Universitat de Girona
Facultat de Medicina

LONG-TERM EFFECTS OF OPPORTUNISTIC SALPINGECTOMY ON THE OVARIAN FUNCTION : A PROSPECTIVE COHORT STUDY

Final Degree Project

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" There are no incurable diseases- only the lack of will. There are no worthless herbs- only the lack of knowledge." Ibn Sina

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1. ABREVIATIONS

AFC: antral follicle count

AMH: anti-Müllerian hormone

CCCT: Citrate clomiphene challenge test

FSH: follicle-stimulating hormone

FTE: Fallopian tube epithelium

GnRH: gonadotropin-releasing hormone

HGSC: High grade serous carcinoma

LH: luteinizing hormone

PSV : Peak Systolic Velocity

OBS : Opportunistic bilateral salpingectomy

OSE: Ovarian surface epithelium

RRSO: Risk-Reducing salpingo-oophorectomy

PSV : Peak Systolic Velocity

STICs: Serous tubal intraepithelial carcinoma

2. ABSTRACT

Background

Ovarian cancer is the most lethal gynecologic malignancy. To date, no effective screening methods have been established to prevent this disease. Bilateral prophylactic salpingo-oophorectomy is indicated in high-risk women, who present mutations related to ovarian cancer. Due to the morbidity associated with the resection of the ovaries, this prevention method is not suitable for low-risk women. Opportunistic salpingectomy is favorable to salpingo-oophorectomy, as it avoids health risks associated with premature menopause after oophorectomy. Salpingectomy has been previously demonstrated to reduce the risk of ovarian cancer. However, whether salpingectomy affects ovarian function or not is controversial.

Objectives

Our main objective is to evaluate wether opportunistic salpingectomy has long-term effects on the ovarian reserve or not.

Study design

Our study is a multicentre prospective cohort study, with a consecutive method of sampling of patients attending Gynecology and Obstetrics Department of four hospitals in the Province of Girona.

Population

The study population will be women attending Gynecology and Obstetrics department, with benign pathology. A consecutive non-probabilistic method of sampling will be used to recruit the 452 patients needed. These patients will be divided into 2 groups, patients with indication of pelvic surgery will be considered as “exposed” and patients with no surgery indication will be considered as “not exposed”.

Methods

The main variable of this study is the ovarian function. We will compare AMH levels and AFC between our study group and our control group. In the case of our study group, both transvaginal ultrasound and blood sample obtention for AMH will be performed prior to surgery and 2 years after surgery. Regarding our control group, we will perform the same tests, in the same time interval.

Keywords

Opportunistic salpingectomy; ovarian reserve; ovarian cancer; STICs; precursor lesions

3. BACKGROUND

3.1 THE OVARY

3.1.1 ANATOMY OF THE OVARY

Macrostructure

The ovaries are glands that lie on each side of the uterus, suspended in the pelvic cavity by a double fold of peritoneum, the mesovarium, which is attached to the upper limit of the posterior aspect of the broad uterine ligament. Their average dimensions are 4x2x3 cm in reproductively mature women.

The lateral surface of the ovary contacts parietal peritoneum in the ovarian fossa. Behind the ovarian fossa are retroperitoneal structures, including the ureter, internal iliac vessels, obturator vessels and nerve, and the origin of the uterine artery. The medial surface faces the uterus and uterine vessels in the broad ligament. Above the superior extremity are the fimbria and distal section of the uterine tube. The mesosalpinx lies below the fallopian tube.

The peritoneal and ligamentous supports of the ovary consist of the unfundibulopelvic ligaments, ovarian ligaments and the mesovarium.

- The suspensory or infundibulopelvic ligament of the ovary is a peritoneal fold attached to the upper part of the lateral surface of the ovary, which contain the ovarian vessels and nerves.
- The ovarian ligament attaches the uterine extremity of the ovary to the lateral angle of the uterus, posteroinferior to the fallopian tube.
- The **mesovarium** is a short peritoneal fold that attaches the ovary to the back of the broad ligament. It carries blood vessels and nerves to the ovarian hilum.

The ovarian arteries are branches of the abdominal aorta and originate below the renal arteries. Each descends behind the peritoneum and enter the true pelvic cavity. Here, the

artery turns medially in the ovarian suspensory ligament and splits into **a branch to the mesovarium** that supplies the ovary, and **a branch** that continues into the uterine broad ligament, below the fallopian tube, and **supplies the tube**.[1]

Microstructure

The surface epithelium of the ovary consists of a single layer of cuboidal cells, which contains some flatter cells. Immediately beneath the epithelium, there is a tough collagenous coat, the tunica albuginea. The ovarian tissue it surrounds is divisible into a cortex, containing the ovarian follicles, and a medulla, which receives the ovarian vessels and nerves at the hilum.

Ovarian cortex

The cortex forms the major part of the ovary, enclosing the medulla, except at the hilum. It contains the ovarian follicles at various stages of development, and *corpora lutea* and their degenerative remnants, depending on age or stage of the menstrual cycle.

Ovarian follicles

Primary follicles develop from primordial follicles. Stroma cells surrounding the follicle begin to differentiate into spindle-shaped cells, which constitute the theca folliculi that will become the theca interna. They are later surrounded by a more fibrous layer, the theca externa.

At the same time, the oocyte increases in size and secrets a thick layer called the zona pellucida, which is important for the process of fertilization. The follicular cells continue to proliferate and so the thickness of the late primary follicle wall increases.

Secondary or antral follicles develop from primary follicles. The number of granulosa cells continues to increase. Cavities begin to form between them and are filled with a fluid containing hyaluronate, growth factors, and steroid hormones, secreted by the granulosa cells. As follicles matures, the theca interna becomes more prominent and its cells produce androstenedione from which the granulosa cells synthesize estrogens. Follicular development is stimulated by follicle-stimulating hormone (FSH).

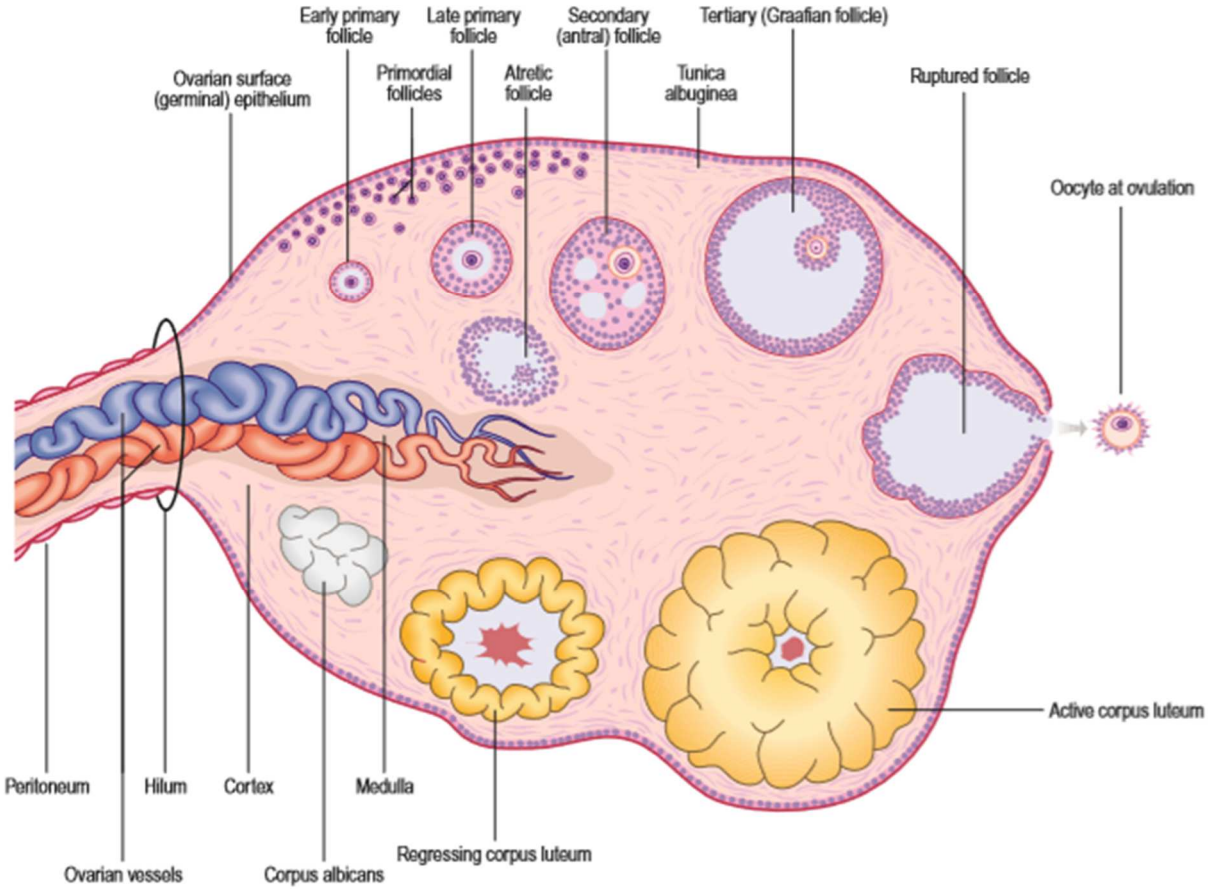


Figure 1. Stages of the folliculogenesis[1]

Although a number of follicles may progress to the secondary stage by about the first week of a menstrual cycle, usually only one *tertiary follicle* develops and increases in size. This follicle moves to the superficial cortex, ruptures and releases its contents into the peritoneal cavity for capture by the fimbria of the fallopian tube.

The remaining follicles become atretic and constitute the corpus luteum. The main function of the corpus luteum is the synthesis and release of progesterone and estradiol. Granulosa and Theca lutein cells respond to FSH and LH respectively. Theca lutein cells also present receptors for β-HCG.[1,2]

Ovarian medulla

The medulla is highly vascular. It contains numerous veins and spiral arteries that enter the hilum from mesovarium and lie within a loose connective tissue stroma.[1]

3.1.2 HYPOTHALAMIC-PITUITARY-OVARIAN AXIS

The female hormonal system consists of three hierarchies of hormones, as follows :

1. An hypothalamic releasing hormone, gonadotropin-releasing hormone (GnRH).

2. The anterior pituitary sex hormones, follicle-stimulating (FSH) and luteinizing hormone (LH), both which are secreted in response to the release of GnRH from the hypothalamus.

3. The ovarian hormones, estrogen and progesterone, which are secreted by both ovaries in response to FSH and LH.[3]

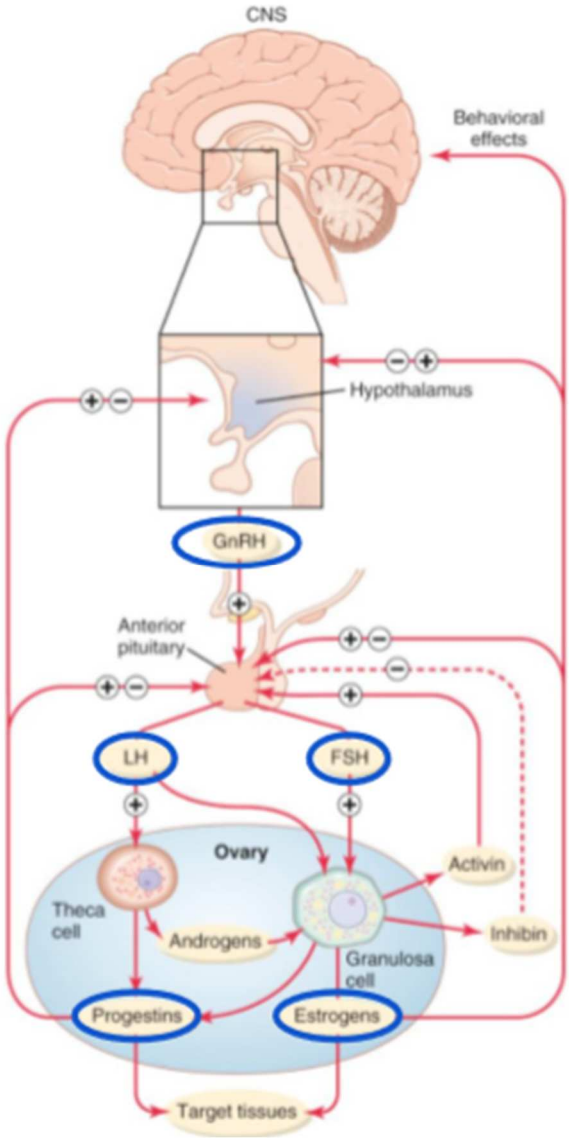
The GnRH pulse generator is the primary structure that drives the menstrual cycle. In the absence of a functional GnRH pulse generator, the gonadotropes remain unestimulated and the ovaries dormant.

