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FACULTY OF MEDICINE

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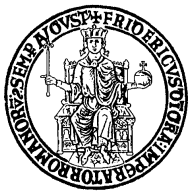

Course coordinator: Prof.ssa Maria Triassi

Tutor: Prof. Attilio Di Spiezio Sardo

"EFFICIENCY AND SAFETY OF OVARIAN STIMULATION WITH LEVONORGESTREL-INTRAUTERINE SYSTEM AND LETROZOLE AFTER COMBINED FERTILITY-SPARING TREATMENT OF WOMEN WITH ATYPICAL ENDOMETRIAL LESIONS"

Candidate

Alessandra Gallo

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ABSTRACT

- **Objective**: The aim of this study was to demonstrate the safety and efficacy of ovarian stimulation (OS) with Levonorgestrel Intrauterine System (LNG-IUS) in situ and co-treatment with letrozole in young patients undergoing fertility-sparing combined treatment for atypical endometrial hyperplasia (AEH) or early endometrial cancer limited to the endometrium.
- **Materials and Methods**: a prospective case-control study was conducted at the Department of Gynecology and Obstetrics of Tertiary Care University Hospital, University of Naples "Federico II", and involved the Hysteroscopy Unit and the Reproductive Endocrinology and Oncofertility Unit. Young women who underwent fertility-sparing "combined" treatment and subsequent OS with letrozole and LNG-IUS in situ were enrolled. Enrolled patients were treated by superficial endometrial resection preserving the basal layer of the endometrium, in case of diffuse atypical lesion, or by "three steps" hysteroscopic resection technique in case of focal development lesions. After achieving a complete response (CR), OS was started, oocytes retrieval was performed, and mature oocytes were cryopreserved. After removal of the LNG-IUS, embryo-transfer was performed. The comparative analysis of the outcomes of assisted reproductive technologies was performed considering two control groups: control group A) healthy infertile women undergoing OS for IVF/ICSI; control group B) patients diagnosed with breast cancer who underwent OS with letrozole.
- **Results**: Seventy-five patients were analysed, divided as follows: 15 patients undergoing oocyte cryopreservation after achieving a CR to fertility-sparing treatment (study group), 30 in control group A and 30 in control group B. No statistically significant differences were observed in

oocyte and mature oocytes retrieved between the study group and the control groups. The clinical pregnancy rate was 55.6%, the cumulative live birth rate was 44.4%, the miscarriage rate was 20%. 3 patients with AEH had recurrence (20%), occurring 3, 6 and 16 months after removal of intrauterine system to attempt embryo transfer, respectively.

- **Conclusion**: This preliminary data demonstrated that fertility-sparing hysteroscopic "combined" treatment and subsequent OS with letrozole and LNG-IUS in situ could be suggested in women with AEH or early endometrial cancer who ask to preserve future fertility. Further studies are needed in order to investigate the effect of OS with letrozole and LNG-IUS in these women.

CHAPTER I

Endometrial carcinoma

1.1 Definition and Epidemiology

Endometrial carcinoma (EC) is one of the most frequent cancers of the female reproductive tract, and ranks sixth in women worldwide, with an estimated 417,000 new cases and 97,000 deaths recorded globally in 2020. Incidence rates has been raising both in developed and developing countries, especially for the increased obesity of the population. Racial disparity and socioeconomic and geographical differences are important determinants of EC incidence and mortality. Several non-genetic risk factors have been associated with an increased probability to have EC, particularly for the most prevalent histological subtype: endometrioid endometrial adenocarcinoma (EEC), which include obesity, physical inactivity, excess exogenous oestrogen, insulin resistance, and tamoxifen use after breast cancer.

Endometrial carcinoma occurs mainly in postmenopausal women, but there has been an increasing trend of precancerous lesions and endometrial adenocarcinomas in women of reproductive age. Notably, 70% of young women (< 40 years old) with endometrial oncologic lesion have not yet completed their reproductive desire.

1.2 Classification

The classification of EC has evolved over time, with the goal of more precisely predicting patient prognosis and guiding management. At the beginning, uterine cancer was subclassified based only on anatomical location, treating tumours from the cervix and uterine corpus as separate entities. With regard to carcinoma of the uterine corpus, in 1983, Bokhman first classified EC on the basis of clinical, epidemiological, metabolic and endocrine features. Two subtypes, Type I and Type II EC, with distinct clinical, pathological, and histological behaviour were identified. Type I EC, are mainly low grade, moderately or highly differentiated with favourable outcomes. Being estrogen-dependent, hormone-receptor-positive adenocarcinomas with endometrioid morphology, they are often referred as endometrioid endometrial cancers. It represents the most common type accounting for approximately 85% of all EC usually diagnosed at an early stage and characterized by a good prognostic. Nulliparity and infertility are frequent risk factors for Type I EC. Estrogen therapy that not balanced by the effect of progestins, estrogen- secreting tumours, early menarche and late menopause (with a risk increased twice), represented other risk factors involved. Conversely, the use of oral contraceptives, and the occurrence of a pregnancy, are protective factors counteracting the onset of disease. Although these risk factors have been extensively described in the literature, there is currently no evidence on the efficacy of a screening test at an early stage to be extended to the asymptomatic female population at medium risk for Type I EC. The only recommended screening is for women with Lynch Syndrome.

On the other side, Type II EC is characterized by non-endometrioid subtypes such as serous, clear-cell, and undifferentiated carcinomas. Not related to hyperestrogenism, they affect non-obese women, often arising in the absence of

endocrine and metabolic disturbances. They generally are high-grade, hormone-receptor negative, poorly differentiated, associated with a higher risk of metastatic spread and poor prognosis. Women with type II EC are often multiparous, smokers with a history of breast cancer. EC is also classified according to histopathological features, with the most common subtypes being endometrioid carcinoma, serous carcinoma, carcinosarcoma and clear-cell carcinoma. Endometrioid adenocarcinomas represent a range of neoplasms, from well to poorly differentiated tumours (i.e. low to high grade), whereas serous and clear-cell carcinomas are high grade by definition.

In support of all previous classifications, molecular data have become an integral component of pathologic evaluation, as EEC (type I) are preferentially associated with mutations in PTEN (Phosphatase and Tensin homolog on chromosome 10), KRAS, CTNNB1 and PIK3CA (Phosphatidylinositol 3-kinase) and MLH1 promoter hyper- methylation, whereas SC (non-endometrioid, type II) show HER2 amplification, inactivation of the TP16 gene, low expression of E- caderina and recurrent TP53 mutations.

In 2013, the Cancer Genome Atlas (TGCA) Research Network introduced a new classification, based on the biomolecular characteristics of the tumour, to improve our understanding of the molecular landscape of EC. The molecular classification identifies four subgroups:

1. POLE: ultra-mutated tumours, characterized by unusually high mutations rates of the exonuclease domain of POLE 58, subunit ε of the DNA polymerase involved in the DNA replication process and a favourable result;

2. MSI hypermutated (microsatellite unstable tumours): a hypermutated group characterized by microsatellite instability secondary to MLH1 promoter methylation and high mutagenicity;
3. copy-number low: generally endometrioid G1-G2, it is a group with lower mutation frequency characterized by microsatellite stability with frequent mutations of CTNNB1;
4. copy-number high tumours: they consists primarily of serous-like cancers characterized by frequent aberrations of the number of gene copies, low mutagenicity, frequent mutations of TP53 and unfavourable outcome.

