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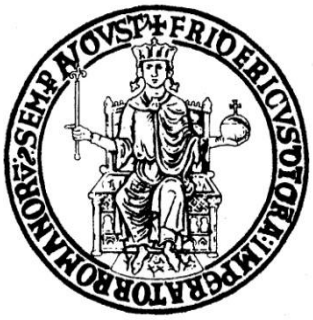

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TESI DI DOTTORATO

"NEW STRATEGIES IN THE APPROACH TO ENDOMETRIAL CANCERS IN YOUNG WOMEN WITH DESIRE OF OFFSPRING"

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Introduction

1.1 Endometrial cancer: characteristics of lesions

Endometrial cancer—a tumour originating in the endometrium—is the most common gynaecological tumor in developed countries, and its prevalence is increasing (1) As the disease is frequently symptomatic at an early stage, endometrial cancer is often diagnosed at stage I.

Endometrial cancer is the fifth most common cancer in women (4.8% of cancers in women), who have a cumulative risk of 1% of developing the disease by age 75 years (1)

Although endometrial cancer is conventionally thought to be a cancer of the postmenopausal period (ie, the sixth and seventh decades of life), 14% of cases are diagnosed in premenopausal women, 5% of whom are younger than 40 years (2-4). The increased incidence of endometrial cancer in Europe and North America could be related to a greater overall prevalence of obesity and metabolic syndromes in these regions, in addition to the ageing of the population (4-7).

The main risk factor is exposure to endogenous and exogenous oestrogens associated with obesity, diabetes, early age at menarche, nulliparity, late-onset menopause, older age (≥55 years), and use of tamoxifen (8-13). The relation between diabetes and endometrial cancer is controversial.

The levonorgestrel releasing intrauterine system might have a protective effect against endometrial malignant transformation (14).

In the past 30 years, endometrial cancer has been broadly classified into two subtypes on the basis of histological characteristics, hormone receptor expression, and grade (table 1) (15). The most common subtype is low-grade, endometrioid, diploid, hormone-receptor-positive endometrial cancer, which has a good prognosis. Type II endometrial cancers are described as non-endometrioid, high grade, aneuploid, TP53-mutated, hormone-receptornegative tumours that are associated with a higher risk of metastasis and a poor prognosis (table 1) (16). Clinical presentation and diagnostic assessment abnormal uterine bleeding—sometimes associated with vaginal discharge and pyometra—is the most frequent symptom of endometrial cancer and is noted in about 90% of patients (usually during menopause). Patients with advanced disease might have symptoms similar to those of advanced ovarian cancer, such as abdominal or pelvic pain and abdominal distension. Disease can easily be diagnosed on the basis of office-based pipelle sampling or other techniques (17, 18). The histological information provided by endometrial biopsy is sufficient for preoperative assessment and planning. However, pipelle sampling can be infeasible in some postmenopausal women because of cervical stenosis. When histological findings from an endometrial biopsy are insufficient to confirm diagnosis, cervical dilation and curettage is recommended, although this investigation necessitates anaesthesia

and has been associated with disease underestimation (19, 20). A biopsy under hysteroscopy remains the gold standard for diagnosis of endometrial cancer and yields higher accuracy than does blind dilation and curettage (20, 21). Results of some studies suggested a higher incidence of malignant peritoneal cytology at the time of hysterectomy in patients who underwent previous hysteroscopy than in those who did not, but no evidence supports an association between diagnostic hysteroscopy and worse prognosis (22). Thus, the standard strategy for investigation of abnormal uterine bleeding is pelvic ultrasonography with an endometrial biopsy in cases of increased endometrial thickness and a hysteroscopy when diagnosis is uncertain (23). A review (24) of 13 studies showed that, in menopausal women, an endometrial thickness cutoff of 5 mm on ultrasonography had sensitivity of 90% and specificity of 54% compared with 98% and 35%, respectively, when the cutoff was reduced to 3 mm (24). Estimated cumulative risk of endometrial cancer is 0.96%; the corresponding mortality risk is 0.23% and mortality to-incidence ratio is 0.24—lower than that of breast cancer (0.32), ovarian cancer (0.63), and uterine cervical cancer (0.55) (1,62). Most endometrial cancers (75%) are diagnosed at an early stage (FIGO stages I or II): 5 year overall survival.

Diabetes mellitus, in particular type II, has long been held as an independent risk factor for endometrial cancer, with an approximate doubling of risk (OR 2.1; 95% CI 1.40–3.41) [10]. However, the fact that people with type II diabetes mellitus (T2DM) tend to be obese is a

confounding factor, and a recent epidemiological study from the United States questioned the independent role of T2DM as a risk factor for endometrial cancer [11].

Nulliparity and infertility are also classical risk factors for endometrial cancer. Among the causes of infertility, polycystic ovarian syndrome (PCOS) seems to be the most important, with an almost threefold increase in risk (OR 2.79–2.89) [12]. However, as with diabetes, obesity seems to be a confounding factor, and the BMI-adjusted OR is lower

Although findings from a recently published meta-analysis have verified the efficacy of the levonorgestrel intrauterine device (LNG-IUD) in preventing *de novo* polyps in breast cancer patients treated with tamoxifen, there was insufficient evidence to ascertain whether the LNG-IUD was associated with any benefit in reducing the incidence of precancerous or cancerous lesions [25]. LNG-IUD but preliminary data using such treatment [added to gonadotropin-releasing hormone (GnRH) analogues] seem to demonstrate similar remission and recurrence rates as oral progestins [26]. Assessment of response must be performed at 6 months with a new D&C and imaging [27].

Response rates associated with the conservative management of endometrial carcinoma are ~75% [28, 29], but recurrence rates are 30%–40% [28, 30, 31]. Standard surgery with hysterectomy should be proposed to non-responders while maintenance treatment for a further 6 months can be considered

in responders who wish to delay pregnancy [32]. Pregnancy is associated with a reduced risk for endometrial cancer recurrence [33]. Findings from recent meta-analyses showed that the pooled live birth rate among women receiving fertility-preserving treatment for endometrial cancer was 28% and reached 39% when assisted reproduction technology was used [28, 34]. Thus, for patients achieving a complete response at 6 months, conception must be encouraged and these patients should be referred to a fertility clinic.

For patients with disease recurrence after an initial response, hysterectomy should be proposed as the first option. Moreover, given the high rate of recurrence, after completion of childbearing (or after the age of potential pregnancy), standard treatment with hysterectomy and salpingo-oophorectomy is recommended. Preservation of the ovaries can be considered in selected cases, depending on the patient's age and genetic risk factors.

Endometrial Hyperplasia (EH) is an irregular proliferative process of the endometrial glands that leads to an increase in the gland to stroma ratio and can progress to type-I EA if left untreated [35]. In the recent *2014 WHO Classification of Tumours of Female Reproductive Organs* [35], EH has been separated into two groups based upon the presence or absence of cytological atypia, in order to highlight the prognostic impact of the presence of atypia on the potentially malignant transformation of the lesion.