LH and FSH are also released in a pulsatile rather than a continuous fashion. Each pulse consists of the abrupt release of the hormone from the gonadotrope into the peripheral circulation.[4,5]

Both gonadotropins act on the ovaries stimulating their ovarian target cells by combining with highly specific receptors, to induce morphologic changes and ovarian steroid action. Morphologic processes include folliculogenesis and the formation of the corpus luteum. These processes occur in sequence conferring a monthly rhythm to the reproductive cycle.

Granulosa and thecal cells within the follicle and luteal cells respond to LH by synthesizing and releasing ovarian steroids, mainly estradiol 17 β and progesterone.[3]

Figure 2. Feedback of the Hypothalamo-Pituitary-Ovarian axis.[3]



Feedback communication between the ovaries and the hypothalamic-pituitary unit is an essential component to the physiology of the reproductive cycle. It is important for the brain to modulate their secretion in response to the activity status of the ovary. Estradiol and progesterone play a major role in these feedback communications.

The major feedback loop is inhibitory whereby the steroid secreted by the ovary regulates the hypothalamic-hypophyseal unit to adjust GnRH and gonadotropin secretion. Estradiol in small amounts is a potent physiologic inhibitor of GnRH and gonadotropin secretion. Its feedback effects operate mainly on the anterior pituitary gland, and to a lesser extent on the hypothalamus to decrease secretion of GnRH, especially by altering the frequency of the GnRH pulses.[5]

In addition to estradiol, inhibin, a hormone secreted by the granulosa cells of the corpus luteum, inhibits the secretion of FSH and LH.[3]

Estrogens and progestins exert both negative and positive feedback effects on the anterior pituitary, depending on stage of the ovarian cycle. The positive feedback effect of estrogen before ovulation causes an increased secretion of LH which conduces to ovulation.[5]

3.1.3 THE OVARIAN CYCLE

The ovarian cycle can be divided into the follicular phase, the ovulation and the luteal phase. Figure 3 depicts the stages of the female sexual cycle .

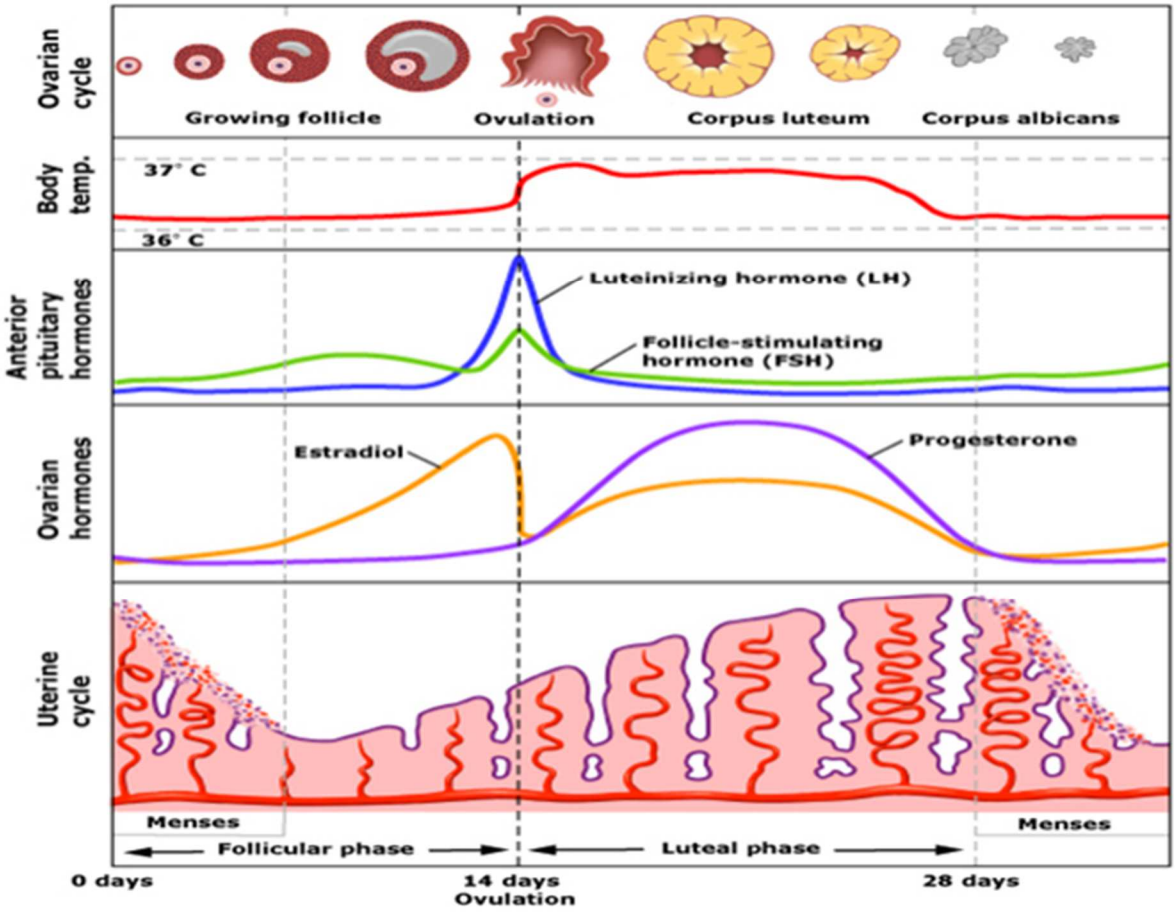


Figure 3. The reproductive cycle.

When a female child is born, each ovum is surrounded by a single layer of granulosa cells; the ovum, with this granulosa cell sheath is called a primordial follicle. After puberty, when FSH and LH begin to be secreted in significant quantities, the ovaries, and the follicles within them begin to grow. The first stage of follicular growth is the enlargement of the ovum, which increases in diameter. Then follows growth of additional layers of granulosa cells in some of the follicles which are known as primary follicles.[3]

Follicular phase

During the first days of each female sexual cycle, the concentrations of FSH and LH increase slightly to moderately and cause accelerated growth of 6 to 12 primary follicles each month.

FSH stimulates mitosis and activates aromatase production in granulosa cells, allowing for growth and increased local estradiol production.

Although several follicles are recruited in the early follicular phase, only one continues into a mature preovulatory follicle. This is referred to as the selection process, which ocurs early in the midfollicular phase.

The selected dominant follicle has a morphologic hallmark which is the acquisition of LH receptors in response to FSH action and the differentiation of an endocrinally active theca layer capable of synthesizing androgens in response to LH stimulation.

These androgens are aromatized to estradiol in the granulosa cell layer, which contains aromatase.

Serum estradiol levels begin to rise as a result of the emergence of the dominant follicle. The **rising estradiol**, through the negative feedback loop, **suppresses FSH levels** to concentrations that are too low to sustain maturation of the remaining follicles with the consequence that these undergo final atresia.

The process of atresia is important because it normally allows only one of the follicles to grow large enough each month to ovulate.

In the late follicular phase, the diameter of the selected follicle increases exponentially, and as a result secretion of estradiol increases exponentially. The increased estrogen milieu in the late follicular phase modifies the genital tract.

As the dominant follicle approaches maturity, estradiol secretion reaches its peak. This peak acts as the crucial ovarian signal that triggers the ovulatory gonadotropin surge (Positive feedback loop)

At the time of ovulation, the mature follicle reaches a diameter of 1 to 1.5 centimetres.[3,4,5]

Ovulation

In a woman who has a 28-day sexual cycle, ovulation occurs 14 days after the onset of menstruation. The estradiol-induced LH surge initiates a chain of events that culminates in follicular rupture and ovulation. Hormonally, drastic changes in ovarian steroid profiles occur after alterations of enzymatic activity. The large increase in LH inhibits androgen production, and as a result estradiol concentrations decrease drastically from the preovulatory peak.[3]

Luteal phase

After ovulation, a new ovarian structure emerges, the corpus luteum. The remaining granulosa and theca interna cells change rapidly into lutein cells. They enlarge in diameter and become filled with lipid inclusions that give them a yellowish appearance.

The granulosa cells form large amounts of the female sex hormones progesterone and estrogen (more progesterone than estrogen).

The theca cells form mainly the androgens androstendione and testosterone rather than female sex hormones. However, most of these hormones are converted by the aromatase into estrogens.

The corpus luteum grows during 7-8 after ovulation. Then, it begins to involute and loses its secretory function about 12 days after ovulation, becoming the corpus albicans, which is replaced by connective tissue and over months is absorved.[3]

3.1.4 EVALUATION OF OVARIAN RESERVE

As women age, their ability to produce oocytes of good quantity and quality decreases. This decline in reproductive function, constituted by both the attenuated size and quality of the primordial follicle pool, has been related to chronological or biological age, which represents the ovarian reserve and its response to stimulation. The decline in number of primordial

follicles determines the onset of menopause. Once the pool is exhausted, growing follicles can no longer be recruited, resulting in menopause.[6,7]

Direct measurement of the primordial follicle pool is not possible. However, the number of growing follicles is proportionally related to the size of the primordial follicle pool.

Although oocyte number and quality decline with age, fertility varies significantly among women of similar age. The concept of ovarian reserve views reproductive potential as a function of the number and quality of remaining oocytes. Ovarian reserve is determined by the interaction of three factors : the *initial follicular pool*, its *atresia rhythm* and *extrinsic factors* which could affect these two circumstances. Ovarian reserve decline is a physiological process, continuous and irreversible, that involves a high ethnic, familiar and inidvidual variability.[8,9]

A number of tests involving biochemical measures (AMH, basal FSH, CCT, estradiol, inhibin B) and ovarian imaging (AFC and ovarian volume), collectively known as ovarian reserve tests, have been proposed to help predict ovarian reserve.[8]

Anti-Müllerian hormone

Anti-Müllerian hormone has long been known for its involvement in the sexual differentiation of the male embryo. AMH is a member of the transforming growth factor β superfamily, which is expressed in granulosa cells as soon as follicles are recruited from the primordial follicle pool. Expression is higher in preantral and antral follicles (4mm) and is nearly lost in follicles larger than 8 mm.

The finding that AMH is expressed in growing follicles prior to FSH-dependent selection, suggested that AMH constitutes an intra-ovarian marker of the number of growing follicles, and thereby indirectly for the quantitative aspect of the ovarian reserve.