Regarding endometrial hyperplasia, historically this has been classified into four categories: simple hyperplasia without atypia, simple hyperplasia with atypia, complex hyperplasia without atypia, and complex hyperplasia with atypia, with a 1-43% risk of malignant progression. The World Health Organization (WHO) approved a new classification of female genital tract cancers in 2014, which distinguishes two categories, non-atypical (benign) hyperplasia and atypical endometrial hyperplasia (AEH), also known as endometrial intraepithelial neoplasia (EIN). EIN is considered the precursor lesion of EEC; all other variants of endometrial hyperplasia are benign variants that can be managed medically. The new binary classification shows more robust prognostic power, reproducibility, and alignment with treatment options and is therefore recommended.

1.3 Grading and Staging

EC is staged according to the 2023 International Federation of Gynecology and Obstetrics (FIGO) System, which reflects new findings and data available for the diagnosis of EC. In fact, since the publication of the latest FIGO staging system for EC in 2009, a considerable amount of new information has emerged that better defines the pathology and molecular findings in relation to the type of EC; in addition, new treatments, clinical trial results, and prognostic and survival data related to pathologic and surgical outcomes have been reported.

Histopathological findings are central features of the 2023 revision of the FIGO staging of EC. All ECs should be classified according to the 5th edition of WHO Classification of Tumors, Female Genital Tumours. The following different histological types have been recognized:

1. EEC, either low-grade (grades 1 and 2) or high-grade (grade 3);
2. Serous Carcinoma (SC);
3. Clear Cell Carcinoma (CCC);
4. Mixed Carcinoma (MC);
5. Undifferentiated Carcinoma (UC);
6. Carcinosarcoma (CS);
7. Other unusual types, such as mesonephric carcinoma;
8. Mucinous-Type Gastrointestinal Carcinomas.

Histologic typing is an important prognostic predictor and is essential in staging. The FIGO staging revision of 2023 divided non-aggressive histologic types (low-grade EEC) and aggressive histologic types (high-grade EEC, SC, CCC, MC, UC, CS, and mesonephric and gastrointestinal mucinous type carcinomas). Molecular classification is particularly useful for high-grade EECs (grade 3) and allows them to be appropriately assigned a risk group, discriminating an excellent prognosis

group (POLE mutated in early-stage disease) from a bad prognosis group (p53 abnormal).

For practical purposes and to avoid undertreatment of patients, if the molecular classification was unknown, high-grade EECs were grouped together with the aggressive histological types in the actual FIGO classification.

In summary, the current modifications to the endometrial staging system have been made to further define the differences in prognosis and survival that have been reported since the 2009 system was published. The following changes have been incorporated into the updated endometrial cancer staging system (Table 1):

- Stage I: (IA1) non-aggressive histological type limited to an endometrial polyp or confined to the endometrium; (IA2) non-aggressive histological types involving less than half the myometrium with no or focal LVSI as defined by the WHO criteria; (IA3) low-grade endometrioid carcinomas limited to the uterus with simultaneous low-grade endometrioid ovarian involvement; (IB) non-aggressive histological types involving one half or more of the myometrium with no or focal LVSI; and (IC) aggressive histological types limited to a polyp or confined to the endometrium.
- Stage II: (IIA) tumors that infiltrate the endocervical stroma, or (IIB) have substantial LVSI or (IIC) aggressive histological types, i.e., serous, clear cell, carcinosarcomas, undifferentiated, mixed, gastrointestinal-type mucinous endometrial carcinoma, and mesonephric-like carcinomas with any myometrial invasion.
- Stage III: (IIIA1) differentiation between adnexal versus (IIIA2) uterine serosa involvement; (IIIB1) vaginal and/or parametrial involvement and (IIIB2) pelvic peritoneal carcinomatosis; refinements are defined within

Stage IIIC to reflect the extent of pelvic and abdominal lymph node metastases with (IIIC1i) micrometastasis and (IIIC2ii) macrometastasis.

- Stage IV: (IVA) reflects locally infiltrative, (IVB) extra pelvic peritoneal metastasis, and (IVC) distant metastatic disease.

When performed, the POLE mutated and p53abnormal molecular groups can increase or decrease the stage of endometrial cancer in Stages I and II. No changes occur through the molecular staging in Stages III and IV. Stage III and IV cases, for which the molecular classification is known, should be recorded as Stage IIIm and Stage IVm with the specification of the molecular class for the purpose of data collection. Based on these molecular assays, an “m” notation is always required to indicate that the stage is modified in case of early stages or recorded in case of advanced stages.

Table 1: 2023 FIGO staging of EC

STAGE	DESCRIPTION
STAGE I	Confined to the uterine corpus and ovary
IA	Disease limited to the endometrium OR non-aggressive histological type, i.e. low-grade endometroid, with invasion of less than half of myometrium with no or focal lymphovascular space involvement (LVSI) OR good prognosis disease IA1 Non-aggressive histological type limited to an endometrial polyp OR confined to the endometrium IA2 Non-aggressive histological types involving less than half of the myometrium with no or focal LVSI IA3 Low-grade endometrioid carcinomas limited to the uterus and ovary
IB	Non-aggressive histological types with invasion of half or more of the myometrium, and with no or focal LVSI
IC	Aggressive histological types limited to a polyp or confined to the endometrium
STAGE II	Invasion of cervical stroma without extrauterine extension OR with substantial LVSI OR aggressive histological types with myometrial invasion
IIA	Invasion of the cervical stroma of non-aggressive histological types
IIB	Substantial LVSI of non-aggressive histological types
IIC	Aggressive histological types with any myometrial involvement
STAGE III	Local and/or regional spread of the tumor of any histological subtype
IIIA	Invasion of uterine serosa, adnexa, or both by direct extension or metastasis IIIA1 Spread to ovary or fallopian tube (except when meeting stage IA3 criteria) IIIA2 Involvement of uterine subserosa or spread through the uterine serosa
IIIB	Metastasis or direct spread to the vagina and/or to the parametria or pelvic peritoneum IIIB1 Metastasis or direct spread to the vagina and/or the parametria IIIB2 Metastasis to the pelvic peritoneum
IIIC	Metastasis to the pelvic or para-aortic lymph nodes or both IIIC1 Metastasis to the pelvic lymph nodes IIIC1i Micrometastasis IIIC1ii Macrometastasis IIIC2 Metastasis to para-aortic lymph nodes up to the renal vessels, with or without metastasis to the pelvic lymph nodes IIIC2i Micrometastasis IIIC2ii Macrometastasis

1.4 Diagnosis

In women of childbearing age, EC is associated with menstrual irregularities, inter-menstrual bleeding (IMB), or occasionally with recent onset menorrhagia. Metrorrhagic symptomatology is early and about 80% of cases are in fact diagnosed at the early stages; in such cases, the 5-year prognosis is more than 80%. Thus, the high survival rate demonstrates the importance of correct diagnosis as early as possible. A small percentage of patients, between 0.5% and 1.5%, however, may remain asymptomatic for a long time.