Ideal candidates for conservative management are young women with grade 1, early stage EC with no myometrial invasion who are highly motivated to maintain their reproductive potential and understand and are willing to accept the risks associated with deviation from the standard of care. Although for this patient population, it is important to note that there are a few encouraging reports of conservative management of grade 1 EC with superficial myometrial involvement (36) and grade 2 or 3 EC with no myometrial invasion (37, 38, 39–44). Given the paucity of data, conservatively managing higher grade disease should be considered with caution. (45)

Women with EA or Atypical Endometrial Hyperplasia (AEH) should undergo a total hysterectomy with bilateral salpingo-oophorectomy. Although most EAs occur after menopause, approximately 25% of EAs arise in premenopausal women, with 5% in women aged less than 40 years, of whom 70% are nulliparous at the time of diagnosis (45-47). In young women, the majority of cases correspond to the type-I EA, since these are of endometrioid type, focal, well differentiated and limited to endometrium or superficial myometrium (stage FIGO IA, *International Federation of Gynecology and Obstetrics*) (45-47). Consequently, the five year Disease-Free Survival (DFS) rate of up to 99.2% in young women is higher than the 5 year DFS rate of 86% observed in women older than 45 [45]. Therefore, given the excellent oncologic outcomes associated with early stage EA in

young women, the fertility-sparing is an important issue to consider when deciding the most proper management to be taken in women diagnosed with EA and AEH who desire pregnancies.

Atypical Polypoid Adenomyoma (APA) is classified as a mixed epithelial and mesenchymal tumor composed of glands showing cytologic atypia and some architectural complexity set in a myofibromatous stromal component (35). Although the majority of APAs are benign lesions, some authors have reported variable rates of coexistence with or progression to EA (48-52), even in the long term (49, 50), thus suggesting recourse to simple hysterectomy in women who do not desire to have their fertility preserved (52, 54). However, in the majority of cases, APA occurs in young nulliparous women (48-54) and for these patients a standard fertility-sparing treatment has not been clearly defined yet. In literature, high rates of recurrence or persistence of the lesion after conventional conservative approaches have been reported numerously (51-53), thus suggesting the importance of defining an innovative safe and effective conservative treatment.

Focal early stage G1-EA, AEH and APA, accordingly to their malignant potential and their histological characteristics, all three may be indicated as Atypical Endometrial Lesions (AELs).

The conservative management of AELs is generally accepted in young women who desire to preserve their fertility or in women having serious surgical risk factors. To date, fertility-sparing treatment guidelines on EA and AEH have been published (55, 56) though uncertainty still exists in regard to

selection criteria and optimal therapeutic and follow-up managements. Furthermore, treatment guidelines on APA are still lacking.

1.2 Clinical presentation and diagnostic assessment

Abnormal uterine bleeding—sometimes associated with vaginal discharge and pyometra—is the most frequent symptom of endometrial cancer and is noted in about 90% of patients (usually during menopause). Patients with advanced disease might have symptoms similar to those of advanced ovarian cancer, such as abdominal or pelvic pain and abdominal distension. Disease can easily be diagnosed on the basis of office-based pipelle sampling or other techniques (57, 58).

A biopsy under hysteroscopy remains the gold standard for diagnosis of endometrial cancer and yield shigher accuracy than does blind dilation and curettage (59,60).

1.3 Preoperative staging

The role of preoperative staging is to establish recurrence risk group, mainly on the basis of assessment of myometrial and cervical invasion and lymph node metastasis, to define the surgical management. MRI is judged the best imaging technique for preoperative staging and has a high interobserver concordance (61). Some studies (62,63) suggest that transvaginal ultrasonography by an experienced radiologist has similar accuracy to that of MRI for assessment of myometrial and cervical

invasion. Transvaginal ultrasonography is less costly than MRI but cannot be used to assess lymph node status. If MRI is not available, CT can be used to determine extrauterine disease (nodal and peritoneal).

Various series have underlined the high accuracy of 18F-fluorodeoxyglucose PET-CT in detection of myometrial and cervical invasion and lymph node metastatic disease. However, although its prognostic value has been shown for advanced stage endometrial cancer, use in preoperative staging in early stage disease remains questionable (64, 65). Emerging molecular imaging techniques, such as hybrid PET/MRI, could improve diagnostic accuracy through superior soft tissue contrast, multiplanar image acquisition, and functional imaging (66).

1.4 Staging classifications, clinical and biological prognostic factors

The main goal of staging classifications is to define groups of patients with similar outlooks to standardise management and allow comparisons of therapeutic strategies. The 2009 International Federation of Gynecology and Obstetrics (FIGO) and the TNM classifications are the most-adopted classifications (table 1) (67, 68). They are based on surgical staging and include assessment of the extent of myometrial invasion and local and distant metastatic disease—overriding prognostic factors in endometrial cancer (67, 69, 70).

Other prognostic factors not included in the FIGO or TNM classifications have also been identified: histological type and grade, the patient's age, tumour size, and lymphovascular space involvement.

Table 1: **FIGO and TNM classification of endometrial cancer, by surgical and histological characteristics**

1.5 Survival

Estimated cumulative risk of endometrial cancer is 0,96%; the corresponding mortality risk is 0,23% and mortality - to- incidence ratio is 0,24 —lower than that of breast cancer (0,32), ovarian cancer (0,63), and uterine cervical cancer (0,55) (71, 72). Most endometrial cancers (75%) are diagnosed at an early stage (FIGO stages I or II): 5 year overall survival anges from 74% to 91%;5,63 for FIGO stage III, 5 year overall survival is 57–66%, and for FIGO stage IV disease is 20–26% (73, 74). 5 year disease-free survival is estimated at 90% in patients without lymph node metastasis, 60–70% in those with pelvic lymph node metastasis, and 30–40% in those with para-aortic lymph node metastasis. However, a substantial proportion of patients with endometrial cancer die from other health conditions as these patients often have several comorbidities.

Survival is dependent on other predictive factors, such as the tumour grade, age, comorbidities, tumour diameter, American Society of Anesthesiologists score, lymphovascular space involvement, and postoperative complications at 30 days. (75, 76–81).

1.6 Principles of surgical treatment

Total hysterectomy and removal of both tubes and ovaries is the standard treatment for apparent stage I endometrial cancer and is effective in most cases. Alternatives to primary hysterectomy in women who want to preserve their fertility have been comprehensively reviewed (82). Hysterectomy and adnexectomy can be done with minimally invasive techniques (laparoscopy or robot-assisted surgery), vaginally, or laparotomically. The safety of laparoscopy has been shown in randomised clinical trials (83, 84) and is associated with shorter hospital stays and fewer postoperative complications than laparotomy.

Laparoscopic or robotic approaches should be avoided in cases of bulky uterine malignant disease that might necessite morcellation, because morcellation can lead to tumour spillage, increasing local or peritoneal recurrence and thereby affecting survival. Although simple total hysterectomy is sufficient for most women, radical hysterectomy is sometimes done in cases of gross cervical invasion or when uncertainty exists about whether the primary tumour is endocervical or endometrial in origin. Surgical staging for endometrial cancer includes careful assessment of the peritoneal surfaces. Omental and peritoneal biopsies are commonly done in high-risk disease.

