theory that extends the previous concepts and is best supported by experimental evidence.

**Keywords:** Ovarian cancer, epithelium, menopause, mouse models, ovarian follicles, pre-malignant lesions, Tp53, ovulation, epidemiology

## Introduction

Epidemiological data consistently confirms a link between reproductive factors and history with the risk of ovarian cancer. The number of full-term pregnancies, years of oral contraceptive use, and nursing or breast-feeding all decrease ovarian cancer risk [1, 2]. In a large combined analysis of twelve U.S. case-control studies, a first full-term pregnancy was found to lower the risk by forty percent, and each birth after the first reduced the risk another fourteen percent [3]. Ever users of oral contraceptives have a thirty percent lower risk compared to non-users [4]. The protection increases with duration of oral contraceptive use, where risk is reduced about fifty percent after five years. The effect of past oral contraceptive use persists for at least ten years after the cessation of use. In addition, tubal ligation and hysterectomy convey protection against epithelial ovarian cancer [5].

The most significant factors influencing ovarian cancer risk are age and menopausal status. Approximately only fifteen percent of ovarian

cancer patients are younger than 50, and many of those cancers are germ cell or granulosa type instead of epithelial-derived malignancies, of which serous carcinomas make up the most common histological subtype [6]. A dramatic increase in ovarian cancer risk occurs in the peri-menopausal and immediate post-menopausal periods, and continues to rise as the ovary ages [7]. Menopause generally occurs between 45-55 years of age, with an average age of 51 years in Western countries. The majority of ovarian cancers are diagnosed in post-menopausal women in their late 50 s and early 60 s. The average age of diagnosis for sporadic ovarian cancer is about 63 years, although women with genetic or familial risk factors tend to be diagnosed at a younger age (average age of diagnosis is 54 years) [8].

Several hypotheses have been proposed to provide a mechanistic explanation for reproductive history and ovarian cancer risk, in particular, the association of ovulation frequency and menopausal status with risk of ovarian cancer [6, 7, 9]. Among these, the incessant ovulation hypothesis and the gonadotropin stimulating

theory are best known. These postulations are based on the idea that ovarian cancer is derived from the surface epithelium. However, recent evidence, based on genetic profiling and immunohistopathology, supports that some or even the majority of serous ovarian cancer may originate not from the ovary itself, but from the fallopian tube fimbria [10, 11]. In this review, we consider experimental support for each mechanism, and also suggest that another idea incorporating the concept of follicle depletion may provide a unified theory that covers the previous concepts and can best be supported by experimental evidence.

### Ovarian function and aging

By the end of the reproductive age, germ cells and follicles are depleted from the ovaries and the ovulatory cycle ceases, resulting in menopause. Menopause is considered the permanent ending of menstruation that results from the depletion of germ cells and loss of ovarian follicular activity [12-14], and has become a women's health issue as women's lifespans have increased. The life expectancy of women in the mid- to late- 1800s was about 40 years of age, such that menopause was not usual and consequently ovarian cancer was rarely experienced. Present day women may reasonably expect to live a third or more of their lives post-reproductively, and face several potential health issues associated with reproductive senescence. The peri-menopausal period commences when the first features of menopause begin until at least one year after the final menstrual period, generally lasting an average of five years. In humans, the transition to menopause is a set of gradual changes, in which ovarian function, reproductive capacity, and hormonal status are altered long before menses stops completely.

Hormonal changes characterize the menopausal transition. In the normal reproductive ovary, following ovulation and release of the ovum, the follicle remnant converts into a corpus luteum, where sex steroids, predominately estrogen and progesterone, are produced and released. The steroid hormones act to inhibit the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary. With the depletion of follicles and cessation of ovulation, estrogen and progesterone levels fall and normal feedback inhibition of

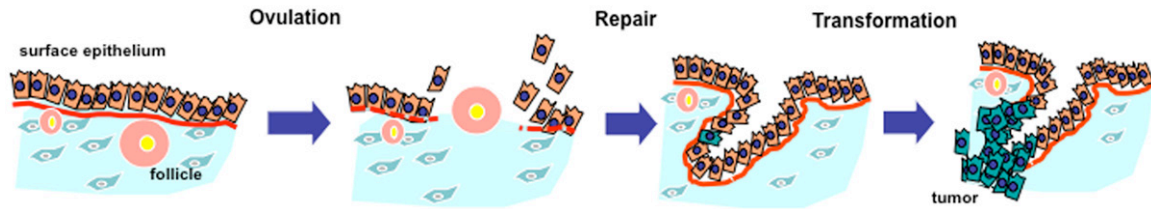
FSH and LH release stops. As a result, FSH and LH reach highest serum levels in peri- and post-menopausal periods and remain elevated for the remainder of a woman's life [12-14]. These changes precipitate a number of menopausal-associated symptoms and disorders. Although the symptoms and the severity accompanying menopause vary among women, they may include hot flashes, bone loss (or osteoporosis), increased body fat deposition and altered metabolism, and cardiovascular diseases, in addition to the increased risk of ovarian cancer [15-17].