At risk for later diagnosis are patients with stenosis of the external/internal uterine orifice and patients in the peri-menopausal age. In fact, at that age, more difficulties are encountered in the interpretation of abnormal uterine bleeding (AUB), since in most cases it is dysfunctional or associated with benign endometrial disease.

A generally late symptom is leucoxantorrhea, consisting of malodorous yellowish-white vaginal discharge due to congestion phenomena associated with the tumor and sometimes necrosis and colliquation phenomena occurring in neoplastic vegetations.

Pain also appears late, when the neoplasm has already involved pelvic or abdominal organs (sigma-rectum, small intestine, or bladder).

Most guidelines recommend either transvaginal ultrasonography or endometrial biopsy as the initial study for the evaluation of EC. Transvaginal ultrasonography is often the first-level investigation in diagnosing AUB because of its availability, cost-effectiveness, and high sensitivity. It allows accurate study of the endometrial rhyme (Figure 1 a, b). Ultrasonographic features suggestive of malignancy include:

- Endometrial thickness: a recent ACOG committee opinion notes that the cut-off value for a normal transvaginal ultrasonography result should be 4 mm in postmenopausal women (95% sensitivity, 55% specificity). An endometrial thickness greater than 5 mm in this kind of patients should be evaluated with a tissue sample, especially if bleeding is present. The American College of Radiology uses a cut-off of 5 mm or less. The optimal cut-off for evaluating premenopausal women has not been defined, but recommendations include a cut-off of 16 mm or less. In women undergoing tamoxifen treatment the recommended cut-off is 8 mm. In all patients, if bleeding persists despite a normal transvaginal ultrasonography result, a tissue biopsy is warranted.

- Heterogeneous endometrial echogenicity.

- Irregularities at the endometrial-myometrial interface.

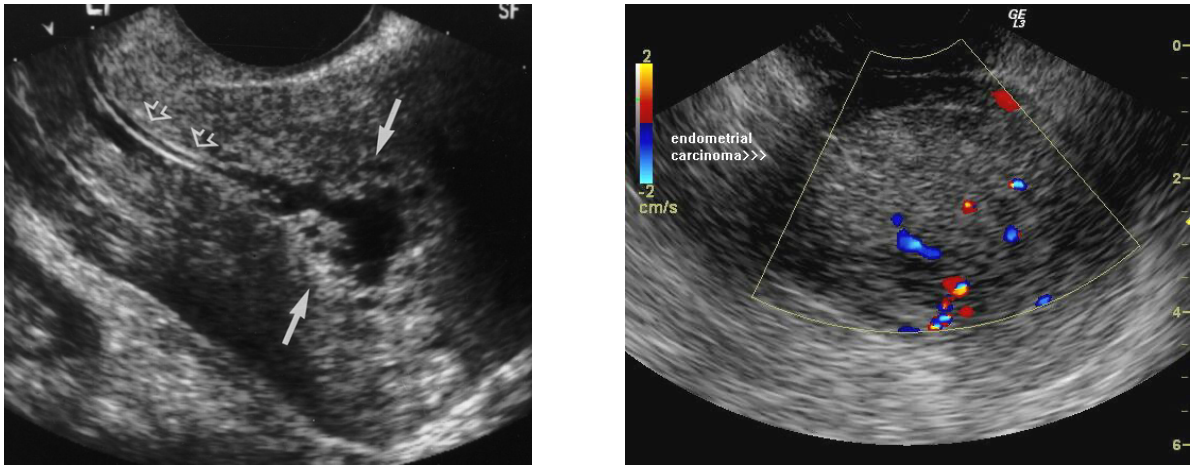
- Characteristic patterns at colour- or power-Doppler, such as multiple vessels with tortuous and irregular pattern, and low resistance velocimetric index.

Transvaginal ultrasound also plays a significant role in the evaluation of myometrial invasion, achieving a diagnostic accuracy of 87%. Preoperative evaluation of myometrial infiltration, cervical invasion, and possible lymph node involvement in EC plays an important role in proper surgical approach.

The ultrasonographic method should be used to select patients for second-level diagnostics.

The validity of MRI in diagnosing endometrial cancer compared with ultrasonographic evaluation and CT scan is well established; the latter method does not seem to show the same reliability in assessing myometrial infiltration, especially in older patients with atrophic myometrium.

Figure 1: AEH (a) and EC (b) at ultrasound.



The cornerstone investigation for the diagnosis of EC is an endometrial biopsy. Several methods to obtain endometrial tissue samples are in use such as curettage techniques using a Pipelle®, Novak®, Vabra® or dilation and curettage (D&C) using metal sharp curettes as well as hysteroscopic guided endometrial biopsy. D&C has long been considered the standard method to obtain a histological diagnosis and despite its many deficiencies is still preferred by many authors. Nevertheless, blind approach can sample less than 50% of the endometrial cavity, and consequently nearly 10% of endometrial lesions could be missed, in particular focal abnormalities, with a high percentage of false negative results.

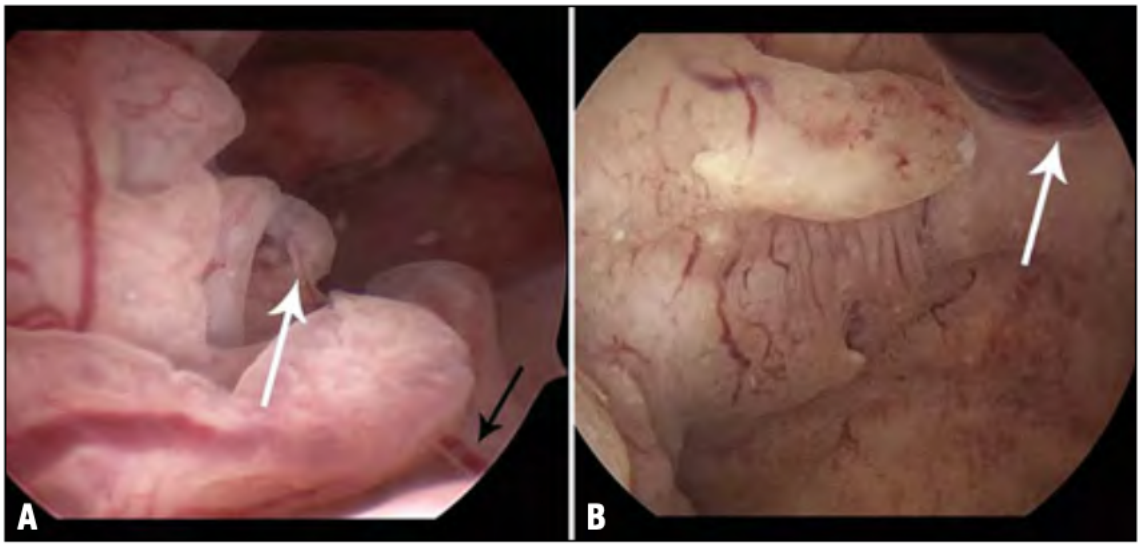
The most recent guidelines from the European Society of Human Reproduction and Embryology (ESHRE), the European Society of Gynaecological Oncology (ESGO), and the European Society for Gynaecological Endoscopy (ESGE) recommend preferring hysteroscopic guided endometrial biopsy over blind biopsy for confirming diagnosis of EC; blind techniques should no longer be offered to obtain endometrial histology.