In adult women, serum AMH levels decline with increasing age to undetectable levels after menopause. AMH levels correlate strongly with the AFC, but also with other markers of ovarian aging such as FSH and inhibin B on cycle day 3.[6]

It is now agreed that circulating **AMH is considered the most reliable test** as it has been found to correlate well with the histological count of ovarian follicles. Furthermore, the stability of serum AMH level throughout the menstrual cycle with minimal variations makes it an ideal marker for detecting relatively small changes in ovarian reserve following salpingectomy.[10]

There is no consensus on the threshold value suggestive of reduced fertility potential. Interpretation of AMH levels is laboratory essay-dependent, and there is no international standard. Clinicians should be guided by their own laboratory's reference ranges.[11]

FSH

Basal FSH determination has been, during a long period, the most used test to determine the ovarian reserve. Although its analytical quantification is satisfactory, FSH presents a significant inter- and intra-cycle variability that limits its reliability.[9]

Basal serum FSH concentrations increase on day 2,3 or 4 of the menstrual cycle with advancing reproductive age.[8]

FSH presents high specificity (83-100%) for predicting poor response to stimulation (defined as <2-3 follicles) using multiple cutpoints above 10 IU/L (10-20 IU/L). However, sensitivity for identifying women who will respond poorly varies widely (10-80%).[9]

The test still is clinically useful, because one can be fairly certain that women having an abnormally elevated FSH value will have decresed ovarian reserve.

The PPV of FSH for poor response to ovarian stimulation or failure to conceive is higher in older women.

Whereas consistently elevated FSH concentrations confer a poor prognosis, a single elevated FSH value in women <40 years of age may not predict a poor response to stimulation or failure to achieve pregnancy.[8]

In conclusion, a single FSH value has very limited reliability because of inter- and intra-cycle variability. An elevated FSH value has good specificity but may represent a false negative, especially when used in a low-risk population. FSH can be used as a screening test, but not as a diagnostic test.

Estradiol

Basal estradiol during the follicular phase has poor reliability due to its high inter- and intra-cycle variability.[8] Although on day 2-4 of the ovarian cycle estradiol value is usually <50 pg/ml, a value of 60-80 pg/ml could be indicating decreasing ovarian reserve.[9] Basal estradiol alone should not be used to screen for diminished ovarian reserve (DOR). The test has value only as an aid to correct interpretation of a "normal" basal serum FSH value. An early rise in serum estradiol concentrations is a classic characteristic of reproductive aging and can lower an otherwise elevated basal FSH level into the normal range, thereby causing a misinterpretation of the test.[8]

Citrate clomiphene challenge test

Citrate clomiphene test is a provocative test for measurement of FSH which involves oral administration of 100 mg clomiphene citrate on cycle days 5 through 9 with measurement of day 3 and day 10 FSH levels and day 3 estradiol level.

The premise of this test is that women with good ovarian reserve have sufficient production of ovarian hormones from small follicles early in the menstrual cycle to maintain FSH at a low level. In contrast, women with a reduced pool of follicles and oocytes have insufficient production of ovarian hormones to provide normal inhibition of pituitary secretion of FSH, so FSH rises early in the cycle.[11]

CCCT presents a high inter- and intra-cycle variability. Although its specificity and sensibility ranges are slightly higher than those of basal FSH, the CCCT does not clearly improve test accuracy for predicting poor ovarian response.[7,8,9]

In conclusion, basal measures of FSH are preferable to the CCT.

Antral follicle count

Antral follicle count is the sum of antral follicles in both ovaries, as observed with transvaginal ultrasonography during the early follicular phase. Most studies have defined antral follicles as those measuring 2-10 mm in mean diameter, however, some have defined antral follicles as those measuring 2-5 mm. Despite the diameter difference, most studies have shown similar results.[9]

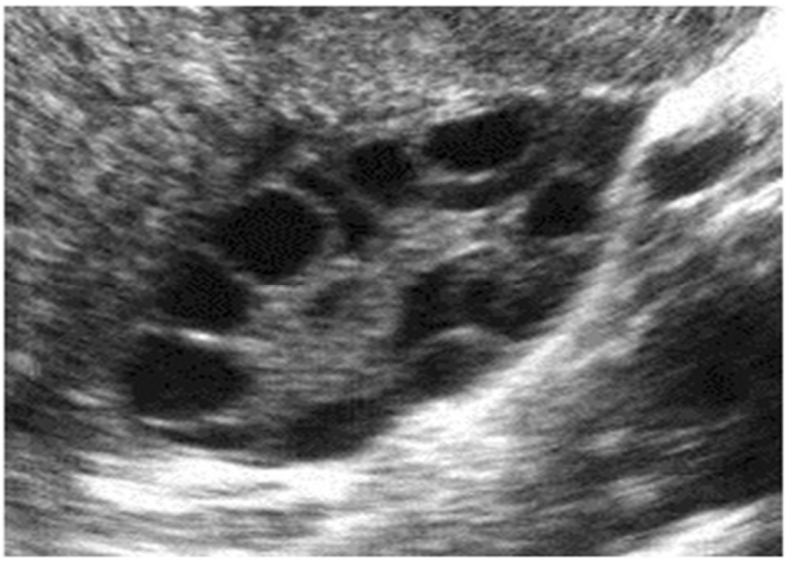


Figure 4. Ultrasound image to determine AFC.[37]

A limitation of AFC regarding AMH, is the fact that its assessment has to be made on a specific day of the ovarian cycle and without the use of oral contraceptive methods. Moreover, its estimation could be harder with the presence of endometriosis or ovarian cysts. Inter- and intra-observer variability may also be limiting, especially in centers having less expertise or lower-quality ultrasound equipment.[8]

The antral follicle count has good predictive values in determining the ovarian reserve, and is superior to FSH in earlier predicting the ovarian reserve.[7]

Inhibin B

Inhibin B levels rise with GnRH or FSH stimulation and therefore exhibit high intra- and inter-cyle variability. The routine use of inhibin B as a measure of ovarian reserve is not recommended (evidence level III, recommendation degree C).[9]

Ovarian volume

Ovarian volume is calculated by measuring each ovary in three planes and using the formula for the volume of an ellipsoid (D1 x D2 x D3 x 0.52 = volume). Mean ovarian volume is the average volume of both ovaries in the same individual. Overall ovarian volume correlates with number of follicles and retrieved oocytes. Studies of ovarian volume have often excluded patients with ovarian pathology, including those with POS, endometriomas and large cysts. Thus, the generalizability is limited.[8]

In conclusion, AFC is a better imaging test to screen for DOR than ovarian volume.

3.2 OPPORTUNISTIC SALPINGECTOMY

Ovarian cancer is still the most frequent cause of death by gynecological malignancy for women in developed countries. To date, all attempted ovarian cancer screening strategies have failed.[12] One of the most important finding of gynecologic oncology in the last decade is the confirmed theory that serous tumors derive from the epithelium of the Fallopian tube, whereas clear cell and endometrioid tumors derive from endometrial tissue that migrate to the ovary by retrograde menstruation.[12,13,14] This is in contrast to the traditional view of ovarian carcinogenesis in which ovarian surface epithelium undergoes metaplastic changes leading to different histologic types of epithelial ovarian cancer.[13] These observations are mainly collected from women that carry BRCA1/2 mutations and underwent prophylactic salpingo-oophorectomy, in which most of the incidentally diagnosed in situ carcinomas or intraepithelial precoursors of cancer (STIC) were detected not in the ovary but in the fimbrial end of the fallopian tube.

As a matter of fact, in the general population, opportunistic salpingectomy might reduce the risk of sporadic ovarian cancer reducing at the same time the risk of premature death due to cardiovascular disease seen in women subjected to salpingo-oophorectomy before the onset of natural menopause.[12,14]

However there has been skepticism about the safety and absolute benefit of this practice as there are no long-term studies confirming that salpingectomy does not compromise ovarian function.[14]

Nevertheless, the available evidence so far suggests that opportunistic salpingectomy is safe, and likely to be effective and cost-effective as an ovarian cancer prevention strategy.

Salpingectomy does not appear to be associated with significant perioperative risks. The average additional operating room time required for salpingectomy was 16 minutes when added to hysterectomy, and 10 minutes when done instead of tubal ligation. While these differences are statistically significant, they are arguably not clinically significant.[13]

Opportunistic salpingectomy is performed at the time of hysterectomy (benign gynecologic disease) or instead of tubal ligation, as a sterilization method. The preservation of the Fallopian tubes during hysterectomy is a surgical strategy with no known benefits and conversely, the blind-ended remnants may give rise to complications, such as hydrosalpinx, which often requires recurring surgeries.

Preservation of the ovarian function is important both in the pre-menopausal age and in the post-menopause, due to the effective prevention of bone resorption, guaranteed by the intact ovaries. Furthermore, surgical menopause increases long-term risk of psychosexual, cognitive and non-fatal coronary diseases.[12]

Ovarian failure is accelerated after hysterectomy even without salpingectomy. Menopause occurs in 20% of women within 5 years of hysterectomy, or about 4 years earlier than without hysterectomy. One of the theories is that hysterectomy affects blood flow to the ovaries, which could compromise their function.[14]

During salpingectomy, a repercussion on the ovarian blood flow could be prevented using a surgical technique that carefully preserves the ovarian blood supply. This was the case of a study done by Morelli et *al*.[12], in which the ovarian function and surgical outcomes were compared between patients affected by benign uterine pathologies submitted to total laparoscopic hysterectomy (TLH) plus salpingectomy and women in which standard TLH with adnexal preservation was performed. Ovarian reserve modification was expressed as the difference between 3 months post-operative and pre-operative values of AMH, FSH, AFC, mean ovarian diameters and Peak Systolic Velocity (PSV).

Operative time, variation of hemoglobin level, postoperative hospital stay, postoperative return to normal activity and complication rate were also recorded as secondary outcomes. The study showed promising results, as no significant difference was observed between the two groups with respect to the ovarian reserve. The explanation of these positive results may be the attention that was given to the ovarian blood supply, which was ensured by an arcade-like anastomosis system formed by the ovarian artery and the tubal artery at the junction of the mesosalpinx and the mesovary. In addition, secondary outcomes (surgical complications) did not show any significant difference.[12]

More studies have been conducted to evaluate the possible effects of opportunistic salpingectomy on the ovary. However, the vast majority of these studies only investigated short-term effects on the ovarian function. A recent meta-analysis of eight studies that analysed changes in serum AMH levels following salpingectomy, yielded good results, as there was no statistically significant change in serum AMH concentration after salpingectomy. This meta-analysis concluded that salpingectomy did not seem to compromise ovarian reserve in the short-term, although the long-term effect remains uncertain and must be taken into account.[13]

Preliminary data on the effects of opportunistic bilateral salpingectomy show that postoperative ovarian function is preserved up to 3 months after surgery.[15] A study conducted by Chan et *al*. on the short-term (3 months) effects of salpingectomy for ectopic pregnancy on the ovarian blood supply found no differences on the ipsilateral side of salpingectomy when compared to the non-operated side. The study included both laparoscopic and laparotomic

approach. However, when they analyzed only patients with laparoscopic salpingectomy, the markers (AFC and ovarian blood flow) were significantly reduced. A possible explanation for this dicrepance is the fact that in each approach, a different mesosalpinx cauterization method was used.[16]

Another study, this time comparing ovarian reserve following C-section with salpingectomy and tubal ligation, presented promising results. The aim of this study was to compare preoperative and post-operative AMH levels between the two groups (C-section + salpingectomy, and tubal ligation). This trial demonstrated that salpingectomy did not affect the ovarian reserve up to 6-8 weeks after surgery.[17]

A study conducted by Venturella et *al*.[18] to estimate the long-term effects of salpingectomy on the ovarian reserve, using OvAge, a statistical model that combines AMH, FSH, 3D AFC, vascular index, flow index, and vascular flow index values, concluded that the addition of OBS to TLH did not modify the ovarian age of treated women up to 3 to 5 years after surgery. However, women included in this study were in their late reproductive years (mean age 49.61 +- 2.15 years), therefore, it would not be proper to extrapolate this data to the general population.