1.7 Lymphadenectomy and sentinel node biopsy

Surgical assessment of lymph nodes for staging at primary surgery remains one of the most varied practices worldwide, ranging from no nodal assessment, to sentinel node mapping, to complete pelvic and aortic lymphadenectomy up to the renal vessels. Most clinicians agree that excision or biopsy of suspicious or enlarged lymph nodes in the pelvic or para-aortic regions is important to exclude nodal metastatic disease. Pelvic nodal dissection and pathological assessment continue to be important aspects of surgical staging for apparent stageIendometrialcancerinmanypractices,andmight be based on preoperative criteria such as histology, grade, or MRI findings, or on intraoperative histology. Para-aortic nodal assessment from the intramesenteric and infrarenal regions is also done for staging selected high-risk tumours, such as deeply invasive lesions, high-grade endometrioid endometrial cancers, and type II disease (85).

The extent of lymphadenectomy varies tremendously between practices and practitioners. However, no survival advantage has yet been associated with staging lymphadenectomy in prospective, randomised, clinical trials. Furthermore, between 8% and 50% of patients develop limb lymphoedema, depending on the number of nodes removed, extent of lymphadenectomy, and use of adjuvant treatment (86). Most available retrospective evidence suggesting a survival advantage with lymphadenectomy is from historical series of selected patients and contrasts sharply with findings from prospective randomised trials (87, 88).

Type II endometrial cancers account for 10–15% of endometrial cancers but cause 40% of deaths because of the high incidence of associated extrauterine disease, especially lymph node metastasis. Surgical manage- ment includes hysterectomy with bilateral salpingo- oophorectomy, pelvic and para-aortic lymphadenectomy, omentectomy, and peritoneal biopsies (89-93).

2. I STUDY: Fertility-sparing treatment of atypical endometrial lesions in young women

We have conducted a retrospective study that assess safety and effectiveness of combined hysteroscopic and medical treatments of AELs in young women who desired to preserve their own fertility.

2.1 Materials and Methods

This was a multi-unit retrospective case series study carried out on young women referred to two Operative Units of the Department of Obstetrics and Gynecology of the "Federico II" University (Naples), diagnosed with AELs and treated by fertility-sparing combined hysteroscopic and medical treatments.

After the approval by the institutional review board, we collected data on the clinical history of patients, and on the diagnostic and therapeutic procedures performed, through querying electronic databases and reviewing individual medical records available from 2007 to 2016.

We analyzed sixty-nine young women diagnosed with AELs and treated by combined hysteroscopic and medical treatment between 2007 and 2016. All patients underwent gynecological examination and Transvaginal-Ultrasonography (TV-USG), and the histological diagnosis of AEL was obtained by endometrial biopsy performed during Office Hysteroscopy (OfH) using miniaturizing instruments (grasping forceps).

Of these sixty-nine women, thirteen were diagnosed with Atypical Polypoid Adenomyoma (APA); forty-two with Atypical Endometrial Hyperplasia (AEH); fourteen with focal well differentiated Endometrial Adenocarcinoma (G1-EA). The patients who had been diagnosed with EA repeated TV-USG and performed contrast-enhanced abdomen and pelvis Magnetic Resonance to exclude

myometrial or cervical invasion and extrauterine metastases. In these patients serum CA-125 and CA19.9 levels were also evaluated.

Every woman who underwent conservative treatment was motivated to preserve her own fertility and was counseled for the risk of recurrence or progression of diseases associated with deviation from demolitive surgical approaches.

Patients diagnosed with APA (n=13) or focal G1-EA (n=14) were treated by Hysteroscopic Resection (HR) in three steps: 1) removal of the exophytic tumor lesion; 2) removal of the endometrium adjacent to the lesion; and 3) removal of the muscle layer beneath the lesion, according to the technique first described by Mazzon et al. [17]. The patients diagnosed with AEH (n=42) were treated by superficial endometrial resection (i.e. preserving the basal layer of the endometrium). All the operative hysteroscopic procedures were performed by expert surgeons (A.D.S.S. and A.M.) under general anesthesia. The cervix was dilated to 10 mm with Hegar's dilator, and a 27 Fr bipolar resectoscope (ETHICON INC, Sommerville, New Jersey, USA) with 12° or 30° lens was introduced. The uterus was distended with normal saline and a 4-mm cutting loop electrode and Versapulse modality were used. Immediately after the Operative Hysteroscopy (OH), a Levonorgestrel-Intrauterine Device (LNG-IUD) (Mirena, Bayer HealthCare, PA, USA.) was inserted in all patients.

For every patient we collected all the pathology reports of hysteroscopic biopsies performed during the follow-up period of 24 months, as well as data on eventual pregnancies. For one patient diagnosed with AEH, who experienced relapse at 24 months and who was submitted to a second cycle treatment (R^{IV}, **Table 1**), pathology reports were collected beyond the 24 months in order to assess her clinical status.

The histological examinations were classified as Complete Response (CR), Partial Response (PR), Stable Disease (SD) and Progression (P). CR was defined as absence of any AEL; PR as regression of EA to AEH and regression of AEH to hyperplasia without atypia; SD as persistence of pre-treatment lesions; P as worsening of the histological grade of lesions. Relapse (R) was defined as the presence of AELs after CR had been previously achieved. In this case series we retrospectively evaluated the regression, relapse, and pregnancy rates during a follow-up time of 24 months.

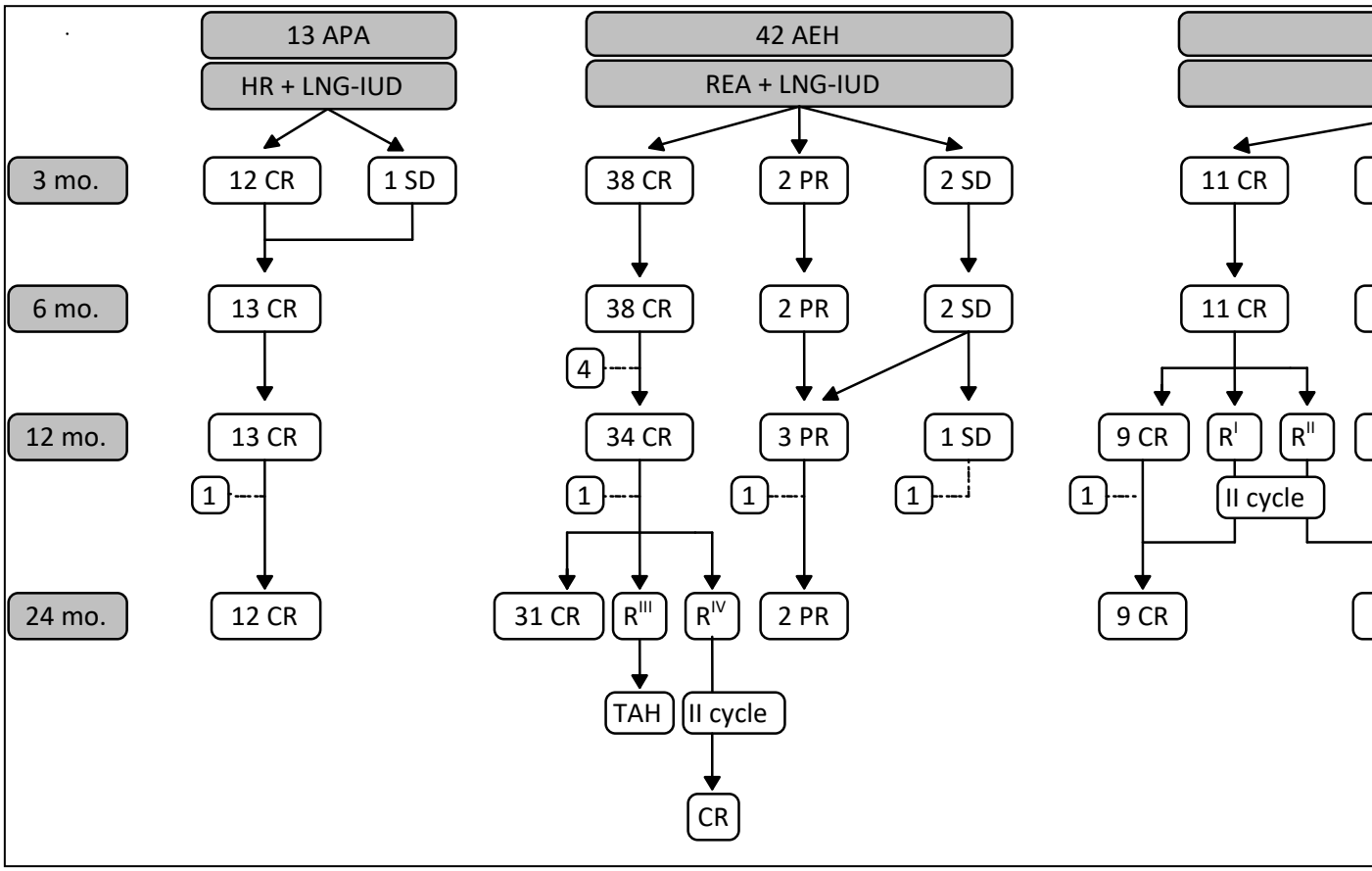
Table 1: Oncologic and reproductive outcomes of patients with AELs conservatively treated