Over the past 25 years, hysteroscopy and directed endometrial biopsy has been recognized as the gold standard in diagnosing endometrial malignancy.

In the literature, there are no hysteroscopic criteria that have been found to be pathognomonic of AEH; however, some authors have attempted to describe the principal morphological hysteroscopic criteria indicative of high-risk endometrial hyperplasia (Figure 2):

- Irregular, mamillated, or polypoid surface, with inter-papillary bridges and haemorrhagic background
- Increased thickness
- Whitish colour
- Marked and irregular vascularity, with atypical, denuded vessels.

Figure 2. Images of AEH under hysteroscopic examination



EC presents hysteroscopically in two types: a circumscribed form and a diffuse form. The circumscribed form most often presents as a polypoid lesion and, more rarely, as an ulcer or nodular relief limited to a specific endometrial area. Such lesions, unlike benign endometrial polyps, are irregular, friable, and have distinct

areas of necrosis and/or haemorrhage. The diffuse form of endometrial pathology usually occupies a large part of the uterine cavity and may be due to the spread of a poorly circumscribed form that begins primarily in the upper third of the cavity, or secondary to a multicentric origin of the tumor. In summary, the specific hysteroscopic features suggestive of endometrial neoplasia are as follows (Figure 3):

- whitish, green-grey coloration: the normal endometrial colour varies from pale pink to yellowish;

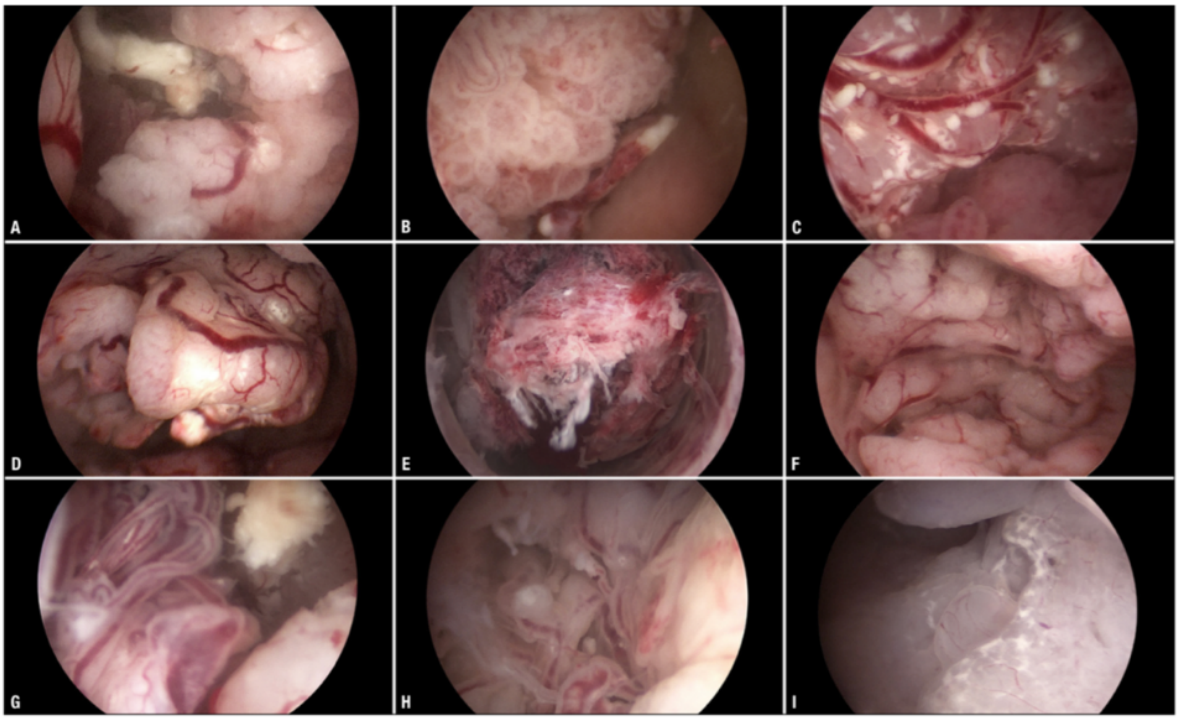
- areas of necrosis, haemorrhage and microcalcification: these findings are strongly suggestive of EC;

- atypical vascularization: diffuse vascular patterns with irregular ramifications or blurred outlines, and inconsistency between the main vascular axis and the lesion’s direction of growth;

- irregular or ulcerated surface: whitish thickened areas or surface irregularities or ulcerations should raise a suspicion for malignancy;

- soft consistency: malignant lesions are generally soft in consistency, friable, and susceptible to bleeding on contact with the hysteroscope.