Among the physiological changes associated with menopause, the ovarian tissues undergo morphological transformation, known as "ovarian aging" [18, 19]. Ovaries from older women contain more defined morphological changes than those from younger women, such as deep invaginations, surface papillomatosis, and inclusion cysts [19], which are thought by some to be the histological precursors of ovarian cancer [20, 21]. Analysis of pre-cancerous ovarian tissues obtained from prophylactic oophorectomies revealed an increased number of morphological changes in high-risk ovaries [21]. We found that the frequency of these histological features, especially inclusion cysts, associates with age or menopausal status rather than BRCA1/BRCA2 status [19]. Presumably, acquisition of oncogenic changes (such as Tp53 mutation) in these proliferative ovarian epithelial cells would promote the development of ovarian cancer.

### Experimental supports for the incessant ovulation hypothesis

Fathalla proposed the theory of incessant ovulation to explain the relationship between ovulation frequency and the increased risk of developing epithelial ovarian cancer [22]. Compared with other mammals, the human female appears to be very extravagant with her ova. Ovulatory cycles are almost continuous from puberty to menopause. The female ovulates monthly throughout her reproductive lifetime. In contrast, most other mammals are more economical with their ova, and ovulations are generally limited to the breeding season, and may even occur only on demand after copulation. Furthermore, the reproductive potential is exercised to the full, allowing adequate physiological rest periods, also known as the

## Incessant Ovulation



**Figure 1.** Incessant ovulation hypothesis. An illustration of the incessant ovulation hypothesis. The release of oocytes damages the ovarian surface epithelium and the associated basement membrane (red line). Following ovulation, ovarian surface epithelial cells proliferate to repair the wound, producing ovarian surface invaginations and inclusion cysts, and the proliferation may lead to precursor cells carrying genetic changes. The accumulated genetic changes lead to transformation and development of ovarian cancer.

non-ovulatory phase. For modern day women and domestic hens, the repeated wounding of the epithelium during the release of the ova and the cell proliferation that occurs after ovulation to repair the ovarian surface epithelium have been proposed to result in an accumulation of mutations in the epithelial cells that ultimately form the tumors [22] (**Figure 1**). The hypothesis substantiates the occurrence of ovarian cancer in modern day women since ovulation occurs monthly throughout her reproductive lifespan, if not punctuated by pregnancy or breastfeeding that reduce ovulation [22].

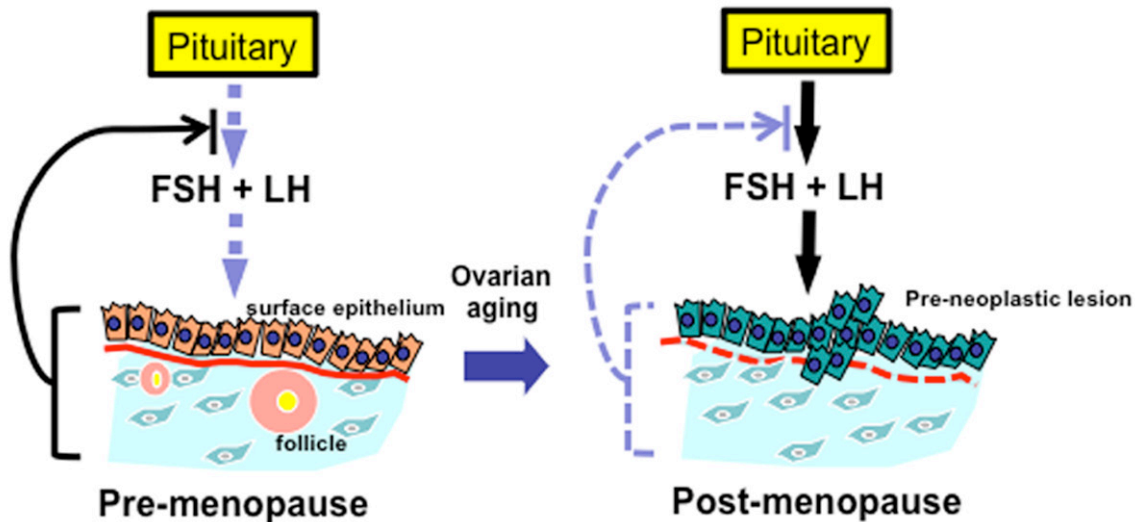
The idea holds that incessant ovulation results in accumulating of mutations in the ovarian surface epithelial cells; subsequently cells with oncogenic mutations give rise to a pre-neoplastic lesion, and ultimately a tumor develops from the lesion [22, 23] (**Figure 1**). Thus, the risk of ovarian correlates with the number of ovulations, and the idea would explain the reduction of risk associated with pregnancy, extended breast feeding, some oral contraceptive formulations, and early menopause, all of which reduce the number of ovulatory events [3, 22, 23].