The confirmation of OBS safety on ovarian function even many years after surgery is essential to reassure the medical community that this new strategy for ovarian cancer prevention, is at least able to avoid the risk of premature surgical menopause.

The absolut benefit from opportunistic salpingectomy is estimated at a number needed to treat (NNT) of 273 to prevent one case of ovarian cancer during hysterectomy, and NNT of 366 for surgical sterilization. While these NNT estimates seem high, they are comparable to the NNT of 324 to prevent one case of cervical cancer with human papilloma virus vaccination, which reflect the relatively low incidence of both ovarian and cervical cancers in our population.[19]

If opportunistic salpingectomy could reduce the number of ovarian cancer cases compared to hysterectomy alone or tubal ligation, this would in turn reduce future health care costs associated with ovarian cancer treatment.

3.3 SEROUS TUBAL INTRAEPITHELIAL CARCINOMAS

Detailed histopathological examination of tubal epithelia in BRCA mutation carriers undergoing risk-reducing surgery led to the discovery of putative cancer precursor lesions in the fallopian tube referred to as serous tubal intraepithelial carcinoma (STIC).[21]

STICs are characterized by a proliferation of non-ciliated epithelium showing nuclear stratification, marked nuclear pleomorphism, prominent nucleoli, and mitotic figures. Inmunohistochemical staining demonstrates **aberrant p53** protein expression (either diffuse nuclear overexpression or complete absence of staining) and an **increased Ki-67** proliferation index in the lesional epithelium.[21]

STIC was first reported by Piek *et al*., who described dysplastic epithelial changes in the fallopian tubes of women with BRCA1 and BRCA2 mutation, who underwent risk-reducing salpingo-oophorectomies (RRSO).

Many studies have now reported the incidence of STIC in the distal fallopian tube. It is estimated that occult invasive and STIC are identified in 0.9-8.5% of women undergoing RRSO (TABLE 1).[20]

The degree of protection of salpingectomy depends, in large part, on the fidelity of the linkage between STIC and high-grade serous tumors. Not all STICs result in invasive cancers, and not all serous tumors have a tubal origin. Better studies using novel methods to determine p53 signatures and the presence of alternative accusative pathways will be needed, such as secretory cell outgrowths (SCOUTS) which are associated with altered PAX2 expression.[22]

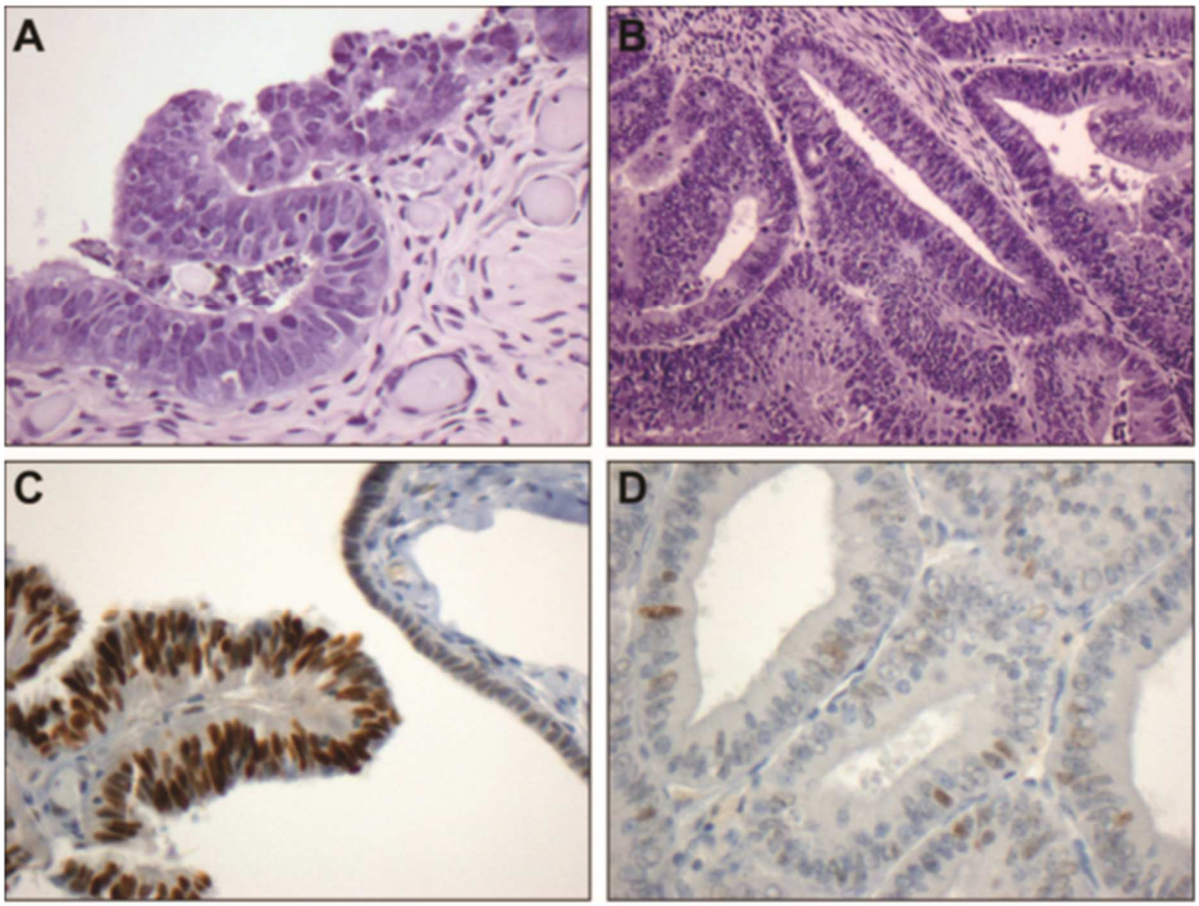


Figure 5. *Hematoxylin-eosin-stained sections of STIC-like lesion in fallopian tube (A), with concomitant endometrioid carcinoma (B). p53 overexpression (C) absence of p53 overexpression (D)*[21]

The exact percentage of ovarian tumors arising from the Fallopian tube is unknown. Some estimates set the association at up to half of these tumors, but recent reports have estimated a much higher proportion. Przybycin et *al*. determined the frequency of STIC in 114 non-uterine gynecologic cancers and determined that STIC was confirmed in 59% of the high-grade serous tumors, with 92% of the lesions found in the fimbriated portion of the tube and the remaining 8% in the ampullary region.[22]

There are currently no standarized criteria for diagnosis of STIC lesions, as the histopathological spectrum is very wide and no two STICs appear exactly the same. However, STIC is traditionally diagnosed with a combination of histopathologic and morphologic evaluation with inmunohistochemical staining.

There is no current consensus among gynecologic oncologists regarding appropriate management for incidental findings of STIC. Proposed management strategies of STIC include close surveillance, surgical staging, or empiric adjuvant chemotherapy.

Close surveillance could be performed with annual review of systems, pelvic exam, CA-125, and/or imaging.

The value of surgical staging may be low, but performing a pelvic washing at the time of RRSO may be beneficial in evaluating for spread of disease. As positive peritoneal washings indicate the presence of circulating premalignant cells in the peritoneal cavity, it may be reasonable to offer patients with positive peritoneal cytology empiric chemotherapy.

For women with only STIC in the absence of positive washings or evidence of malignant spread, empiric adjuvant chemotherapy similar to what would be recommended for stage I ovarian or fallopian tube cancer (3-6 cycles of paclitaxel/carboplatin chemotherapy) could be given.

However, given the possibility of adverse effects, a risk-benefit ratio should be performed between the physician and patient prior to initiating chemotherapy.[23]

REFERENCE	Number of RRSO cases	Incidence of STIC or occult carcinoma in the distal end of the fallopian tube
Colgan et al.	60	5 (8.3%)
Piek et al.	12	5 (41.6%)
Leeper et al.	30	3 (10%)
Powell et al.	67	4 (6%)
Carcangiu et al.	50	4 (8%)
Finch et al.	159	7 (4.4%)
Callahan et al.	122	7 (5.7%)
Shaw et al.	176	15 (8%)
Hirst et al.	45	4 (8.9%)
Powell et al.	111	6 (5.4%)
Manchanda et al.	117	10 (8.5%)
Mingels et al.	226	16 (7.1%)
Reitsma et al.	303	3 (0.99%)
Wethington et al.	593	12 (2%)
Cass et al.	78	9 (11.5%)
Sherman et al.	966	25 (2.6%)

Table 1. STIC incidence in fallopian tubes of women with BRCA1/2 mutations.[20]

3.3 OVARIAN CANCER

Ovarian cancer is a neoplasm that forms in or on an ovary. It results in abnormal cells that have the ability to invade or spread to other parts of the body. Most ovarian cancers are either ovarian epithelial cancers or malignant germ cell tumors. Fallopian tube cancer and primary peritoneal cancer are similar to ovarian epithelial cancer and are staged the same way.[24,25]

3.3.1. EPIDEMIOLOGY

Although ovarian cancer has a lifetime risk of only 1.5% in the general population, it is the fifth-leading cause of cancer-related deaths in women and the most lethal gynecologic cancer. It has a mediocre survival rate, as only 40% of patients survive five years after the diagnosis.[26,27,28]

Around 3300 cases are diagnosed each year in Spain (3412 cases in 2017), wich represents 5,1% of all cancers in women. It is the fifth most commonly diagnosed cancer in women, after breast, colorectal, endometrial and lung cancer.[27] More than 75% of affected women are diagnosed at an advanced stage because early-stage disease is usually asymptomatic, and when there is a clinical manifestation of the cancer, the symptoms are non-specific.[26] Consequently, ovarian neoplasms account for a disproportionate number of deaths from cancer of the female genital tract. This makes the prevention and early screening of this disease a challenging task.