Figure 3. Images of EC under hysteroscopic examination

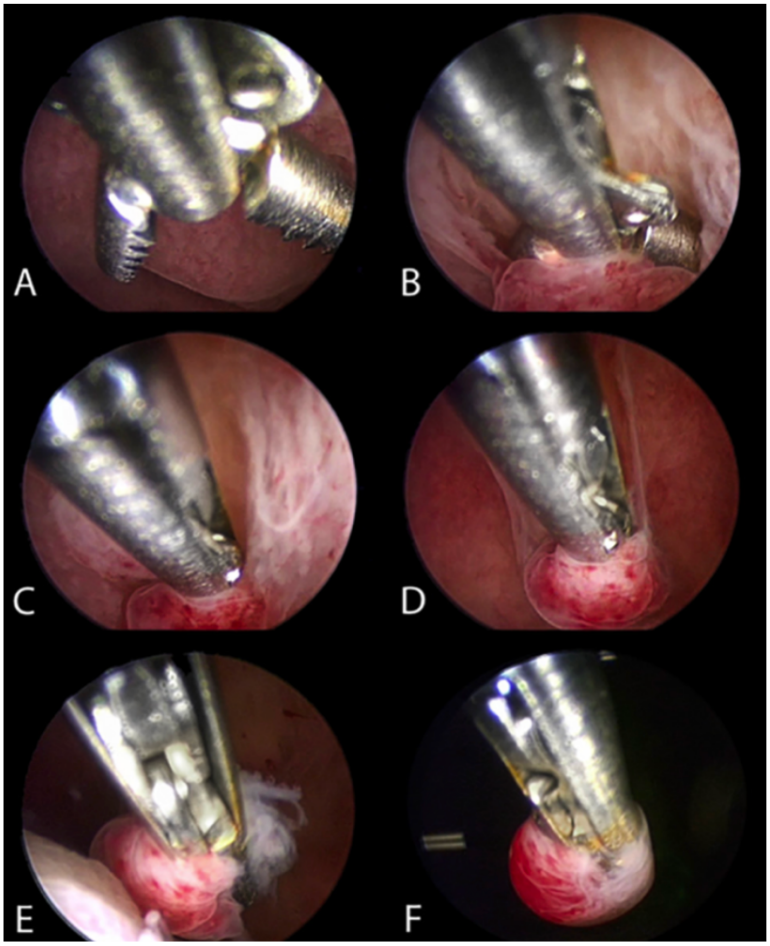


The proper and complete description of a lesion suggestive of malignancy must also take into account the following aspects: intracavitary tumoral extension, invasion of the cervical canal and patterns of tumoral growth (nodular, polypoid, papillomatous).

The targeted endometrial biopsy can give a sensitivity of 97.5%, and a specificity of 100%; the "grasp" technique has replaced the traditional hysteroscopic "punch" biopsy, as it allows removal of larger portion of endometrial tissue. This technique achieves a high concordance of histologic type and tumour grade, especially in presence of an endometrioid-type tumour (Figure 4). Once the area to biopsy has been identified, the alligator forceps is positioned with the jaws opened at the level of the endometrium to be sampled (A). Next, the jaws are dragged across the tissue for about 0.5-1 cm (B). At this point, the jaws are closed, grasping the piece of

tissue to be examined (C-D), which is then retrieved - together with the hysteroscope - from the uterine cavity, without retracting the tip of the forceps into the operating channel of the hysteroscope (E-F).

Figure 4. Hysteroscopic endometrial biopsy with "grasp technique"



1.5 Treatment

The standard treatment for endometrial carcinoma and its precursors is total hysterectomy with bilateral salpingo-oophorectomy, and surgical staging; while achieving excellent survival outcomes, this radical treatment is devastating for women interested in future fertility. However, fertility-sparing treatment approaches for women who desire offspring are now well established worldwide.

Young patients are more likely to have atypical endometrial lesions or focal, well-differentiated endometrioid tumours, confined to the endometrium or superficial myometrium (stage IA according to the FIGO 2023 staging system). In these patients the prognosis is good, with a 95% 5-year survival rates. Considering that the average age of first pregnancy is significantly delayed worldwide (predominantly over 30 years age), planning a fertility preservation strategy in young women diagnosed with EC becomes crucial.

The latest evidence-based guidelines of ESGO/ESHRE/ESGE provided recently comprehensive guidelines on fertility-sparing management of EC in a multidisciplinary setting.

The cornerstone of the fertility-sparing treatment for EC and its precursor EIN has traditionally been continuous progestin-based therapy. The recent ESGO/ESHRE/ESGE guidelines state for the first time clearly that a combined approach consisting of hysteroscopic tumor resection, followed by oral progestins and/or Levonorgestrel-Intra-Uterine System (LNG-IUS), is the most effective fertility-sparing treatment both for complete response rate and live birth rate compared with other treatment options.

Since the goal of conservative treatment is to achieve pregnancy, women should be encouraged to actively aim for conception as soon as complete response is achieved; several authors and more recent guidelines have suggested the

importance of considering assisted reproductive technologies (ART) to improve the success rate and shorten the time to conception, while minimizing the risk of relapse and disease progression.

To date, there is no clear protocol for ovarian stimulation (OS) in these patients. Although there are reassuring data on recurrence rates in patients undergoing ART, OS protocols are associated with increased serum estradiol (E2) levels; and it is well established that unopposed estrogen, whether endogenous or therapeutic, can induce endometrial hyperplasia and potentially EC. To balance these negative effects, some authors have shown that the use of letrozole with gonadotropins during OS provides endometrial protection (9), with comparable results in terms of oocyte recovery and maturation, and that less cancer recurrence is obtained in women undergoing OS with LNG-IUS.

CHAPTER II

Objectives

The aim of our study was to demonstrate the efficacy and the safety of OS with LNG-IUS *in situ* and co-treatment with letrozole in patients undergoing fertility-sparing combined treatment for AEH or well-differentiated (G1) EEC, limited to endometrium (FIGO stage IA1).

The primary outcome of the study was the number of oocytes retrieved and their maturation (number of metaphase II oocytes retrieved). To establish the safety or our stimulation protocol, we evaluated the recurrence rates after OS in the study group.

The following secondary outcomes were also assessed: total gonadotropin dose, number of stimulation days, E2 levels at day 5th and at ovulation induction, progesterone levels at ovulation induction, recurrence rates (RR) after OS, clinical pregnancy rate (CPR), cumulative live birth rate (C-LBR), miscarriage rate (MR). CPR was defined by the presence of intrauterine sac with fetal heart beath; C-LBR was defined as the number of deliveries with at least one live birth resulting from one completed ART cycle; MR was defined as the spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age in relation to clinical pregnancy achieved.