OfH diagnosis	n°	Treatment	Response at 3 months			Response at 6 months			Response at 12 months				Response at 24 months				Pregnancies (n° of patients)
			CR	PR	SD or P	CR	PR	SD or P	CR	PR	SD or P	R	CR	PR	SD or P	R	
APA	13	HR + LNG-IUD	12	-	1 SD	13	-	-	13	-	-	-	12 CR, 1†	-	-	-	3 NSFTD 1 ECS
AEH	42	REA + LNG-IUD	38	2	2 SD	38	2	2 SD	34, 4†	3	1SD	-	31, 5†	2, 1†	1†	R^{III}, R^{IV}	4 NSFTD 2 ECS
Focal G1-EA	14	HR + LNG-IUD	11	1	1SD, 1 P*	11	1	1SD	9	1	1SD	R^{I}, R^{II}	9, 1†	1	2 SD**	-	0

OfH: Office Hysteroscopy; APA (Atypical Polypoid Adenomyoma); AEH (Atypical Endometrial Hyperplasia); G1-EA (well differentiated Endometrial Adenocarcinoma); HR (Hysteroscopic Resection) in three steps; REA (Resectoscopic Endometrial Ablation); LNG-IUD (Levonorgestrel - Intrauterine Device); CR: Complete Response; PR: Partial Response; SD: Stable Disease; P: Progression; R: Relapse; R^{I}: patient underwent re-treatment with HR and LNG-IUD and achieved a second CR, response maintained at 24 months; R^{II}: patient underwent re-treatment with HR and LNG-IUD, however she showed SD and was consequently submitted to demolitive surgery at 24 months; R^{III}: definitive surgery at 24 months; R^{IV}: patient underwent re-treatment with REA and LNG-IUD and achieved a second CR, response maintained at 3 and 6 months after second-cycle treatment; NSFTD: Normal Spontaneous Full Term Delivery; ECS: Elective Caesarean Section at 39 weeks.

† data not available because patients had not reached the follow-up target time; * Definitive surgery at 3 months; ** Definitive surgery at 24 months (two patients with focal G1-EA and SD; one patient with G1-EA/AEH and P)

Figure 1: Schematic representation of patients



APA (Atypical Polypoid Adenomyoma); AEH (Atypical Endometrial Hyperplasia); G1-EA (well differentiated Endometrial Adenocarcinoma); HR (Hysteroscopic Resection) in three steps; REA (Resectoscopic Endometrial Ablation); LNG-IUD (Levonorgestrel - Intrauterine Device); CR: Complete Response; PR: Partial Response; SD: Stable Disease; P: Progression; R: Relapse. TAH: Total Abdominal Hysterectomy (with bilateral salpingo-oophorectomy).

Dotted line: patients who had not reached the follow-up target time.

2.2 Results

A total of sixty-nine patients were analyzed in this retrospective study. The mean age was 32.6±3.5 yrs (range 20-44) and the mean Body Mass Index was 25.9±5.3 Kg/m^2. Twenty-nine patients (42%) had not had previous pregnancies and fifteen of them (51.7%) referred primary infertility. None of the enrolled patients had any complications or adverse effects related to the endoscopic surgery or the hormonal therapy.

Histological analysis of surgical specimen obtained by OH confirmed in all patients pre-treatment hysteroscopic diagnosis and, in patients who had been diagnosed with G1-EA, the absence of myometrial infiltration.

The oncologic and reproductive outcomes are detailed in **Table 1**. The schematic representation of patients according to their treatment and follow-up is given in **Figure 1**.

At 3 months of follow-up, data on sixty-nine patients were collected. Sixty-one patients (88.4%) obtained a CR, three patients (4.3%) obtained a PR, four patients (5.8%) showed SD, while one patient (1.5%), who had been diagnosed with focal EA-G1, showed P of the lesion and underwent definitive surgery: the pathology report of the surgery specimen indicated Stage FIGO IA G3-EA (lesion invaded less than half of myometrium).

At 6 months of follow-up, one patient diagnosed with APA, who had previously shown SD, achieved a CR.

At 12 months of follow-up, one patient diagnosed with AEH, who had previously shown SD, achieved a PR. Two patients first diagnosed with focal G1-EA, both of whom had previously achieved a CR, showed R and were re-treated with a second-cycle HR and LNG-IUD: the first patient (R^{I}, **Figure 1**) achieved and maintained a CR at 3, 6 and 12 months after second-cycle treatment; the second one (R^{II}, **Figure 1**) showed SD at 3, 6 and 12 months after second-cycle treatment and underwent definitive surgery at 24 months of follow-up.

At 24 months of follow-up, two women, first diagnosed with focal G1-EA, were submitted to definitive surgery: one had shown SD since the third month of follow-up; the other (R^{II}, **Figure 1**) has been already described above. One patient (R^{III}, **Figure 1**) first diagnosed with AEH, who had previously achieved a CR, showed R and underwent definitive surgery. Another patient (R^{IV}, **Figure 1**) first diagnosed with AEH, who had previously achieved a CR, showed R and underwent re-treatment with a second-cycle REA and LNG-IUD; she achieved and maintained a CR at 3 and 6 months after re-treatment.

Follow-up data on nine patients were incomplete. These patients were detailed as follows: one APA patient with CR at 12 months; four AEH patients with CR at 6 months; one AEH patient with CR at

12 months; one AEH patient with PR at 12 months; one AEH patient with SD at 12 months; one focal G1-EA patient with CR at 12 months.