This mechanism is supported experimentally by the gain of tumorigenic potential following a serial passaging of isolated rat ovarian surface epithelial cells in culture. Godwin and colleagues observed that spontaneous transformation of rat ovarian surface epithelial cells occurred in vitro in a passage-dependent manner [23]. It was reasoned that passaging and growth of the cells in culture resemble repeated ovulations, and the results support the hypothesis that repeated cycles of ovulation, surface wounding, and proliferation to close

the ovulatory site contribute to the etiology of human ovarian cancer [23]. Further evidence was presented in a molecular analysis of Tp53 mutations, a control study where women with a greater mean number of ovulatory cycles had a significantly increased risk of developing Tp53 protein-positive (mutated) but not Tp53-negative ovarian cancers [3, 24]. These findings concur with the genetics of serous ovarian carcinoma reported by the Cancer Genome Atlas Project, that the tumor suppressor Tp53 is frequently mutated, but no other somatic mutation is consistently or frequently found [25]. The Tp53-positive cancers overexpress mutant Tp53 protein and reflect DNA damage to the Tp53 tumor-suppressor gene. The loss of the normal p53 protein may increase the risk for propagation of DNA-damaged cells and malignant transformation.

Moreover, the combination of estrogen and progesterone found in oral contraceptives acts by suppressing ovulation and has been shown to decrease the risk of ovarian cancer by about forty percent after three years of use [26, 27]. Pregnancy and oral contraceptives provide long-term protection; however, the effect may be more convoluted than by simply limiting ovulation. These observations suggest that progesterone, which is the most abundant and perhaps most essential hormone required to maintain pregnancy, likely provides benefits beyond anovulation [7]. During pregnancy, circulating maternal progesterone levels may increase 10-fold or more, and the levels are even higher with twins or multiples [28]. Similarly short-term oral contraceptive benefits are greater than predicted based on the period of ovulation suppression, and progestin-only contraceptives reduce ovarian cancer risk as

## Gonadotropin Stimulation



**Figure 2.** Gonadotropin stimulation hypothesis. In pre-menopausal women, the pituitary-ovarian axis is under the control of a feedback mechanism to achieve the monthly cycle. Gonadotropins, LH and FSH, are synthesized and released from the pituitary gland into the circulation. LH and FSH stimulate ovulation; consequently the ovaries produce hormones and factors including estrogen, progesterone, and inhibin alpha. The ovarian factors in turn reach pituitary to suppress the synthesis and releases of gonadotropins. In postmenopausal women, the ovaries no longer produce hormones and factors, and the feedback loop is missing. Hence, the pituitary gland produces increased gonadotropins, which stimulate and promote the transformation of ovarian surface epithelial cells. The degradation of basement membrane (red line) is a result of gonadotropin stimulation.

well as or greater than other formulations, though they appear to exert their contraceptive effect by reducing the endometrial receptivity to implantation [29].