CHAPTER III

Methods

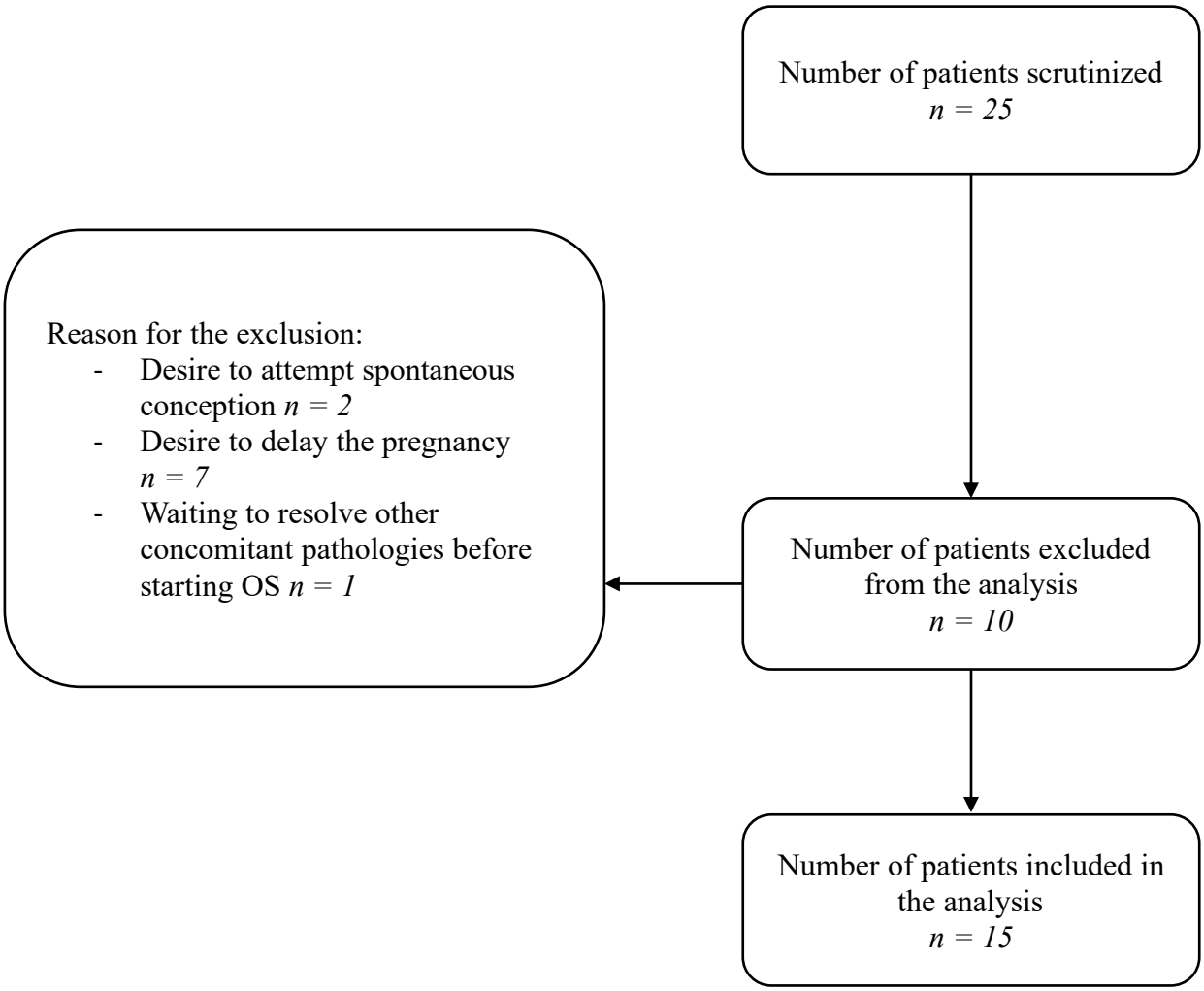
This prospective case-control study was conducted following the Declaration of Helsinki (1975) and Good Clinical Practice guidelines and approved by the Internal Institution Board.

The study group involved women with AEH or EEC who underwent fertility-sparing combined treatment and OS with letrozole plus LNG-IUS after complete remission. Inclusion and exclusion criteria for the study group are shown in Table 1. All patients underwent counselling to inform them that fertility-sparing option is not the standard of care, and that the primary treatment is total hysterectomy, bilateral salpingo-oophorectomy and surgical staging. Both oral informed consent and written informed consent to anonymized data collection was obtained by all patients included.

In order to assess the efficacy and safety of OS conducted in the study group we selected two control populations, paired 2:1 with cases and comparable in age, AMH and BMI. The first one consisted of infertile women without endometrial pathology and undergoing OS for in vitro fertilization/ICSI for male factor (Control group A) and the second one of patients undergoing fertility preservation with Letrozole for breast cancer (Control group B). The first group was selected to compare the results of OS with healthy patients without neoplasm who had no other causes of infertility apart from severe male factor of the partner. The second group was selected to compare patients with an estrogen-dependent neoplasm and receiving letrozole.

The flowchart of the diagnostic-therapeutic process is shown in Figure 5.

Figure 5. Flow chart of analysis process



3.1 Hysteroscopic treatment

In the study group, the diagnosis was obtained by hysteroscopic endometrial biopsy. Hysteroscopic procedures were performed in ambulatory outpatient setting by vaginoscopic approach, without cervical dilatation nor analgesia or anesthesia, with Bettocchi continuous-flow operating hysteroscope (KARL STORZ, Germany) with a diameter of 5 mm and a 5-Fr operating channel. Uterine cavity distension was achieved with saline solution. Using the "Hamou Endomat®" pump (KARL STORZ, Germany), the mean intrauterine pressure was kept constant at 30-40 mmHg, with a flow rate of 200 ml/min, an irrigation pressure of 50-75 mmHg and a suction pressure of 0.25 bars. Endometrial biopsy was performed with 5-Fr grasping forceps using the "grasp biopsy" technique. If the body mass index (BMI) was >30 kg/m2, consultation with the bariatric surgery department was suggested, which initially provided psychological, nutritional, and endocrinological support to modify the diet and lifestyle of these patients, and/or actual bariatric surgery when these measures were not sufficient.

Women underwent fertility-sparing combined treatment, consisting of hysteroscopic resection followed by medical therapy with the insertion of a 52 mg LNG-IUS.

According to Giampaolino et al., 2019, patients with diffuse atypical lesion were treated by superficial endometrial resection preserving the basal layer of the endometrium, with an endouterine Tissue Removal Device (Medtronic, Italy) with an outer diameter of 5.5 mm or 6 mm or with a 15-Fr bipolar resectoscope. Patients with focal development lesions were treated with the “three steps” hysteroscopic resection technique by Mazzon et al., 2005, under loco-regional anesthesia, with a 27-Fr bipolar resectoscope, after progressive dilatation of the cervix with Hegar dilator up to 10 mm, or with a 15-Fr bipolar resectoscope

(KARL STORZ, Germany), with cutting loop; resection of the focal tumor lesion (Step 1), of the endometrium adjacent to the lesion (4–5 mm outside) (Step 2) and the myometrium underlying the lesion (3–4 mm) (Step 3) was performed; multiple endometrial biopsies were also added. After both surgical procedures, a 52 mg LNG-IUS was inserted.

Responses to treatment are classified and explained below:

- **Complete Response (CR):** two successive negative biopsies

- **Partial Response (PR):** regression from EEC to AEH

- **Stable disease (SD):** persistence of the initial lesion

- **Progressive disease (PD):** progression from AEH to EEC or further worsening

- **Relapse (R):** presence of AEH or EEC after obtaining a CR.

Follow-up was based on multiple endometrial hysteroscopic biopsies, performed in ambulatory setting, without removing the LNG-IUS, after 3 and 6 months, then, if negative, every 6 months for 2 years and finally once a year.

3.2 Fertility preservation strategy

Only women with CR, who underwent multidisciplinary counselling with fertility counsellors, gynaecologic oncologists, biologists, and psychologists, on reproductive strategies were admitted in our study. Young women (<35 years old) with no previous history of infertility were encouraged to attempt spontaneous conception and follow-up with multiple endometrial biopsies after 6 months was planned if pregnancy was not achieved. However, women were also informed that performing ART could be beneficial because it has been shown to improve success rates and reduce the interval to conception without an increased risk of recurrence (10).