Ovarian cancer affects women of all ages but it is usually diagnosed in women between 45-65 years old, eventhough, this range of age can vary depending on the hystological type.[29]

There are many types of ovarian malignant neoplasm, according to the histology of the tumor. About 90% of tumors are epithelial ovarian cancers that occur primarily in postmenopausal women. Germ cell tumors, which occur primarily in women in their early 20s, comprise 5% of tumors, and sex cord–stromal tumors, which secrete sex steroids can occur at any age (most commonly in a patient’s 50s).[26]

3.3.2 ETIOLOGY

Many risk factors have been associated with ovarian cancer. Familial genetic syndromes are the strongest known risk factors, accounting for about 10% to 12% of ovarian cancers. BRCA gene mutations are involved in about 10% of cases of ovarian cancer, and hereditary nonpolyposis colorectal cancer (Lynch syndrome) is involved in 2% to 3% of cases. (Table 2)[26]

Syndrome	*Gene mutations*	*Features/epidemiology*	*Lifetime OC risk*
Hereditary breast And ovarian cancer Syndrome	BRCA1 and BRCA2 tumor suppresors, possibly others	10 times more common in Ashkenazi Jews; associated with breast, ovarian, fallopian tube, peritoneal, and pancreatic cancers	BRCA1 : 25% to 65%
Hereditary nonpolyposis Colorectal cancer (Lynch syndrome)	MLH1, MLH3, MSH2, MSH6, TGFBR2, PMS1, and PMS2	Increased risk of colon cancer, as well as endometrial and ovarian cancers	10%
MUTYH-associated Polyposis	MUTYH	Polyps in the colon and small intestine; increased risk of colon and other cancers, including ovarian and bladder cancers	No good data available
Peutz-Jeghers syndrome	SKT11	Polyps in the stomach and intestine in teenagers, increased risk of esophageal, stomach, small intestine, and colon cancers, as well as epithelial ovarian cancer and stromal tumors	No good data available
PTEN hamartoma tumor Syndrome	PTEN	Increased risk of thyroid disorders and thyroid, breast and ovarian cancers	No good data available

Table 2. Hereditary syndromes related to ovarian cancer.[26]

BRCA1/BRCA2 tumor suppressor gene mutations are the cause of hereditary breast and ovarian cancer syndrome. These are two tumor-suppressor genes, whose protein products are BRCA1 and BRCA2. These two proteins interact with recombination/DNA repair proteins to preserve intact chromosomal structure. Mutations of BRCA1 and BRCA2 genes lead to these proteins' dysfunction, which results in genetic instability and subject cells to a higher risk of malignant transformation.[30]

The BRCA1 gene is located on chromosome 17q21. Patients with a proven mutation have a dramatically elevated risk of developing ovarian cancer, 39% to 46%. BRCA2 is located on chromosome 13q12 and in general is less likely to lead to ovarian cancer (12% to 20%).[31]

Hereditary breast and ovarian cancer syndrome affects one in 300 to 800 women, but the prevalence may be higher in some ethnic groups as Ashkenazi Jews. In families with a history of ovarian or breast cancer, BRCA mutations are responsible for about 90% of cases of ovarian cancer. Because of incomplete penetrance, however, 35% to 85% of BRCA carriers do not develop ovarian cancer and about 20% to 30% never develop breast cancer.[26] Both genes are inherited in an autosomal dominant fashion, with variable penetrance.[32]

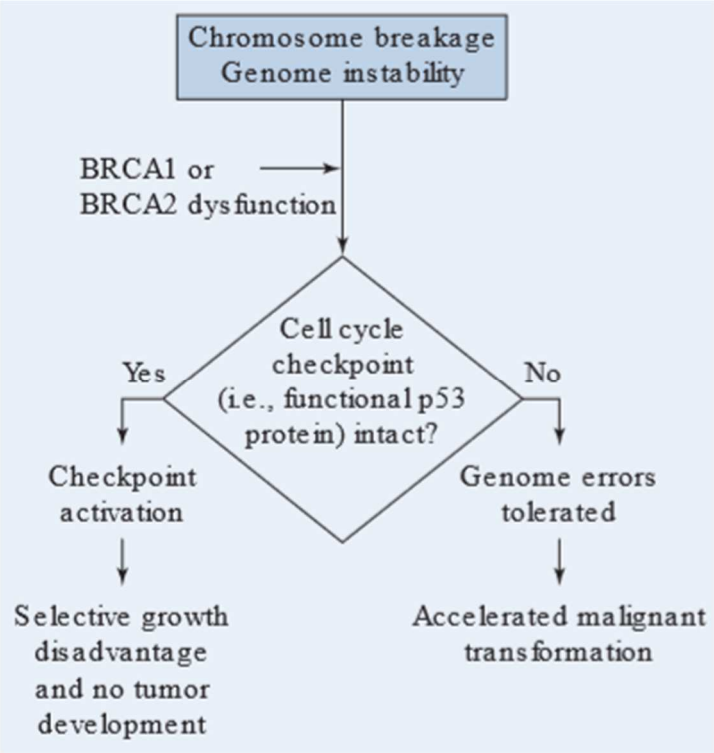


Figure 6. Role of BRCA mutations in tumor development.[31]

TABLE 2. *WOMEN WHO SHOULD UNDERGO GENETIC TESTING*
Epithelial ovarian cáncer
Breast cancer diagnosed at age 45 or younger
Breast cancer with two distinct and sequential primaries, first one diagnosed at age 50 or younger
Breast cancer tat is triple-negative diagnosed at age 60 or younger
Breast cancer at any age, with at least one close relative diagnosed at age 50 or younger
Breast cancer diagnosed at any age, with two or more close relatives with breast cancer, on close relative with EOC, or two close relatives with pancreatic cancer or aggressive prostate cancer
Breast cancer, with a close male relative with breast cancer at any age
Breast cancer and Ashkenazi Jewish ancestry
Individuals from a family with a known deleterious BRCA1 or BRCA2 mutation

Table 2. Women who should undergo genetic testing.[31]

Genetic testing identifies women with deleterious BRCA1 and BRCA2 mutations, leads to intervention with prophylactic surgery, and thereby prevents ovarian cancer.[32]

Almost all reported cases of ovarian carcinomas arising in women with BRCA1 or BRCA2 mutations are high-grade serous carcinomas and commonly have p53 mutations. Close examination to these tumors has suggested that a significant percentage of BRCA1 and BRCA2-related tumors arise form the epithelium lining the fimbriated end of the fallopian tube.[29]

For the other 90% with no identifiable genetic link for their ovarian cancer, most risks are related to a pattern of uninterrupted ovulatory cycles during the reproductive years. Repeated stimulation of the ovarian surface epithelium is hypothesized to lead to malignant transformation.[31]

Nulliparity is associated with long periods of repetitive ovulation, and patients without children have double the risk of developing ovarian cancer. Among nulliparous women, those with a history of infertility have an even higher risk.[33]

TABLE 3. *Risk factors for developing Epithelial Ovarian Cancer*
Nulliparity
Early menarche
Late menopause
White race
Increasing age
Residence in North America and Northern Europe
Family history
Personal history of breast cancer
Ethnic background (European Jewish, Icelandic, Hungarian)
Postmenopausal hormone therapy
Pelvic inflammatory disease

Table 3. Ovarian cancer risk factors. [31]

Early menarche and late menopause are also associated risks. In contrast, breastfeeding has a protective effect, perhaps by prolonging amenorrhea. Presumably by also preventing ovulation, long-term combination oral contraceptive use reduces the risk of ovarian cancer by 50 percent, however, it increases the risk of endometrial and breast cancer. The duration of protection lasts up to 25 years after the last use. In contrast, hormone replacement therapy after menopause has an elevated associated risk.[28,34]

Tubal ligation and hysterectomy are each associated with a substantial reduction in risk. Theoretically, any gynecologic procedure that precludes irritants from reaching the ovaries via ascension from the lower genital tract might plausibly exert a similar protective effect. In turn, women who regularly use perineal talc may possibly have an elevated risk.[28]

3.3.3 OVARIAN NEOPLASMS TYPES

There are numerous types of ovarian tumors, and overall they fall into benign, borderline and malignant categories. About 80% are benign, and these occur mostly in young women between the ages of 20 and 45 years old. Borderline tumors occur at slightly older ages.[29]

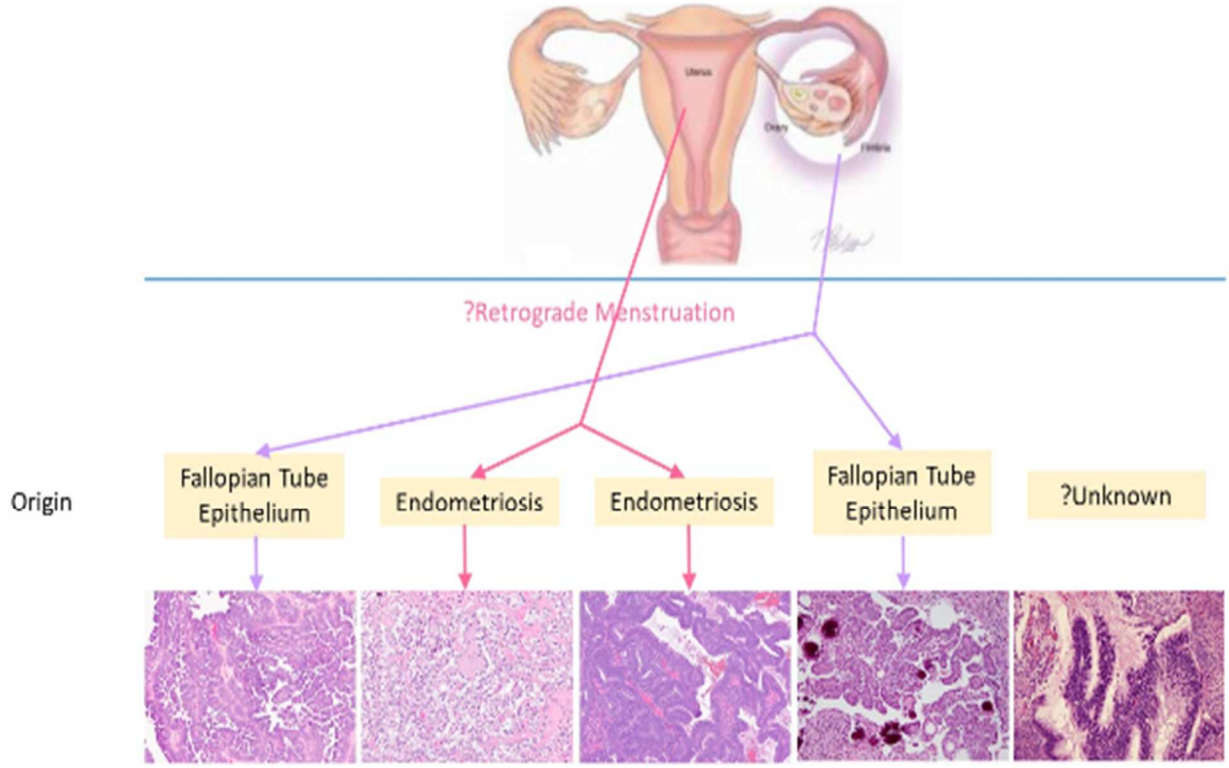
The histological classification of the WHO separates ovarian neoplasms according to the most probable tissue of origin. It was believed that tumors of the ovary arise ultimately from one of three ovarian components; (1) surface epithelium derived from coelomic epithelium; (2) the germ cells, which migrate to the ovary from the yolk sac and are pluripotent; and (3) the stroma of the ovary, including the sex cords, which are forerunners of the endocrine apparatus of the postnatal ovary.[29]

Currently, this theory is losing importance, whereas the **fallopian tube as a site of origin** of ovarian cancer is gaining more weight.[35] There is also a group of tumors that defy classification, and finally there are secondary or metastasic tumors to the ovary.

Some of the specific tumors have distinctive features and are hormonally active, however, most are nonfunctional and tend to produce relatively mild symptoms until they reach a large

size. Malignant tumors have usually spread outside the ovary by the time a definitive diagnosis is made.[29]

There are three main types of ovarian tumors : *epithelial tumors*, *germ-cell tumors* and *sex cord-stromal tumors*. We will focus on epithelial serous carcinoma which is the most common and relevant type to our study.



	High-Grade serous carcinoma	Clear Cell Carcinoma	Endometrioid Carcinoma	Low-Grade Serous Carcinoma	Mucinous Carcinoma
% Of all Ovarian carcinomas	70%	10%	10%	<5%	<5%
Precursor lesions	Serous tubal intraepithelial carcinoma (STIC)	Clear cell borderline tumor	Endometrioid borderline tumor	Serous borderline tumor	Mucinous borderline tumor
Inherited Syndromes	BRCA1/2, Hereditary Breast and Ovarian Cancer (HBOC)	Lymch Syndrome	Lynch Syndrome	?	?
Common mutations and molecular aberrations	TP53 BRCA1/2 and HRD chromosomal instability Aneuploidy (100%)	ARID1A PIK3CA CTNNB1 PPP2R1A MSI	PTEN CTNNB1 ARID1A PPPR2R1A MSI	KRAS BRAF	KRAS HER2 amplification
Potential Molecular Targeted Therapies	PARP inhibitors, inmune checkpoint inhibitors	Tyrosine kinase inhibitors	mTOR inhibitors	MEK1/2 inhibitors	Trastuzumab

Table 4. Main types of ovarian epithelial cancer.[29]

EPITHELIAL OVARIAN TUMORS

Most primary neoplasms in the ovary fall within this category. The classification of epithelial ovarian tumors is based on both differentiation and extent of proliferation of the epithelium.