Till the end of follow-up period, out of the sixty-two patients who had achieved a CR, 8.2% (4/49) of patients with AEH or EA and 0% (0/13) of patients with APA showed relapse of lesions.

At 12 months of follow-up, the complete responders were proposed to remove the LNG-IUD and attempt to conceive. Out of the fifty-six patients who had achieved and maintained CR, twenty-five (44.6%) removed LNG-IUD. The remaining thirty-one patients (55.4%), although had been motivated to preserve their fertility, did not wish to get pregnant at the time of survey; therefore, they decided to leave the LNG-IUD in situ as contraceptive device. Among patients who had removed LNG-IUD, ten patients (40%) became pregnant after natural conception in the following twelve months. Three babies were born by caesarean section at 39 weeks of gestation, and seven were born at term spontaneously. No complications occurred during pregnancy and delivery.

2.3 Discussion

In this retrospective study we analyzed the oncologic and reproductive outcomes of patients who had been diagnosed with AELs and treated by combined OH and hormonal therapy.

The efficacy and safety of oral and intrauterine-released progestins as fertility-sparing treatments in patients with early stage G1-EA and AEH have been evaluated in many studies, even recently (94-98). The most contemporary meta-analysis demonstrated that the women with EA or AEH managed with progestins had a pooled CR rate of 71%, though the authors did not report the separate CR rates of the two atypical lesions (99). A previous meta-analysis on women with EA or AEH conservatively treated reported a pooled regression rate of 76.2% for EA and 85.6% for AEH (100), although the authors did not perform a subgroup analysis for each of several treatments included (oral progestins, LNG-IUD, HR). However, the major effectiveness of LNG-IUD compared to oral progestins in women with AEH was observed in a meta-analysis (101) and in a comparative cohort study (102) by the same author. By contrast, in women with early stage EA the use of LNG-IUD was found to be similarly effective compared to oral progestins (103), although the use of LNG-IUD alone in EA patients has not been extensively reviewed as oral progestins. More recently, HR of EA and AEH followed by oral or intrauterine-released progestins has been demonstrated to be an effective fertility-sparing treatment (104-108).

In our series, all women with focal early stage G1-EA had undergone HR of the lesion in three steps followed by insertion of LNG-IUD. This approach resulted in an overall response rate of 85.7% (12/14) and in a CR rate of 78.6% (11/14), while two patients did not respond to the first-cycle treatment and were consequently submitted to definitive surgery: the first one had shown progressive disease at 3 months; the second one had shown SD after 24 months of conservative therapy. At our knowledge, this is the second largest series of women with focal early stage G1-EA treated by such a combined conservative technique, after Falcone et al. (108), and our results, although not as noticeable as those reported by other authors (104-108), essentially confirmed the major effectiveness of such combined approach. In fact, the CR rate achieved in our series (78.6%) was within the range of 78% to 100% CR reported by Falcone et al. (108) in their Literature review of the studies on combined therapies, and was higher than those reported in most recent studies and meta-analyses on progestin therapies alone (97, 99, 100), as partly detailed above.

In contrast with EA, limited data are available in literature on the combined surgical and medical treatment of AEH (106-110). Shan et al. (110) reported a CR rate of 83.3% in patients with AEH treated by extensive endometrial hysteroscopic curettage followed by Megestrol Acetate. More recently, De Marzi et al. (106) reported a CR rate of 100% in patients with AEH treated by HR of hyperplastic areas and subsequent hormonal therapy. In our series, all patients diagnosed with AEH

underwent superficial endometrial resection followed by insertion of LNG-IUD. As already described by Shan et al. (110), this technique allows the removal of most, if not all, cancer tissue, thus probably increasing the responsiveness to progestin treatments. Furthermore, the preservation of the basal layer of the endometrium allows the complete regeneration of endometrium after the end of the medical treatment, thus preserving women's childbearing potential. Our combined approach resulted in an overall response rate of 95.2% (40/42) and in a CR rate of 90.5% (38/42). At our knowledge, this is the largest series of patients with AEH treated by such a combined treatment and the CR rate achieved was higher than those reported in recent studies on women with AEH treated by oral progestins alone (range 46.2%-81.8%) (117,118) and by LNG-IUD alone (range 67%-76.2) (117,118). However, the high CR rates, up to 100%, observed in women with AEH treated by LNG-IUD alone and reported in older and more recent studies (111-113), would indicate the need for further comparative cohort studies.

Unlike other AELs, there are few data available in Literature on conservative treatment of patients with APA. In the past, blunt curettage had represented the conventional conservative treatment but some authors described its limitations (119-120). HR had been therefore recommended by many authors (119, 54, 114, 115) as conservative treatment for APA. The oncologic and reproductive outcomes of a hormonal therapy for APA have been recently reported (121). At the best of our

knowledge, this is the first case series of patients with APA treated by HR of the lesion and subsequent insertion of LNG-IUD. At 12 months of follow-up, 100% of patients (13/13) diagnosed with APA achieved a CR. Matzumoto et al. (52) had already described the superiority of HR over the blunt curettage after having compared respective recurrence rates (10% for HR and 36.4% for blunt curettage), and our results also confirmed the higher effectiveness of hysteroscopic surgery.

With regard to pregnancy outcome, a meta-analysis of women with AE and AEH, managed with fertility-sparing treatments, found pooled live birth rates of 28% and 26.3% respectively (100). In recent studies on women treated by hysteroscopic and medical treatment, De Marzi et al. (106) and Falcone et al. (108) reported a pooled live birth rate of 21.7% and 50% respectively. In our series no patient with EA got pregnant while 14.3% (6/42) of patients with AEH did, although this rate would have been higher (17.1%) if we had considered only the patients who had reached the 24-month follow-up (6/35). These low rates could be explained by the more restrictive follow-up time retrospectively analyzed in our series compared to the mean follow-up times of other authors (104, 108). Furthermore, in our series 30.7% (4/13) of patients diagnosed with APA got pregnant within 24 months. This was consistent with the live birth rates reported by Nomura et al. [8] (22.2%) and Grimbizis et al. (49) (22.2%) in most recent studies on patients with APA conservatively treated.

With regard to recurrences, the most contemporary meta-analysis (99) showed an overall Relapse Rate (RR) of 20% in women with AEH or early-EA successfully treated by progestins, over a median follow-up time ranging from 0.3 to 98 months, and the RR was higher in women treated by oral progestins than those treated by intrauterine-released progestins. This RR seems to be higher than the 8.2% (4/49) RR observed in our series of patients with AEH or early-AE.

Concerning only the AEH, in our series two patients first diagnosed with AEH (5.3%, 2/38), who previously achieved CR, were found to have a Relapse of the lesion at 24 months (R^{III}, R^{IV}, **Table 1**). In a cohort study on patients with AEH medically treated, relapse of hyperplasia occurred in 27.3% of women treated by LNG-IUD and 50% of women treated by oral progestins (116), and a consistent number of patients relapsed during the first 24 months. Moreover, the risk of relapse of AEH is especially high in the first 2 years from diagnosis (104). In the most recent study on women with AEH treated by combined approach, De Marzi et al. (106) reported a RR of 0% (0/20) after a mean follow-up time of 25 months. Taking into account all the previous observations, our results might confirm the major safety of the combined treatment compared to the progestin therapies alone.