In cases where ART was chosen, the OS protocol and oocyte retrieval in the study group were performed as follows:

- beginning on day 1-2 of the menstrual cycle (even if it was spotting) Letrozole 5 mg/day was prescribed; in case of amenorrhea from LNG-IUS, OS was started after ultrasound evaluation (synchronous follicular wave, absence of ovarian cyst);

- the day after starting letrozole, recombinant FSH (r-FSH) were administrated based on age and ovarian reserve (from 150 to 300 IU daily);

- all patients were treated with flexible GnRH antagonist protocol (Ganirelix, subcutaneous injection 0.25 mg/day) in case of dominant follicle at the ultrasound (mean diameter >13 mm);

- in women with at least three follicles of mean diameter >17 mm, letrozole was discontinued and 0.2 mg of GnRH agonist (GnRH-a, Triptorelin) was administered subcutaneously to induce ovulation;

- oocyte retrieval (PU) was performed by ultrasound-guided transvaginal aspiration 34-36 hours after the induction of ovulation;

- after PU, Letrozole was re-administered until E2 values < 50 pg/ml were reached;

- the obtained mature oocytes were then subjected to vitrification (Irvine® or Vitrolife® oocyte vitrification kit).

Patients underwent further hysteroscopy with multiple endometrial biopsies within one month after oocyte retrieval to verify the presence of any recurrence. If biopsies were negative, the LNG-IUS was removed. Endometrial preparation was performed by adopting the modified natural cycle protocol. In case of adequate endometrium, the oocytes were fertilized, and the embryo transfer (ET) scheduled on the fifth day of progesterone intake. In all cases we performed a single blastocyst embryo transfer.

Women who were not ready to conceive immediately and chose to postpone pregnancy were advised to maintain LNG-IUS while continuing to have follow-up hysteroscopic endometrial biopsies every 6 months.

Patients of both control groups were stimulated with r-FSH using flexible antagonist protocol and were induced with GnRH-a. Patients of Control Group B also underwent OS with Letrozole, while Control Group A did not use it.

3.3 Statistical Analysis

Continuous and categorical data are presented in mean ± standard deviation and percentage, respectively Shapiro normality test to quantify the distribution of all continuous variables. The two-sided t-test for independent samples was used to assess inter-group differences concerning parametric data. The two-sided Mann-Whitney U test was used to test inter-group differences for non-parametric data, whereas the Chi-square test was adopted to verify differences in terms of categorical data between groups. Results were analysed using the statistical package SPSS 22 for Windows (Statistical Package for the Social Sciences, IBM, New York). A p-value < 0.05 was considered statistically significant.

CHAPTER IV

Results

A total of 25 patients meeting the inclusion criteria were included in the study group: 22 had been diagnosed with AEH and 3 with EEC G1. For 23 patients the first diagnosis was made at our hospital during an office hysteroscopy performed in our unit for several reasons: infertility, endometrial thickness, or abnormal uterine bleeding. Only two patients came to our observation with a histological diagnosis made at another centre and later confirmed by another biopsy and histological analysis in our clinic. Among the women included in the study, the mean age was 34.28 ± 5.88 years (Range 22 - 46) and mean BMI was 28.98 ± 7.43 (21.2 - 53.6) kg/m^2. Six women (24%) were obese, with a body mass index (BMI) >30 kg/m^2. All women were nulliparous. The main characteristics of the patients are shown in Table 2.

Table 2: Characteristics of patients scrutinized.

CHARACTERISTICS	**N = 25**
AGE (mean ± SD) Range	34.28 ± 5.88 22 - 46
BMI Kg/m2 (mean ± SD) (Range)	28.98 ± 7.43 21.2 - 53.6
% Obese women (BMI >30)	24%
% Born in Land of Fire	20%
% Living in Land of Fire	40%

All the patients analysed, underwent hysteroscopic removal of the pathology according to the protocols provided for their diagnoses, and then underwent

insertion of 52 mg LNG-IUS. Histopathological examinations and magnetic resonance confirmed endometrioid histotype, Grade 1, and absence of myometrial infiltration, respectively. None of the patients had complications or adverse effects related to hysteroscopic surgery or hormone therapy. Patients' compliance with follow-up was good, with no refusal of scheduled hysteroscopies.

Of the 25 women enrolled:

- 15 underwent oocyte cryopreservation (Study Group);
- 2 patients decided to try a spontaneous conception;
- 7 patients, although motivated to preserve their fertility, did not wish to get pregnant at the time of survey, and decided to leave the LNG-IUS in situ as a contraceptive device;
- 1 patients postponed fertility treatment or spontaneous conception for endocrinological or metabolic issue.

The comparison between the Study Group and the Control Groups A and B is shown in the Tables 3 and 4, respectively. No statistically significant differences between the study group and the control group A in terms of oocytes and MII oocytes retrieved were found (Table 3). Control group A showed significantly higher fifth day and peak E2 levels and lower total gonadotropin dose and number of days of stimulation than the study group. No statistically significant differences between the study group and the control group B in terms of oocytes and MII oocytes retrieved, total gonadotropin dose and number of days of stimulation and estradiol levels were found (Table 4). Control group A showed significantly higher fifth day E2 levels than the study group.

Table 3: Comparison of characteristics and OS response between the study group and healthy women undergoing ART

	Patients with AEH N=15	**Control group A n= 30**	**p-value**
Age (mean ± SD)	32.93 ± 5.44	33.17 ± 3.88	0.76
BMI (Kg/m2) (mean ± SD)	29.55 ± 7.02	29.12 ± 4.84	0.9
AMH (ng/ml) (mean ± SD)	4.89 ± 4.8	4.29 ± 2.03	0.46
Total oocytes retrieved	10.64 ± 3.99	9.43 ± 3.2	0.25
MII oocytes obtained	9.08 ± 4.09	7.73 ± 2.6	0.43
% mature oocytes	0.82 ± 0.1	0.83 ± 0.14	0.87
Days of stimulation	10.86 ± 2.73	9.3 ± 0.95	0.15
Total gonadotropin dose (IU)	2262.5 ± 462.84	1867.77 ± 391.28	0.09
E2 day 5 (pg/mL)	84.55 ± 69.65	364 ± 301.17	0.04
E2 Peak (pg/mL)	480.76 ± 330.67	1718.57 ± 828.22	<0.001
P Peak (ng/mL)	1.06 ± 0.9	1.01 ± 0.8	0.84

Table 4: Comparison of characteristics and OS response between the study group and women diagnosed with breast cancer undergoing OS with Letrozole

	Patients with AEH N=15	**Control group B n= 30**	**p-value**
Age (mean ± SD)	32.93 ± 5.44	33.44 ± 3.7	0.66
BMI (Kg/m2) (mean ± SD)	29.55 ± 7.02	26.5 ± 3.01	0.06
AMH (ng/ml) (mean ± SD)	4.89 ± 4.8	3.33 ± 2.24	0.58
Total oocytes retrieved	10.64 ± 3.99	10.52 ± 5.58	0.7
MII oocytes obtained	9.08 ± 4.09	8.12 ± 4.98	0.12
% mature oocytes	0.82 ± 0.1	0.81 ± 0.26	0.97
Days of stimulation	10.86 ± 2.73	10.17 ± 1.6	0.58
Total gonadotropin dose (IU)	2262.5 ± 462.84	2113,48 ± 572.58	0.5
E2 day 5 (pg/mL)	84.55 ± 69.65	141.05 ± 87.24	0.12
E2 Peak (pg/mL)	480.76 ± 330.67	525.26 ± 394.8	1
P Peak (ng/mL)	1.06 ± 0.9	1.75 ± 1.57	0.49

Of the 15 stimulated patients, 9 underwent ET. The CPR was 55.6% (5/9), the C-LBR was 44.4% (4/9), the MR was 20 % (1/5). No pregnancy-related disorders occurred during pregnancy.