There are three major histologic types based on the differentiation of the neoplastic epithelium : ***serous****, mucinous and endometrioid tumors.* The extent of epithelial proliferation is associated with the biologic behaviour of the tumor and is classified as **benign** (minimal epithelial proliferation), **borderline** (moderate epithelial proliferation) and **malignant** (marked epithelial proliferation with stromal invasion). The tumors can be relatively small, or they can grow to fill the entire pelvis before they are detected.[29]

Several recent studies suggested that ovarian carcinomas may be broadly categorized into two different types based on pathogenesis: (1) those that arise in association with borderline tumors, and (2) those that arise as “de novo” carcinomas.

Clinicopathological studies have shown that well-differentiated tumors, often contain areas of borderline tumors of the same epithelial cell type, whereas this association is rarely seen for moderately to poorly differentiated serous carcinoma.[29]

Serous tumors

Serous tumors are cystic neoplasms lined by columnar ciliated and non-ciliated epithelial cells and are filled with clear serous fluid. Serous carcinomas account for approximately 40% of all cancers of the ovary and are the most common malignant ovarian tumors. [29]

Based on clinicopathologic and molecular studies, serous ovarian carcinoma can be divided into two major groups : (1) low-grade (well-differentiated) and (2) high grade (moderately to poorly differentiated) carcinoma.

This distinction can be made on the basis of nuclear atypia and correlates with patient survival. It is now widely accepted that high-grade serous carcinomas arise in association with serous tubal intraepithelial carcinoma.[35,36]

Molecular studies of low and high-grade serous carcinoma have revealed dictinct molecular genetic changes in the two types of carcinoma. The low-grade tumors arising in serous borderline tumors have mutations in *KRAS* or *BRAF* oncogenes with only rare mutations in *p53.* In contrast, high-grade tumors have a high frequency of mutations in the *p53* gene but lack mutations in either KRAS or BRAF oncogenes.[29]

High-grade serous carcinoma

It is the most aggressive and most common subtype of EOC, accounting for 75% of cases. Most women present at advanced stages (stage III or IV) at diagnosis, at which point the 5-year survival rate ranges between 20 and 40%. However, for patients with stage I disease, the 5-year survival rate exceeds 90%.[20]

Of the patients diagnosed with a HGSC, 15-20% will have a known germline mutation in the highly penetrant homologous repair pathway genes, BRCA1 and BRCA2. Almost all reported cases of ovarian carcinomas arising in women with BRCA1 and BRCA2 mutations are high-grade carcinomas.[20]

Molecular and genetic data indicate that HGSC of the ovary may have similar origin to HGSC of the fallopian tube and peritoneum, and therefore it has been suggested that all the three be described collectively as HGSC.[25]

Prior to the reported observation of *in situ* carcinoma in the distal end of the fallopian tube of women undergoing prophylactic surgery, the ovary was thought to be the etiological site of high-grade serous ovarian cancer. Now, there are two candidates for the cell of origin, namely, the fallopian tube epithelium (FTE) and the ovarian surface epithelium (OSE). Both share common mesodermal embryological origin and close anatomic proximity.[20]

The "*incessant ovulation"* hypothesis, proposed by Fathalla[34], suggested that continuous ovulatory cycles during the reproductive lifespan of a woman increase her risk of developing HGSC. He proposed that ovulation resulted in an increase in inflammation through which the secretion of cytokines, chemokines, bradykines, and hormones induce DNA damaging *via*

oxidative stress in the cortical inclusion cysts observed in the ovary. These events, along with proliferation of the OSE, promote metaplastic changes leading to neoplastic transformation.

During the last years, abundant histological and molecular evidence has emerged which suggests strongly that the vast majority of ovarian and peritoneal high-grade serous carcinomas probably **originate in the fallopian tube**, specifically from a **STIC**, and therefore implies that the ovarian/peritoneal tumors are metastases.[35]

A series of transcriptional studies by Tone et *al*. and George et *al*. have shown that phenotypically normal fallopian tube epithelia from BRCA1 and BRCA2 mutation carriers show transcriptional differences when compared to epithelial cells with a normal BRCA genotype.

These differences have been shown to impact different molecular pathways. Consequently, these pathways are implicated in tumor initiation, progression, and recurrence. As a result of these studies, the authors proposed that chronic inflammatory states through cyclical ovulation in the presence of a mutated BRCA allele could predispose the normal FTE to undergo neoplastic transformation, which may lead to serous carcinoma.

This would primarily occur through deregulation of DNA damage response genes and synergistically through upregulation of cytokines, proinflammatory and proliferation genes.[20]

The biologic behaviour of serous tumors depends on the degree of differentiation.

Predictably, unencapsulated serous tumors of the ovarian surface are more likely to extend to the peritoneal surfaces, and prognosis is closely related to the histologic appearance of the tumor and its growth pattern on the peritoneum.[29]

Low-grade carcinomas

These tumors can arise in borderline carcinomas and may be associated with “invasive implants”, which demonstrate destructive, infiltrative growth, similar to metastasic

carcinoma. However, low-grade carcinomas, even when spread outside the ovary, often progress slowly, and patients survive for relatively long periods before dying of disease.

In contrast, high-grade tumors are often widely metastasic throughout the abdomen at the time of the diagnosis. Consequently, careful pathologic classification of the tumor, even if it has extended to the peritoneum, is relevant to both prognosis and selection of therapy.[29]

4. JUSTIFICATION

Ovarian cancer is one of the most mortal cancers in women. It is usually diagnosed in advanced stages and most tumors are high grade serous tumors. There are no effective screening methods to detect ovarian cancer, which explains why most of ovarian OC cases are detected in advanced stages and why the survival rate does not exceed 40%.

The new ovarian carcinogenesis theory proposes that premalignant cells in the Fallopian tubes are the precursor of high grade serous ovarian carcinomas, as well as low grade carcinomas. Following the new discoveries in the ovarian carcinogenesis field, many studies were conducted to see if an opportunistic salpingectomy could prevent ovarian cancer in low-risk women.

These studies have showed that the incidence of OC is much lower in the group of women who underwent an opportunistic surgery than in the group of women who did not.

The rising trend in salpingectomy has been associated with a rising concern over its potential damaging effect on ovarian reserve due to possible concomitant damage of ovarian blood supply, given the close proximity of the median ovarian artery and the medial tubal artery at their origins. Preliminary data on the effects of opportunistic bilateral salpingectomy show that postoperative ovarian function is preserved up to 3 months after surgery. The confirmation of OBS safety on ovarian function even many years after surgery is essential to

reassure the medical community that this new strategy for ovarian cancer prevention, is at least able to avoid the risk of premature surgical menopause.

It has been hypothesized that salpingectomy could interrupt ovarian blood supply, thereby compromising ovarian blood flow with a consequent decline in ovarian reserve. This theory is supported by a study of the ovarian blood flow and follicle count following laparoscopic salpingectomy for ectopic pregnancy. This study showed a post-salpingectomy impairment of the ovarian blood flow on the operated side.

The majority of studies that evaluated the possible effects of salpingectomy on the ovarian function concluded that salpingectomy does not seem to compromise ovarian function in the short-term. Nevertheless, the long-term effect of salpingectomy on ovarian reserve remains uncertain.

One recent long-term study revealed no change in circulating AMH up to 5 years after salpingectomy. However, these data are inconclusive due to relatively old age of all participants, with very low AMH levels at baseline.

In conclusion, more long-term studies are needed to assess the possible effects of opportunistic salpingectomy on ovarian function.

5. HYPOTHESES

5.1 MAIN HYPOTHESIS

Opportunistic salpingectomy in women at low risk group, does not have long-term effects on the ovarian function.

6. OBJECTIVES

6.1 MAIN OBJECTIVE

We want to determine if bilateral opportunistic salpingectomy has effects on the ovarian reserve. We will use the Anti-Müllerian hormone and the antral follicle count to compare the ovarian reserve between the study group and control group.

6.2 SECONDARY OBJECTIVE

Detection of serous precursor lesion or inmunohistochemical abnormalities in the resected fallopian tubes, such as serous tubal intraepithelial carcinomas, tubal intraepithelial lesions in transition or TP53 aberrations.

7. METHODOLOGY

7.1 STUDY DESIGN

The study design is a multicentric prospective cohort study, with a consecutive method of sampling of patients attending the Gynecology and Obstetrics department of 4 hospitals. Data will be collected in the OBGYN department of the main hospitals of Girona Province, which are the following :

-*Hospital Universitari Dr Josep Trueta*

-*Hospital de Figueras*

-*Hospital de Palamós*

-*Hospital de Blanes*

7.2 STUDY POPULATION

The study population will include patients with indication of elective pelvian surgery (study group), and patients with no indication of surgery (control group). Both cohorts must fulfil inclusion criteria and none of the exclusion criteria.

-*Study group* : Opportunistic bilateral salpingectomy will be performed in this group of women.

-*Control group* : this is the non-exposed group. We will create a normogram of the ovarian function based on AMH levels and the AFC of these women.

-*Exposure* : Bilateral opportunistic salpingectomy (as a sterilization method, instead of tubal ligation; during a hysterectomy due to benign gynecologic pathology).

Inclusion criteria

- Women aged 18-45 years old
- Regular menstrual cycle
- Presence of the ovaries
- Parous

Exclusion criteria

- Patients with BRCA1/BRCA2 mutations or other mutations linked to ovarian cancer
- Patients with pelvic iflammatory disease
- Patients with tubal pathology
- Patients with malignant gynecologic pathology
- Personal history of infertility
- Polycystic ovary syndrome
- Endocrinologic disease
- Use of oral contraceptive pill over the last 6 months
- Previous salpingectomy or salpingo-oophorectomy

7.3 SAMPLE

Sample size

Accepting an alpha risk of 0.05 and a power of 80% in a two sided-test, we will need 226 patients in each group to achieve a difference statistically significant. A drop-out rate of 15% has been anticipated.

The computations were carried out with Prof. Marc Saez' software based on the library 'pwr' of the free statistical environment R (version 3.5.1).

Sampling method

A non-probabilistic consecutive sampling method will be performed for a year.

The patients will be recruited at the Gynecology and Obstetrics Department of 4 hospitals. Patients of the study group will be informed about the study before surgery. Patients from the

control group will receive information about the diagnostic tests that we will perform. The information document (annex 1 and 2) and the informed consent (annex 3) of the study will be given to all participants. They will only be included in the study if they sign and agree with the conditions of the research.

7.4 DATA COLLECTION

Ovarian reserve

The aim of our study is to compare the ovarian reserve between our study group and our control group. To do this, we will run tests prior to surgery, and 2 years after surgery in both groups.

Antral follicle count

The AFC describes the total number of follicles measuring 2–10 millimeters in diameter that are observed during an early follicular phase. A transvaginal ultrasound will be performed between day 2 and 3 of the menstrual cycle to measure the AFC. Each time, the test will be carried out by the same physician in each hospital.

Anti-Müllerian Hormone

To determine AMH serum levels, we will run a blood test on any day of the menstrual cycle, as this hormone is not ifluenced by the hypothalamo-pituitary-ovarian axis. All AMH samples will be analyzed using the same kit in a central laboratory (in order to prevent any bias).