Considering only the early-EA, in our series two patients first diagnosed with G1-EA (2/11, 18.2%), who had previously achieved CR, were found to have a Relapse of the lesions at 12 months (R^{I}, R^{II}, **Table 1**). A meta-analysis of seven studies (103) reported a 24.6% RR in women with EA treated by

progestins alone during a mean follow-up of 27 months. Another contemporary meta-analysis (116) on women with early EA showed a pooled recurrence rate of 40.6% after successful fertility-sparing therapy, although the median follow-up time of the studies included ranged from 11 to 76.5 months, and thus it was longer than ours. Our findings suggest that the combined treatment might be safer than progestin therapies alone, thus confirming what was described by other authors. In fact, Falcone et al. (108) had already observed that the pooled recurrence rate of EA in studies on women treated by combined therapy (16%) was lower than that reported in most recent studies on progestin therapies alone (32%).

In regard with APA, among most recent studies, Nomura et al. (121) reported a RR of 44.4% and the median time to recurrence was 5 months; Grigoris et al. (122) reported two relapses after 5 months and 5 years respectively; Matzumoto et al. (119) observed a RR of 23.8% during a mean follow up of 38.9 months. In our series after 24 months of follow-up no patient experienced recurrence of lesion, suggesting that HR followed by hormonal adjuvant therapy might be proposed as a safe conservative treatment.

In conclusion, taking into account the oncologic and reproductive outcomes, we believe that OH with subsequent insertion of LNG-IUD could be considered an efficient and safe approach in the management of AELs in young women who wish to preserve their own fertility. However larger

series and randomized clinical trials are needed to further assess the effectiveness of such combined treatments.

3. Endometrial cancer and PCOS

A state of hyperestrogenism with endometrial hyperplasia are the etiopathogenetic causes of endometrial cancer, both in early and advanced disease stages, (ie excessive proliferation of the endometrial mucosa). Endometrial hyperplasia is mostly the result of persistent and prolonged estrogenic stimulation of endometrium (absolute or relative hyperestogenesis). Endogenous absolute hyperestrogenic conditions are determined by obesity, the presence of polycystic ovary syndrome (PCOS) and the presence of granular or adrenal cell tumors.

A growing scientific interest is emerging in endocrinology and gynecologic oncology in assessing the risk of endometrial carcinoma in women with PCOS. Since the risk of endometrial cancer (CE) is strongly associated with high circulating levels of estrogen, all pathological conditions leading to this hormone may promote proliferation of endometriosis. This is the case for women with PCOS who show a high risk of developing atypical endometrial hyperplasia (EH), considered precancerous lesion, which may progress toward the development of endometrial cancer. However, an assessment of the prevalence of atypical endometrial hyperplasia in women with PCOS remains a widely debated

matter. The existing biological relationship between PCOS and CE, though still unclear, refers to a combination of complex reproductive and metabolic disorders: in fact, chronic anovulation, obesity and hyperinsulinemia also cause progesterone deficiency. As a result, the endometrium tends to remain in a proliferative state of estrogen dependent, thus increasing the risk of developing endometrial cancer (123). In addition, insulin resistance, other metabolic impairment in PCOS, results in an increase in IGF-I, and synergistically play a key role in the proliferation and differentiation of endometrial cells, promoting EC development (124,125). Many studies in the literature confirm a higher risk of developing an endometrial cancer in women with PCOS (124-128). Indeed, observational studies have reported that women with PCOS have a threefold higher risk of developing a well-differentiated endometrial carcinoma and this has recently been reported in a meta-analysis by Haoula et al (124) which confirmed that the risk of developing an endometrial cancer is three times higher than in the general population.

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders of the reproductive age. It is a complex, multidimensional endocrine-metabolic disorder that has profound repercussions on women's health not only in fertile ages but throughout the entire life span (129). PCOS affects about 4 to 8% of women of childbearing age. According to the European Society of Human Reproduction and Embriology (ESHRE) and the American Society of Reproductive Medicine

(ASRM), the diagnosis is based on the presence of at least two of the following three symptoms: oligo-anovulation or anovulation, hyperandrogenism and/or hyperandrogenemia, polycystic ovaries. One of the major disorders caused by PCOS is anovulatory infertility. There are several methods to treat anovulatory infertility by PCOS, and these include both simple interventions such as lifestyle changes, and more complex interventions such as laparoscopic ovarian drilling (LOD). Reduction in body weight is considered first-line therapy in patients with overweight and obese PCOS associated with oral sensitizers such as metformin (129). Currently, the high-grade drug for induction of ovulation in patients with PCOS anovulatory infertility remains clomiphene citrate (CC) (130) and has been around for nearly 50 years. The CC has good management, is inexpensive and has minimal side effects. Acting as an estrogen receptor antagonist in the hypothalamus and at the anterior pituitary, increases FSH secretion and induces ovulation in 75-80% of patients, but only 35-40% of patients are able to get pregnant (131). The discrepancy between the percentage of response to induction of ovulation and pregnancy rates is thought to be mainly related to its anti-estrogenic activity on cervical function and endometrial receptivity. In addition, increased CC secretion induced by CC, which does not occur with FSH administration, may also reduce the probability of conception (132).

In addition, although limited, there are some studies that show how CC can, in the long term, double the risk of developing a endometrial cancer (133). Indeed, studies claim that the CC has a structure similar to tamoxifen (SERM) (134, 135) and the latter is recognized as a potentially predisposing factor to endometrial hyperplasia and therefore a precursor to endometrial cancer. CC resistance (ovarian failure with maximal doses, 150 mg per day for 5 days for at least 3 consecutive cycles) or failure of CC, there is a second line of treatment for ovarian stimulation with injectable gonadotropins (FSH). However, their use is burdened with some disadvantages such as OHSS (Ovarian Hyper-Stimulation Syndrome) or multiple pregnancies (132). Laparoscopic Ovarian Drilling (LOD) has been shown to be a viable therapeutic alternative for patients with PCOS-resistant patients, for patients who are hyper-responsive to gonadotrophin therapy and for patients who need diagnostic laparoscopy for infertility (136). A recent Cochrane has demonstrated the efficacy of LOD in terms of pregnancy rate in the absence of increased risk of multiple pregnancies or ovarian hyperstimulation (137). The mechanism of action is that the destruction of ovarian follicles and part of ovarian stroma results in local and systemic reduction of androgens and inhibition levels followed by an increase in FSH levels that promotes follicular maturation and subsequent ovulation (138). However, two major disadvantages do not allow laparoscopic ovarian drilling to be considered the first line of PCOS

treatment: the risk inherent in surgery as such and the risk associated with the formation of post-operative adherence that may further interfere with fertility .

Therefore, considering the interest addressed to PCOS in a study by the title “Post-operative ovarian adhesion formation after ovarian drilling: a randomized study comparing conventional laparoscopy and transvaginal hydrolaparoscopy”, we conducted a review of the literature and described a new mini-invasive surgical approach to ovarian drilling in patients with PCIs anovulatory infertility, using the Transvaginal Hydrolaparoscopy technique to reduce the rate of ovarian adherence. From the results we have shown that patients undergoing ovarian drilling by THL showed a reduction in adhesion formation compared to patients treated with classical technique (LOD) (15.5% vs. 70.2%).