A number of mechanisms have been examined to determine how progesterone reduces ovarian cancer risk. The high levels of progesterone in pregnancy and oral contraceptive use could influence the clearing of transformed cells from the ovarian surface, thus reducing the risk of ovarian cancer [30]. Experimental studies in animals suggest that synthetic progesterones up-regulate expression of the tumor suppressor Tp53 and have a potent apoptotic effect on ovarian epithelial cells [31]. In this respect, as the likelihood of transformed cells increases with age, the age at the last full-term pregnancy or last regular use of oral contraceptives is a protective factor, and the benefits decrease with time. Progesterone has been shown to suppress the tumorigenicity of ovarian carcinoma cells in xenografted mice [32], but has little effect on established tumors. Progesterone has also been reported to enhance the DNA repair capability of the ovarian surface epithe-

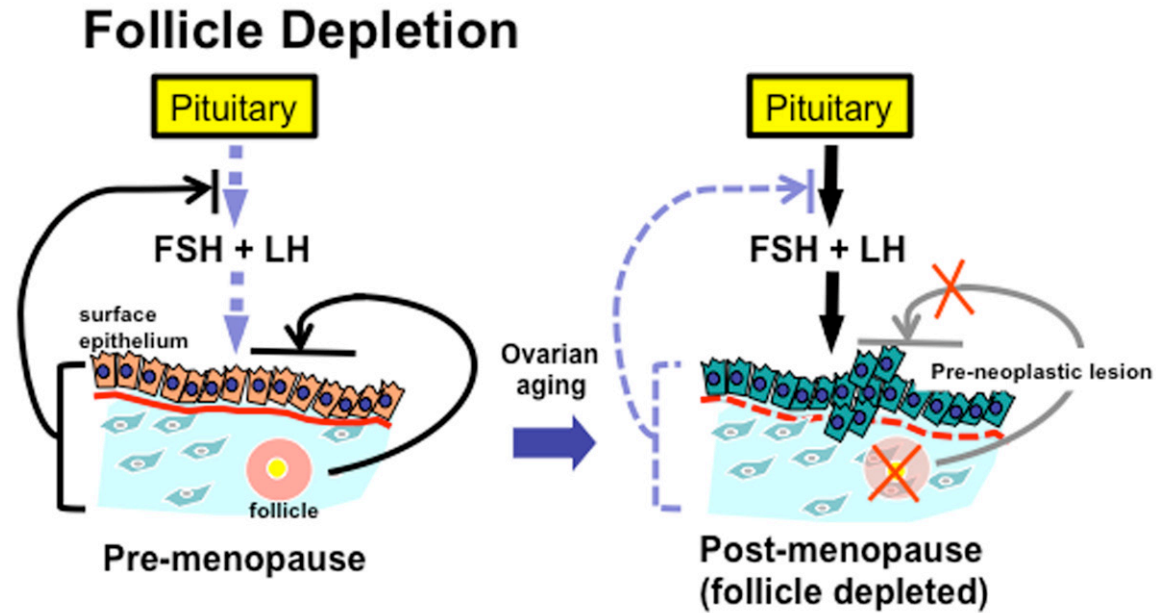
lial cells [33]. Moreover, progesterone could also exert its effect by suppressing gonadotropin levels. Thus progesterone may act by limiting ovulation, clearing transformed cells either on the ovarian surface or fallopian or uterine epithelia, enhancing DNA repair, or by influencing gonadotropin levels.

### Experimental evaluations of the gonadotropin stimulation theory

Supported by the same epidemiological evidence that suggests the incessant ovulation hypothesis, the gonadotropin stimulation hypothesis postulates that the surges of pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), that initiate each ovulation also stimulate the ovarian surface epithelium and induce cell transformation [34-36] (**Figure 2**). The speculated role of gonadotropins is consistent with the fact that ovarian cancer occurs most frequently in postmenopausal women, when ovulation ceases yet plasma gonadotropins are elevated [1, 8].

Several epidemiological observations seem to be consistent with the gonadotropin theory.





**Figure 3.** Follicle depletion hypothesis. The consequences of follicle depletion in the development of ovarian tumors are illustrated. The idea is similar to the gonadotropin stimulation theory shown in **Figure 2**. The depletion of ovarian follicle causes increased gonadotropins, which stimulate the ovarian surface epithelial cells. Furthermore, the follicle depletion hypothesis suggests that in addition to suppressing gonadotropin levels, ovarian follicles may have a local, paracrine role in suppressing ovarian epithelial cells. The idea that ovarian structure integrity is important in suppression tumor development has been suggested previously (Vanderhyden, 2005). Upon follicle depletion, both the increased gonadotropin stimulation and the loss of a local suppressive function promote the transformation and tumorigenesis of ovarian epithelial cells.

One such is the close temporal association that exists between the increased incidence of ovarian epithelial cancer and the rise of circulating gonadotropins in transition to menopause [34-36]. The complete cessation of ovarian function, which occurs during menopause, results in the loss of negative feedback of ovarian steroids on gonadotropins. In approximately two to three years after menopause, the gonadotropin levels are particularly high, where concentrations of both FSH and LH peak and are about ten to twenty times higher than found during the proliferative phase of the menstrual cycle and after which there is a gradual decline in both levels. Coincidentally, the incidence of ovarian cancer climbs dramatically around the age at which most women reach menopause [35].