Precursor lesions

An anatomopathological examination will be performed on the resected fallopian tubes to evaluate two main lesions : serous tubal intraepithelial carcinoma (STIC) and P53 signature. All AP examination will be performed in Hopital Josep Trueta.

We will use a validated diagnostic algorithm from Vang et *al*[35]. This algorithm combines histologic features and inmunohistochemical expression of p53 and Ki-67 to classify precursor lesions in STIC and non-STIC (STIL, p53 signature, normal). (Annex 4)

7.8 MEASUREMENTS : VARIABLES

Independent variable

Bilateral salpingectomy

Salpingectomy is a surgical procedure which consists in removing the fallopian tubes. Bilateral salpingectomy will be performed in our first cohort, sparing the ovaries. Three types of surgical approaches will be accepted, vaginal, laparotomic and laparoscopic approach. The choice of the surgical approach will depend on the requirements of the main surgery.

A study made by Chan et *al.*, suggested that the cauterization method used during salpingectomy might affect the antral follicle count and ovarian blood flow.[16]

To avoid possible biases, bipolar diathermy will be used to cauterize the mesosalpinx in laparoscopic as well as laparotomic approach.

Dependent variable

Ovarian reserve

Ovarian reserve will be assessed using two diagnostic tests : anti-Müllerian hormone and transvaginal ultrasound (AFC).

Covariables

-Age
-Hypertension
-Body mass index
-Smoking
-Socioeconomic variables proxied by education and occupation (both, polytomous qualitative variables).

7.9 FEASABILITY, SCHEDULE AND CHRONOGRAM

The study is expected to last around 3 years. All the activities carried out during this period of time will be organized into 4 stages that are detailed below.

Stage 1: Preparation, coordination and formation

- Elaboration of the protocol.
- Coordination and formation of the research team
- Protocol evaluation and its approval by the Ethics Committee (CEIC)

Stage 2 : Participant inclusion, evaluation and data collection

The data collection will be carried out during 3 years.

Patient recruitment and evaluation will be carried out by the co-investigators during a year.

Patients attending Gynecology and Obstetrics department during this period, who fulfill the inclusion criteria and none of the exclusion criteria will be offered to enter the study. They will be included after reading and signing the informed consent.

During this period patients will be recruited, evaluated and put into the “exposed” or “not exposed” groups.

To evaluate the ovarian reserve, a blood test and a transvaginal ultrasound will be performed twice. In the case of patients undergoing opportunistic salpingectomy, both tests will be carried out during the month prior to surgery and 2 years after surgery. In the case of patients

of the control group, the diagnostic tests will be performed as we recruit the patients. The second evaluation will be done two years after the date of the first one.

Stage 3: Data analysis

Once the data collection is finished according to our sampling, the whole data will be organized in the database by the study coordinator. The necessary univariate, bivariate and multivariate analyses will be performed by the statistician during this period.

Stage 4: Interpretation and publication of results

A meeting will be done with all the research team to discuss the results, once the data has been analyzed.

The final article will be published in a medical journal in order to properly disseminate the results of the study.

As the study is longitudinal and will last about three years, the researchers will meet all way through the data collection period, at the end of data collection period, after data analysis and after result interpretation. The objective of these meetings is to identify deficiencies in the study and to correct methodological errors.

Members of the team :

Main investigator : responsible for, elaboration of the protocol, overseeing the study, coordination and formation of the research team, results interpretation, writing of the conclusions and results publication and dissemination.

Study coordinators : Responsible for overseeing the study (according to the study protocol) and coordination and formation of the research team. There will be a study coordinator in each hospital.

Co-investigators:

- Gynecologists : Responsible for performing the ultrasound to determine the antral follicle count. The test must be performed by the same gynecologist in each hospital.

Expert statistician: Responsible for the statistical analysis of the study.

Nursing staff : responsible for taking the blood samples.

	ASSIGNMENT	STAFF	2019				2020				2021				2022				2023			
			March-May	May-June	July	August	Jan-March	Apr-June	July-Sept	Oct-Dec	Jan-March	Apr-June	July-Sept	Oct-Dec	Jan-March	Apr-June	July-Sept	Oct-Dec	Jan-March	Apr-June	July-Sept	Oct-Dec
STAGE 1	PROTOCOL ELABORATION	MAIN INVESTIGATOR																				
	COORDINATION AND FORMATION OF THE RESEARCH TEAM	MAIN INVESTIGATOR AND STUDY COORDINATOR																				
	PROTOCOL APPROVAL BY THE CEIC	Ethics Committee																				
STAGE 2	PATIENTS RECRUITMENT AND EVALUATION	ALL RESEARCH TEAM																				
	DATA COLLECTION	Gynecologists Nursing staff																				
	MEETINGS	ALL RESEARCH TEAM																				
STAGE 3	Introduction of data database	Main investigator																				
	Statistical Analysis	Statistician																				
	MEETINGS	All research team																				
STAGE 4	RESULTS DISCUSSION	ALL RESEARCH TEAM																				
	ARTICLE WRITING AND PUBLICATION	MAIN INVESTIGATOR																				
	MEETINGS	ALL RESEARCH TEAM																				

8. STATYSTICAL ANALYSIS

Statistical analysis will be performed usin Statistical Package for Social Sciences (SPSS) software:

8.1 UNIVARIATE ANALYSIS

In the univariate analysis, variables will be defined as qualitative or quantitative :

-For quantitative dependent variables and covariables, we will use mean (standard deviation), median (interquartile range), stratificating by the groups of the independent variable (exposed and not exposed).

-For qualitative variables, the results will be expressed in percentages, proportions or frequencies (STIC prevalence).

-For qualitative covariables, the results will be expressed in proportions, stratificating by the groups of the dependent variable.

8.2 BIVARIATE ANALYSIS

Comparison of the qualitative covariables proportions between the 2 groups (exposed and not exposed) will be carried out using Chi-square or Fisher's exact Test.

Comparison of the dependent variable and quantitative variables' mean and median between the groups of the independent variable will be carried out using Student-t test and Mann-Whitney U respectively.

8.3 MULTIVARIATE ANALYSIS

A multivariate analysis will be performed to adjust for the covariables, trying to avoid potential confounders that could modify the results.

To analyze the relationship between the Anti-Müllerian hormone and the independent variable, a lineal regression model will be used, adjusting for the covariables.

Since the antral follicle count might present a high variability among our patients, a lineal regression model will be used to analyze its relationship with the independent variable, adjusting for the covariables.

The adjusted values of the AMH and the AFC in the linear regressions will be cathegorized into percentiles to evaluate its distribution in correlation to the independent variable.

9. ETHICAL CONSIDERATIONS

This research protocol will be presented to the Clinical Research Ethical Committee (CEIC) of Hospital Universitari Dr. Josep Trueta for its assesment and approval. Moreover, the recommendations given by the committee will be taken into account to carry out the study.

The study will be conducted according to the requirements expressed in the Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects signed by the World Medical Association in 1964 (last actualization, october 2013).

A patient will not enter the study until she has been properly informed, has been given time to contemplate participation and has freely given her consent by reading and signing the informed consent. This will be done prior to performing any study related procedures. Patient autonomy will be respected, not only before entering the study, but at all times.

Patient data anonymity will be guaranteed to preserve patient confidentiality. Patient anonymity and rights will be based on the Organic Law 15/1999 of the 13th of December on the Protection of Data, the Basic Law 41/2002 on the autonomy of the patient and rights and obligations with regard to clinical information and documentation and the Royale Decree 1090/2015, of the 24th of July, on Biomedical Research.

The patient's confidentiality will be preserved by using codes to identify the patient instead of their names. Only the principal investigator, study coordinator and physicians will know the relation between the inclusion codes and the patient's name.

All investigators of this study will have to declare conflict of interest if they exist.

10. LIMITATIONS OF THE STUDY

-Intrinsic limitations related with the design of the study, although we believe that a prospective cohort is the best choice for our objectives.

-As we use a consecutive sampling method, we could have a selection bias that we will try to avoid using inclusion and exclusion criteria. Only the patients that fulfill the inclusion criteria and none of the exclusion cirteria will be invited to participate in our study.

-Withdrawal and losses during the follow-up period could cause a selection bias. They will be registered. To avoid this bias the sample size has been calculated with expectations of future losses and withdrawals.

-The impossibility to randomize the patients of the study. We have tried to minimalize the effects of possible confounding bias by defining the plausible confounding factors described in the literature as covariables, with the use of multivariate logistic regression analyses.

-Inter- and intraobserver variability of measurement of the antral follicles. All measurements will be obtained following current guidelines.

-Variability of the antral follicle count

-We won't be able to extrapolate our study's results to ethnically different populations.

- A dicrepancy between AMH levels and AFC might be possible. This discrepancy was reported by Ye et *al*. and Şahin Ersoy et *al*., however, it could be attributed to the fact that patients included in this study had adnexal/tubal pathology, which could have already compromised AFC before surgery, rather than a consequence of the salpingectomy.[10] Adnexal and tubal pathology are exclusion criteria in our study, nevertheless, a previous assymptomatic tubal or ovarian disease could have gone unnoticed, and therefore, it could compromise our results.

11. IMPACT ON THE NATIONAL HEALTH SYSTEM

Ovarian cancer has an important economic and social impact, due to its late diagnosis and high mortality. Opportunistic salpingectomy for ovarian cancer prevention is a relatively new cancer risk reduction strategy in low-risk population. We dispose of evidence that support the new ovarian carcinogenesis theory, which stipulates that the serous ovarian carcinoma originates in the fallopian tubes.

However, we have no information about the possible long-term effects of the salpingectomy on the ovary, as most studies have evaluated only short-term effects of the technique.

If we could verify that opportunistic salpingectomy has no potential effects on the ovarian function, the reluctance regarding the opportunistic salpingectomy could dissipate, and more women who have completed their childbearing desire, would ask for a salpingectmy instead of a tubal ligation.

Moreover, this study could solve some issues regarding the treatment of infertility. Salpingectomy is indicated in patients with ectopic pregnancy, with hydrosalpinx or with endometriosis who are proceeding to in vitro fertilization (IVF) treatment. However, there is a concern about the possibility of ovarian function impairment after the procedure. If the results of our study are conclusive, this matter would be solved.

12. BUDGET

The budget includes all the possible expenses that will be needed to realize the study. Diagnostic tests performed in daily clinical practice for patients undergoing pelvic surgery are not considered additional costs of the study as they are part of normal clinical practice. Since the standard procedure will be achieved (salpingectomy), no additional personnel will be required for surgery nor anatomopathological exam.

The research team will assume the tasks related to patient recruitment, coordination, data collection and interpretation of resulsts as part of their normal activities.

	Price per unit	Time/Quantity	Subtotal
PERSONNEL			
Statistician	40€/h	20h	800€
Nurse	15€/h	75h	1.125€
MATERIALS AND SERVICES			
Anti-Müllerian Hormone	30€	904	27.120€
Transvaginal ultrasound	50€	904	45.200€
Blood extraction kit	5€	904	4.520€
MEETINGS			
Coordination meetings	300€	7	2.100€
PUBLICATION AND DISSEMINATION			
Publication	1000€	1	1000€
National congress	1500€	2	3000€
		TOTAL	**84.865€**

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14. ANNEXES

ANNEX 1.

HOJA INFORMATIVA PARA LA PARTICIPANTE (Control group)

Nos dirigimos a usted para informarla sobre la realización de un estudio de investigación al cual la invitamos a participar. El presente estudio ha sido aprobado por el Comité de Ética y de Investigación Clínica (CEIC), de acuerdo con la legislación vigente, Real Decreto 1090/2015, de diciembre, sobre la investigación biomédica.