3.1 II STUDY: Post-operative ovarian adhesion formation after ovarian drilling: a randomized study comparing conventional laparoscopy and transvaginal hydrolaparoscopy

Laparoscopic ovarian drilling (LOD) is an alternative method to induce ovulation in CC-resistant anovulatory patients affected by PCOS, as an alternative to gonadotropins (136, 139). However, there are concerns regarding the long-term effects on ovarian function of LOD, in particular iatrogenic adhesions and decreased ovarian reserve (DOR), which may potentially jeopardize future fertility (140). Fernandez et al. (141) suggested a new approach for ovarian drilling using Transvginal

hydrolaparoscopy (THL). They showed THL to be a safe and feasible alternative procedure to LOD. Since THL could be considered less invasive than laparoscopy, infertile women with PCOS may benefit from ovarian surgery performed using a mini-invasive approach that may limit post-operative ovarian adhesions and return to daily activities.

Aim of our study was to compare conventional LOD with THL ovarian drilling in terms of ovarian adhesion formation, evaluated using office THL during follow up.

3.2 Materials and methods

This open, randomized study was performed at the Department of Obstetrics and Gynecology of the University of Naples "Federico II". During the period from December 2009 and July 2015, 286 patients CC-resistant with PCOS were considered for inclusion in the study. Inclusion criteria were: age 18-40 years; PCOS diagnosis according to 2003 Rotterdam criteria (142). Exclusion criteria: endocrine anomalies other than PCOS (congenital adrenal hyperplasia, Cushing syndrome, previous administration of androgens, and androgen secreting tumor - AST); any disease potentially responsible of ovarian adhesion (i.e., ovarian cyst, endometriosis, pelvic infection, pelvic inflammatory disease), previous abdominal or pelvic surgery, presence of adhesions, fixed retroverted uterus; lateral displacement of the cervix; suspect pelvic tumor; vaginal infection; abnormalities at vaginal examination and transvaginal ultrasound; psychiatric condition impairing the ability of the

patients to cooperate, obliteration of the pouch of Douglas or inability to perform the vaginal exam and any other contraindication to THL or laparoscopy.

The study design was approved by the institutional review board (IRB), and written informed consent was obtained from each patient.

The flow of patients throughout the study is illustrated in Figure 2.

Two hundred and forty- six patients satisfied the inclusion criteria. Forty patients were not included in the study: twelve refused to undergo the randomization process and twenty-eight did not meet the inclusion criteria. Two hundred and forty- six patients were randomized into two groups in a 1:1 ratio by use of a randomization list generated by a computer with blocks of 4. One hundred and twenty-three patients were scheduled to undergo LOD and 123 to undergo THL ovarian drilling. Before the procedure, all patients underwent a clinical examination, routine preoperative biochemical testing and a transvaginal pelvic ultrasound scan. The allocation sequence was concealed from the researchers, who enrolled and assessed the participants and attached a sequentially numbered, opaque, sealed, and stapled envelope containing the allocated treatment to the clinical record of the patient after having signed the informed consent. The envelope was opened on the morning of the procedure. Patients and surgeon were not blinded to the procedure performed because concealment was not possible due to the differences in the procedures.

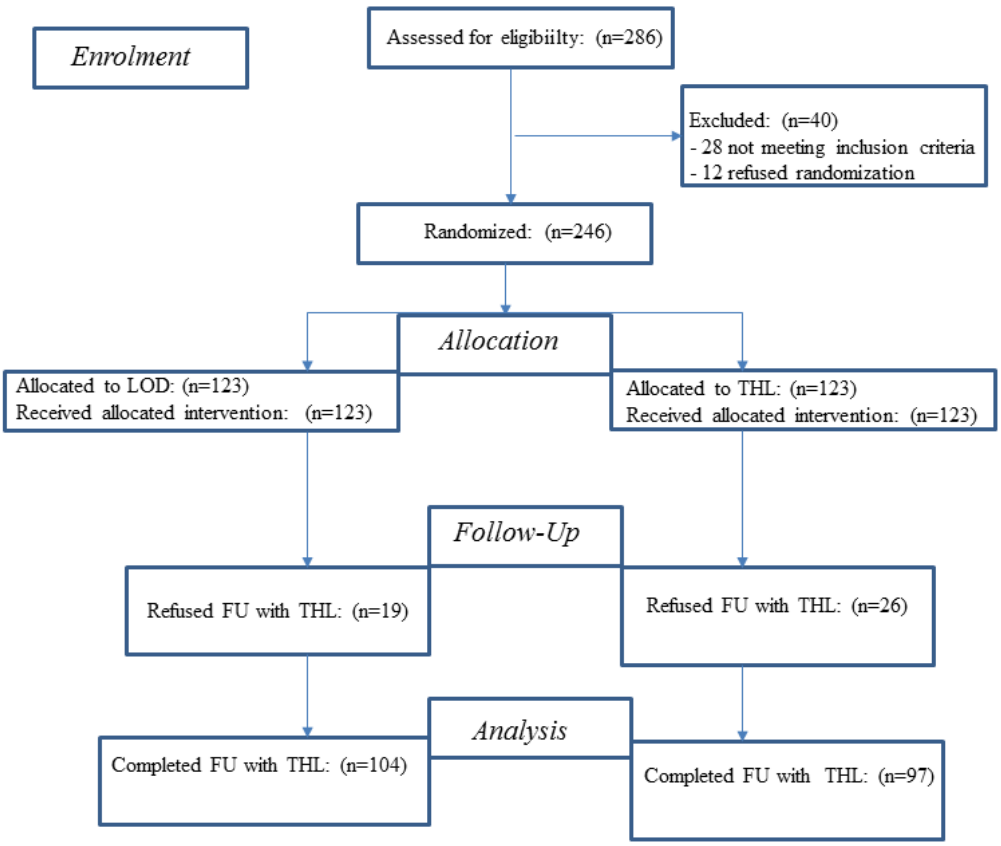


Figure 1. Flow of patients enrolled in the study

All procedure were performed by a single surgeon who already performed more than 50 procedures before the beginning of the study. Patients in group A (n = 123) underwent ovarian drilling by laparoscopy under general anesthesia, while patients in group B (n = 123) underwent ovarian drilling with THL under spinal anesthesia.