Recent laboratory research also suggests the relationship between gonadotropins and tumorigenicity. The growth of many gonadal, ovarian cancer, and extragonadal, breast, uterus, prostate and adrenal tumors is stimulated by gonadal sex hormones. Since the gonadal hormone production is regulated by the pituitary gonadotropins, FSH and LH can be considered

as indirect promoters. Also, both gonadal and extragonadal tumors express gonadotropin receptors, suggesting the possibility of a direct tumorigenic role of both hormones. There is also a direct involvement of gonadotropins in the induction and growth of gonadal and extragonadal tumors [37].

Another possibility is that gonadotropins may stimulate the cells to produce proteolytic enzymes, which will then remove the basement membrane [38]. The loss of basement membrane in pre-neoplastic ovarian epithelia, and the possible mechanism in promoting ovarian epithelial transformation has been explored in several studies [3-43].

However, a number of studies have failed to identify a correlation between serum gonadotropin level and ovarian cancer risk [44-46], and there is no good clinical evidence to support the gonadotropin hypothesis of epithelial ovarian carcinogenesis [45]. Moreover, laboratory research also negates a direct association between gonadotropins and ovarian tumorigenicity [47]. In a study that targeted ovarian sur-

face epithelial cells harvested from both premenopausal and postmenopausal women, FSH was found to lower the ovarian surface epithelial cell proliferation under non-confluent conditions. The inhibitory effect was most pronounced in cells from postmenopausal women. In the confluent culture condition, only cells from postmenopausal women showed significantly decreased proliferation. No effects of LH on ovarian surface epithelium cells were detected. These unexpected results do not support the theory that gonadotropins are directly involved in ovarian carcinogenesis through an enhanced proliferation of ovarian surface epithelial cells [47]. Since the hormones have unremarkable effects on growth of ovarian surface epithelial cells in culture, a direct effect of the hormones on ovarian epithelial transformation is unlikely to be sufficient [37]. Thus, the theory fails to explain completely or satisfactorily the epidemiological observation of an association between ovarian cancer incidence and peri-/postmenopausal period.

### Experimental evidences for the follicle depletion hypothesis

A more recent idea puts forth that the depletion of ovarian follicles disrupts ovarian epithelial homeostasis and may be the true cause of an increased cancer risk in menopause [9] (**Figure 3**). The idea that loss of ovarian function may underlie the link between reproductive factors and ovarian cancer was also proposed previously [48].

The depletion of ovarian follicle causes increased gonadotropins, which stimulate the ovarian surface epithelial cells. Furthermore, the follicle depletion hypothesis suggests that in addition to suppressing gonadotropin levels, ovarian follicles may have a local, paracrine role in suppressing transformation of ovarian epithelial cells. Upon follicle depletion, both the increased gonadotropin stimulation and the loss of a local suppressive function promote the transformation and tumorigenesis of ovarian epithelial cells (**Figure 3**).

The follicle depletion hypothesis was postulated based on results from a series of studies examining a germ cell deficient mouse model [49-51], the white spotting variant (Wv) mice [49, 52, 53]. The Wv mice are characterized by having a point mutation in the c-Kit gene that greatly decreases the tyrosine kinase activity of

the c-Kit receptor, which consequently affects the development of germ cells, pigment cells, and mast cells [54, 55]. Homozygous female Wv-mutant mice are sterile due to an early exhaustion of ovarian follicles, though they have a similar lifespan as wild type littermates. Thus they have an extended post-reproductive lifespan and exhibit several physiological changes that are common in menopause, such as decreased steroid hormones, increased gonadotropin levels, changes in cardiac function and bone density, and altered serum lipid levels [49, 50, 56].

Following follicle depletion, Wv mice develop ovarian tubular adenomas, a benign epithelial tumor that resembles preneoplastic lesions caused by ovarian aging in women [49, 53]. Additional oncogenic mutations convert these benign tumors into lesions resembling human ovarian carcinomas, and the Wv tumor bearing mice are considered models for postmenopausal ovarian cancer [57].