Nuestra intención es que usted reciba la información de manera correcta y que ésta sea suficiente para que pueda decidir si quiere participar o no en este estudio. Por este motivo, le agradeceríamos que leyera atentamente esta hoja informativa y posteriormente nosotros le aclararíamos las dudas que pudieran surgirle.

En primer lugar, usted ha de saber que su participación en este estudio es totalmente voluntaria. Si decide participar en el estudio tiene que saber que podría abandonarlo en cualquier momento sin que eso repercuta en sus cuidados médicos.

Título del estudio: *Efectos a largo plazo de la salpingectomía oportunista en la función ovárica*.

Explicar salpingectomía

Lugar de realización: Servicios de Ginecología y Obstetricia de los siguientes hospitales

-Hospital Universitario Dr Josep Trueta
-Hospital de Figueras
-Hospital de Palamos
-Hospital de Blanes

Finalidad: Evaluar la repercusión de la salpingectomía (resección de las trompas de Falopio) en las hormonas ováricas y la reserva folicular. Prevalencia de lesiones precursoras del cáncer de ovario.

Confidencialidad, protección de datos y derechos del paciente: se adoptarán las medidas necesarias para garantizar la confidencialidad de sus datos en cumplimiento de la Ley Orgánica 15/1999 y la información recogida será gestionada de forma anónima y sólo se utilizarán con fines de investigación. También se garantizarán los principios establecidos por la Ley de Investigación Biomédica 14/2007 y por la Ley 41/2002 básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica.

Pruebas diagnósticas : Se le hará una primera analítica sanguínea (para valorar las hormonas ováricas) y una ecografía transvaginal (para realizar el recuento de los folículos ováricos). Al cabo de dos años se le harán las mismas pruebas de nuevo.

Resultados y beneficios de la participación : La paciente está en su derecho de ser informada de los resultados de la investigación, así como de no ser informada acerca de estos. Los resultados de las pruebas diagnósticas que se realizarán le permitirán conocer el estado de su reserva ovárica. Los beneficios de la investigación pueden beneficiar a la participante como a otras personas, y estos serán adecuadamente utilizados para conseguir los objetivos del estudio y servirán de base para futuras investigaciones en este ámbito. Conocer su función ovárica y posibles lesiones precursoras…

Riesgos asociados : Se trata de riesgos leves relacionados con la extracción sanguínea.

- Sangrado excesivo, formación de un hematoma
- Infección del sitio de punción

Le agradecemos su participación.

ANNEX 2.

HOJA INFORMATIVA PARA LA PARTICIPANTE (Study group)

Nos dirigimos a usted para informarla sobre la realización de un estudio de investigación al cual la invitamos a participar. El presente estudio ha sido aprobado por el Comité de Ética y de Investigación Clínica (CEIC), de acuerdo con la legislación vigente, Real Decreto 1090/2015, de diciembre, sobre la investigación biomédica.

Nuestra intención es que usted reciba la información de manera correcta y que ésta sea suficiente para que pueda decidir si quiere participar o no en este estudio. Por este motivo, le agradeceríamos que leyera atentamente esta hoja informativa y posteriormente nosotros le aclararíamos las dudas que pudieran surgirle.

En primer lugar, usted ha de saber que su participación en este estudio es totalmente voluntaria. Si decide participar en el estudio tiene que saber que podría abandonarlo en cualquier momento sin que eso repercuta en sus cuidados médicos.

Título del estudio: *Efectos a largo plazo de la salpingectomía profiláctica en la función ovárica*.

Lugar de realización: Servicios de Ginecología y Obstetricia de los siguientes hospitales

-Hospital Universitario Dr Josep Trueta

-Hospital de Figueras

-Hospital de Palamos

-Hospital de Blanes

Finalidad: Evaluar la repercusión de la salpingectomía (resección de las trompas de Falopio) en las hormonas ováricas y la reserva folicular.

Confidencialidad, protección de datos y derechos del paciente: se adoptarán las medidas necesarias para garantizar la confidencialidad de sus datos en cumplimiento de la Ley Orgánica 15/1999 y la información recogida será gestionada de forma anónima y sólo se utilizarán con fines de investigación. También se garantizarán los principios establecidos por la Ley de Investigación

Biomédica 14/2007 y por la Ley 41/2002 básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica.

Pruebas diagnósticas : Se le hará una analítica sanguínea (para valorar las hormonas ováricas) y una ecografía transvaginal (para realizar el recuento de folículos ováricos) previamente a la salpingectomía y al cabo de dos años. Se realizará también un estudio anatomopatológico de las trompas extirpadas. Si se detectara una anomalía relevante procederíamos a informarla.

¿Qué es la salpingectomía oportunista? Es un procedimiento quirúrgico que consiste en extirpar las trompas de Falopio durante una histerectomía o como método de esterilización definitiva (en lugar de la ligadura de trompas). El objetivo de la salpingectomía es prevenir el cáncer de ovario, ya que se ha visto que los tumores ováricos más agresivos se originan en las trompas uterinas. Esta intervención no supone más riesgos que los relacionados con la cirugía principal (histerectomía/ligadura de trompas).

Resultados y beneficios de la participación : La paciente está en su derecho de ser informada de los resultados de la investigación, así como de no ser informada acerca de estos. Los resultados de las pruebas diagnósticas que se realizarán le permitirán conocer el estado de su reserva ovárica. Los beneficios de la investigación pueden beneficiar tanto a la participante como a otras personas, y estos serán adecuadamente utilizados para conseguir los objetivos del estudio y servirán de base para futuras investigaciones en este ámbito.

Riesgos asociados : Se trata de riesgos leves relacionados con la extracción sanguínea.

- Sangrado excesivo, formación de un hematoma
- Infección del sitio de punción

Le agradecemos su participación.

ANNEX 3.

CONSENTIMIENTO INFORMADO DEL ESTUDIO

TÍTULO DEL ESTUDIO : *Long-term effects of opportunistic salpingectomy on the ovarian reserve : a prospective cohort study*

Yo, Sra. …….. Con DNI …….

Afirmo que,

- He recibido y leído la hoja informativa que se me ha entregado.
- He podido hacer todas las preguntas necesarias respecto al estudio y han sido respondidas de manera satisfactoria.
- He recibido suficiente información acerca de las características y objetivos del estudio, los posibles riesgos y la importancia de mi contribución para el avance de la medicina.
- He estado informado por el investigador…….. de las implicaciones y la finalidad del estudio.
- Entiendo que mi participación es voluntaria
- Estoy de acuerdo con que mis datos sean utilizados por el estudio indicado de forma anónima.
- Doy mi permiso para que los datos de mi historia clínica sean utilizados por el equipo de investigación para fines relacionados con el estudio, entendiendo que después de haberlos comprobado se eliminará del registro toda la información que me pudiese identificar.
- Doy mi permiso para que el equipo de investigación pueda consultar los resultados del examen anatomopatológico.
- Sé que se mantendrá la confidencialidad de mis datos.
- Otorgo mi consentimiento de manera voluntaria y sé que soy libre de retirarme del estudio en cualquier momento del mismo.

_____ _____ _____

(Fecha) (Nombre y apellidos de la participante) (Firma de la participante)

Confirmo que he explicado a la paciente el carácter y el propósito del estudio.

…….. (Firma de un miembro del equipo del proyecto)

REVOCACIÓN DEL CONSENTIMIENTO INFORMADO

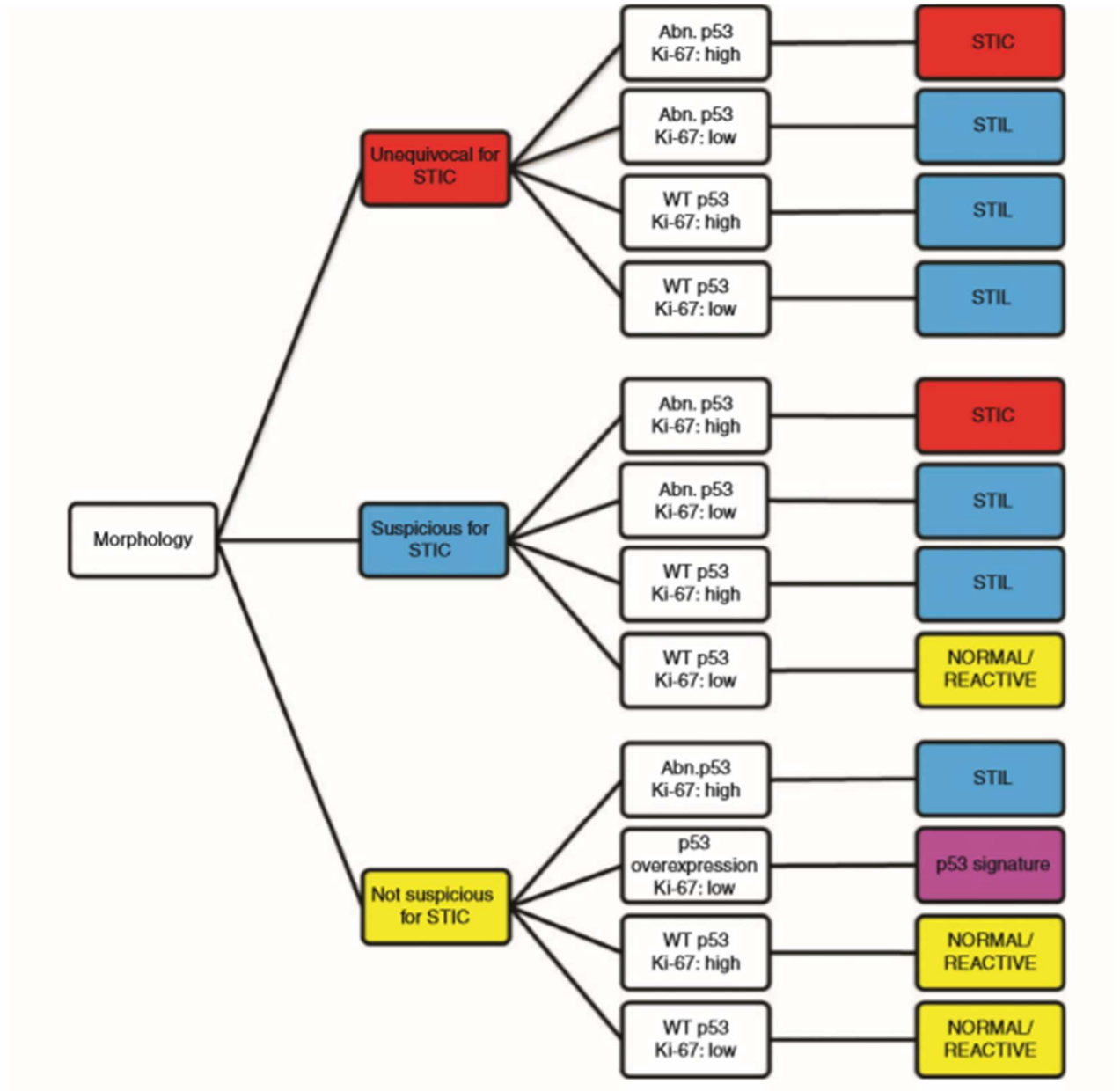
Yo,……., revoco el consentimiento informado firmado para la participación en el estudio "*Long-term effects of opportunistic salpingectomy on the ovarian reserve : a prospective cohort study"*

_____ _____

(Firma de la paciente) (Firma del investigador)

Fecha y lugar : ……., …….. de ……. Del 20……

ANNEX 4.


Morphology
Unequivocal for STIC
Suspicious for STIC
Not suspicious for STIC
Abn. p53 Ki-67: high
Abn. p53 Ki-67: low
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STIC
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