The treatment did not cause complications affecting fertility (e.g., Asherman's syndrome). The time from LNG-IUS removal to ET averaged 2.3 months, with a range of 1 to 3 months.

No patient had a PD or SD or recurrence during OS. We experienced recurrence only after the removal of LNG-IUS. In detail, three patients diagnosed with AEH had recurrence, occurring 3, 6 and 16 months after removal of LNG-IUS, respectively. We achieved a CR in all of them with the re-insertion of LNG-IUS.

CHAPTER V

Discussion

In this study, we demonstrated that fertility-sparing hysteroscopic combined treatment and subsequent OS with LNG-IUS *in situ* and Letrozole lead to an efficient ovarian response comparing with breast cancer and infertile women with similar ovarian reserve and basal demographic and anthropometric characteristics, while allowing the protection on the endometrium provided by LNG-IUS to be maintained.

Fertility-sparing treatment was managed with hysteroscopic resection in combination with intrauterine progestin therapy with LNG-IUS. This approach was adopted because it is significantly associated with a shorter treatment duration to achieve CR and a longer time to recurrence, compared with progestins alone. This type of combined treatment has also resulted in higher live birth rates than progestin therapy alone. In addition, the hysteroscopic approach is able to provide an accurate diagnosis of the histology type of endometrial tumor and tumor grade, but also a useful cytoreduction and reliable subsequent follow-up through periodic targeted biopsy sampling. In this way, we can not only keep the oncologic disorder under control but also better plan the fertility desire of the women.

In our study, the primary endpoint was to evaluate the ovarian response with Letrozole and LNG-IUS *in situ* comparing with two control groups composed by breast cancer women and infertile women. According to our findings, the stimulation protocol combining the use of Letrozole and LNG-IUS produced a mean number of 9.58 oocytes, which is in line with the recommended parameters to reasonably achieve procedural success. Furthermore, our protocol resulted in good number of clinical and live birth rate, with 44.4% of cumulative live births

observed. We observed also very satisfactory results in terms of safety considering that no women experience recurrence after the OS.

We detected only three recurrences, all of which occurred after LNG-IUS removal. Despite this, the fertility-sparing approach was maintained with the re-insertion of LNG-IUS in these women. This finding is important because it underscores that the risk of recurrence upon discontinuation of therapy is high and therefore all efforts should be aimed at shortening the time these women remain free from therapy.

The high rate of recurrence in the absence of therapy emphasizes the fact that fertility-sparing treatment is by definition a temporary treatment, exclusively aimed at achieving pregnancy, and that when the reproductive desire is exhausted, it is mandatory to proceed to definitive surgical demolitive treatment. For women who do not plan a second pregnancy immediately after the first one, or who wish to maintain their reproductive potential despite recurrence, it is necessary to maintain close surveillance; it is also possible to repeat progestin therapy with a good chance of success, as was also the occurrence in our case.

Although studies directly comparing ART with expectant management in women with endometrial cancer are scarce and inconclusive, there is currently a trend to encourage the use of ART. The main reason is that the use of ART shortens the time to conception and avoids prolonged and uncontrolled estrogen stimulation, resulting in oncologic safety and reduced risk of disease recurrence and progression. In addition, it should be considered that women with AEH or EEC could present conditions that could impair spontaneous conception, such as obesity, chronic anovulation, and polycystic ovary syndrome (PCOS). Notably, hysteroscopy diagnosis of AEH was accidentally carried out in our series even in infertile women seeking medically assisted reproductive treatment. Thus, despite

we cannot provide sufficient data comparing immediate OS plus ART versus expectant management, we believe that the first option could represent the best solution in women with endometrial oncologic lesions candidates for fertility preservation.

The main concern in women with AEH or EEC lies in the elevated estrogen levels caused by gonadotropin administration during OS, which in turn could induce endometrial hyperplasia and potentially endometrial cancer. To counteract this effect, we used Letrozole during OS, which is a valuable strategy to protect against estrogen-sensitive cancers. In fact, estrogen levels in women undergoing Letrozole were drastically and significantly lower than in infertile women undergoing routine OS without Letrozole (Table 3). Apart from the safety profile, the use of Letrozole has a recognized ability to increase ovulatory response by a central mechanism, through reduction of estrogenic negative feedback, but also peripherally, by increasing follicular sensitivity to gonadotropins. Administration of Letrozole during OS is associated with comparable or even better oocyte recovery than traditional protocols, without increasing serum E2 levels. Together with letrozole, the LNG-IUS in situ could balance the OS-related intrauterine exposure to estrogen without affecting oocyte retrieval and improving oncological response. Previous studies of women undergoing ovodonation protocols had demonstrated a normal follicular response to stimulation with exogenous gonadotropins in patients carrying 52 mg LNG-IUS for contraceptive purposes, with no detrimental effects of LNG on oocyte quality, fertilization, and subsequent embryo development.

Even our data confirm that the presence of LNG-IUS in situ during controlled OS does not affect oocyte retrieval and maturation and may help balance the OS-related hyperestrogenism in these patients. Furthermore, the effect of LNG-IUS

on the embryo implantation does not persist beyond its removal, allowing embryo transfer to not be postponed further; in fact, the endometrium of all these patients, up to three months after IUS removal, did not show dysfunctional characteristics and was receptive for embryo implantation.

However, it is necessary to continue close surveillance and to recommend maintenance therapy with LNG-IUS to women who refuse surgery after delivery and who do not plan a second pregnancy immediately after the first one, because of the high risk of recurrence, estimated in literature at 14.1% after hysteroscopic resection followed by progestin therapy. Because of that recurrence rate, the recent ESGO/ESHRE/ESGE guidelines recommend definitive surgical treatment after completion of childbearing age and repeat fertility-sparing treatment in women who wish to maintain their reproductive potential despite recurrence.

CHAPTER VI

Conclusion

Fertility-sparing hysteroscopic combined treatment and subsequent OS with letrozole and LNG-IUS *in situ* could be suggested in women with AEH or EEC who ask to preserve future fertility.

The limitation of our study resides in the lower sample size and single center design. In addition, we do not have enough data to evaluate time to live birth comparing of study group with women who opt for spontaneous conception. A greater number of well-designed studies are required to investigate the effect of OS with letrozole and LNG-IUS in women with AEH or ECC who undergo combined fertility sparing approach, with hysteroscopic resection in combination with intrauterine progestin therapy with LNG-IUS.

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