The studies of the Wv mice indicate the relationship between the depletion of ovarian germ cells and follicles may explain the foundation of ovarian cancer risk. Follicle depletion demonstrates the relationship between the age-dependent risks and ovarian cancer, especially that the development of ovarian cancer is usually seen in the immediate postmenopausal years, when the follicles are depleted. As part of the consequence, depletion of the follicles precedes and causes the increased serum gonadotropins, which likely stimulate an inflammatory environment in the ovary, and promote the transformation of surface epithelial cells and tumor development [9]. Additionally, the loss of follicles and compromise of the homeostasis of cell community likely foster an environment that allows for the growth and transformation of ovarian epithelia through a local or paracrine mechanism [9, 48] (**Figure 3**).

Furthermore, protective factors such as oral contraceptives, birth control, and use of aspirin (cyclooxygenase inhibitors) may preserve the ovarian follicles. The cyclooxygenases have important roles in ovulation and other ovarian functions, and their inhibition may slow follicle maturation and delay their depletion. Clinical and epidemiological research established that lifetime ovulation correlates with ovarian cancer incidence in the premenopausal stage and

not the post-menopausal stage [2]. Suggestively, a study using the Wv mouse models shows that Cox-1 and/or Cox-2 suppression reduces ovarian tumor development [49]. The study has shown that the reduction in Cox-1 or Cox-2 activity, rather than complete inhibition is sufficient to abate ovarian cancer epithelial morphological transformation, since Cox-1 expression is compensatory in the ovarian tissues when Cox-2 is deleted [49]. Furthermore, Cox-1 inhibition or gene deletion prolongs the presence of ovarian follicles and delays ovarian tumor development in the mice [51]. Whether these findings in mouse models can be extended and applied to women is not yet clear.

### Conclusion and perspectives

The follicle depletion hypothesis seems to accommodate ideas in both the incessant ovulation and the gonadotropins theories, since “incessant ovulation” leads to an accelerated follicle depletion, which subsequently induces menopausal increase of gonadotropins. The ideas are not necessarily incompatible, but the emphasis differs. In addition to the possible carcinogenic stimulation of gonadotropins, the follicle depletion hypothesis also implies a potential local suppressive function of an intact ovarian structure [9, 48] (**Figure 3**).

The above hypotheses concerning the link between reproductive factors and ovarian cancer risk are based on the idea that ovarian cancer arises from ovarian surface epithelial cells. However, recent findings suggest that some or a majority of ovarian cancer may instead originate from fimbria of the fallopian tube [58, 59]. In relation to the new idea that ovarian cancers are derived from fallopian tube fimbria, these hypotheses would need to be re-tailored. One possibility is that like the ovarian surface epithelial cells, the fallopian tube fimbria epithelial cells may also respond to gonadotropin stimulation. Another idea is that the changes in ovarian surface structure following menopause may allow for the easier implantation of epithelial cells derived from the fallopian tube fimbria. Additionally, follicle depletion may also remove the growth suppressive local signal to the fimbria epithelial cells deposited in the ovary. These ideas have not been investigated in clinical tissues or in laboratory systems yet.

The fallopian tube origin of ovarian cancer suggests that tubal epithelial cells from the normal

fimbria, which envelops the ovary and contacts the ovarian surface, dislodge and seed, or implant on, the surface of the ovary [60]. Thus, the tumor that establishes and appears to arise from the ovary is, in fact, fallopian tube-derived. It may be that age and follicle depletion alter both the receptivity of the ovarian surface to seeding by fallopian fimbria-derived cells and make it a more permissive substratum for engulfment or proliferation of the seeded cells. Thus, the idea may also be adaptable to the fallopian tube cell of origin, in addition to that originally proposed considering only cancer derived from ovarian surface and derived inclusion cysts. Additionally, follicle depletion may also encourage the proliferation of non-surface epithelial cells (such as rete ovarii) of Müllerian origin, which have also been considered to be possible cells of origin of ovarian serous carcinomas [61, 62]. Similarly, though purely speculative for other histological subtypes of ovarian carcinomas such as clear cell or endometrioid, the presence of follicles also may constrain the transformation of the precursor cells of other ovarian cancer histological subtypes, which may arise within or implant onto the ovary.

The follicle depletion hypothesis predicts that follicle preservation and delaying reproductive aging may prevent or decrease the risk of developing ovarian cancer, and several possible agents such as cyclooxygenase inhibitors and progestin may be explored. Also, the idea implies a local follicle-epithelial cell regulation in maintenance of homeostasis and suppressing neoplastic growth, which will need further experimental evidence for verification.

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## Disclosure of conflict of interest

The authors declare no conflict of interest.

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