All subjects received antibiotic prophylaxis immediately before the procedure (cefazolin 2 g intravenously). Laparoscopic ovarian cautery was performed using a traditional 10- mm endoscope inserted through the primary sub umbilical trocar, with two additional 5-mm trocars in the lower

abdomen. A grasping forceps was introduced through one of the lower abdominal 5 mm trocars to grasp the utero-ovarian ligament and lift the ovary away from the bowel. The other access was used to introduce the unipolar needle. The whole length of the needle was inserted with a 90° angle into the ovary using an unipolar needle electrode with a power setting of 40 W for 4-5 seconds set at 30 W per ovary (the lowest recommended dose) (143). The ovarian punctures were uniformly distributed throughout the ovarian surfaces. Three to six punctures per ovary were performed depending on the size of the ovary, since it was demonstrated that these numbers yield statistically significantly higher ovulation and pregnancy rates (144). THL was performed with the patients in gynecologic position, under spinal anesthesia. Using a Verres needle, 300 mL of normal saline solution was instilled into the peritoneal cavity through the posterior vaginal fornix. A specially designed needle dilating trocar system with a total diameter of 3.9 mm was placed 10–20 mm below the insertion of the posterior vaginal wall to the cervix. A 2.7 mm diameter semi rigid endoscope with a 3.5 mm sheath was used with an optical angle of 30° to visualize the pelvic cavity (Karl Storz, GmbH & Co., Tuttlingen, Germany). The posterior aspect of the uterus was examined first, followed by the ovary and fallopian tube on each side. A bipolar electrosurgical probe (Versapoint- Gynecare Inc., menlo Park, CA) was introduced through the auxiliary channel of the sheath. By using this probe, the ovarian cortex was drilled in 3 to 6 points per ovary, using a power setting of 110 to 130 W.

The informed consent that the patients signed before being included in the study stated the possibility to undergo laparoscopy or THL with the same probability. Moreover, the informed consent states the possibility of converting the procedure to laparotomy (or laparoscopy for the patients allocated to the THL group).

Six months after the procedure, all patients underwent trans-vaginal ultrasound scan and evaluation of any complication. Moreover, all patients were offered to undergo office transvaginal hydrolaparoscopy (THL) under local anesthesia to evaluate the presence of adhesions. After office THL follow-up, the participants were instructed to monitor their menstrual cycles for the next 6-12 months for a spontaneous pregnancy.

The primary end-point of the study was the proportion of women without ovarian adhesions as evaluated by THL 180 days after the primary surgical procedure. Secondary end-point were the grade of ovarian adhesions and any peri- and post-operative complication. Grading of adhesions was as follows: grade 1: filmy avascular; grade 2, dense or vascular; grade 3, cohesive (145).

Adhesions not involving the ovaries were not the object of this study.

Considering an adhesion formation rate of 60% in the laparoscopic group (146) and hypothesizing a reduction of 20% of this rate in the THL group, we calculated that a sample of 194 patients would

have been sufficient to reject the null hypothesis (i.e. adhesion formation equal in the two groups) with a statistical power of 80% and an alfa error of 5%. Hypothesizing that 25% of patients would have refused to undergo the follow-up THL procedure, we recruited 123 patients per group.

Statistical analysis was performed using the Social Package for Social Sciences (SPSS, Chicago, II, version 17.0). Data distribution for continuous variables was assessed with the Shapiro-Wilk's test. Student's t test for paired was used to compare these variables, which all showed a normal distribution. Differences in proportions between groups for categorical variables (presence of adhesions, grading of adhesions) were analyzed with the χ^2 test.

3.3 Results

The flow of patients is reported in Figure 1. All patients received their allocated intervention. Analysis was carried out on an intention-to-treat basis. Baseline demographic, clinical and hormonal data of the patients included in the study are given in Table 1. No significant differences were observed between the two groups. Duration of the procedure was significantly shorter in the THL group in comparison with LOD group ($p < .0001$) (Table 2). No intra- or post-operative complication was observed in any patients in both groups.

Table 2. Demographic, Clinical, and Hormonal Data. Values are given as mean ± SD

Variable	**THL** (***n*** **= 123)**	**LOD** (***n*** **= 123)**	**p**
Age (years)	27.5 ± 6.8	30.1 ± 7.5	ns
BMI (kg/m^2)	27.3 ± 5.6	25.9 ± 7.1	ns
Procedure duration (mins)	20 ± 10	40 ± 20	.0001
Duration of infertility (years)	1.95 ± 0.8	2.15 ± 0.6	ns
Pre-treatment with metformin (%)	45.8 ± 2.1	53.6 ± 1.3	ns
CC cycles (months)	7.6 ± 1.36	6.9 ± 1.32	ns
AMH (ng/ml)	5.84 ± 1.16	6.06 ± 1.18	ns
Testosterone (ng/ml)	1.2 ± 0.3	1.6 ± 0.2	ns
Androstenedione (ng/ml)	1.5 ± 0.2	1.6 ± 0.2	ns
DHEA sulphate (ng/ml)	6.0 ± 0.8	6.2 ± 0.9	ns

Post-operative THL evaluation of ovarian adhesion formation 6 months after the original procedure was performed on 104 patients in group A and 97 patients in group B because 45 patients (19 group A and 26 group B) refused follow-up with THL. The analysis showed that 15 (15.5%) patients in the THL group and 73 (70,2%) in the LOD group showed the presence of any type of ovarian adhesion. This difference was highly significant with a p value < .0001 and a relative risk of 0.22 [95%IC 0.133-0.350]

Among patients with post-operative ovarian adhesions observed at follow-up THL, 8 (53.3%) and 44 (60.3%) showed filmy adhesions, 5 (33.3%) and 22 (30.1%) dense adhesions, and 2 (13.3%) and 7 (9.6%) cohesive adhesions in the THL and LOD groups, respectively. Cumulative pregnancy rate was defined as percentage of pregnant patients, per total patients. The cumulative pregnancy was calculated according to time to the first event. In our study cumulative pregnancy rate after ovarian drilling was 68 %; no multiple pregnancy was observed and no differences in cumulative pregnancy was detected between groups underwent to the different surgical techniques.

3.4 Discussion

Our study showed that ovarian drilling using THL reduces post-operative ovarian adhesion formation.

Postoperative adhesions , fibrous connections developing between tissue and organs as a sequel to surgical trauma, have become the commonest complication of surgery and a source of major concern because of their potentially dramatic consequences. Adhesions may produce disruption of the normal anatomy, thus altering normal tubal performance. Thus, follicular growth, pick-up of the oocyte after ovulation and spermatozoa or embryo transport may be impaired (147). Several women develop post-operative adhesions after laparoscopy surgery. The most common site of post-operative adhesions formation is the ovary (147). Although several surgical measures and systemic pharmacologic treatments for adhesions prevention have been proposed the rate of peri-ovarian adhesion formation was not significantly reduced (148). The high incidence of post-operative adhesions and their clinical significance underline the importance of modifying the surgical technique in order to reduce potential adhesion formation (148).

Laparoscopic ovarian drilling (LOD) is a common surgical approach currently to treat anovulatory infertility in clomiphene (CC)-resistant women with polycystic ovarian syndrome (PCOS). The mechanism of action is still uncertain but likely lies in the reduction of ovarian androgen production through a decrease in stromal mass, or in the disruption of parenchymal blood flow (138). Nevertheless, two main drawbacks prevent LOD from being considered a primary approach to the treatment of PCOS: the risk inherent to any surgical procedure, and the risk of

adhesion formation potentially interfering with fertility (149). Indeed, one of the main shortcomings of LOD is postoperative adhesion formation due to bleeding from ovarian surface or premature contact between the ovary and the bowel after cauterization (146).

A number of studies have been designed to evaluate the rate of adhesion formation. As a result, currently, the incidence of postoperative adhesion formation range from 19% to 43% in some studies and up to 82 % in other studies, but such wide variation possibly reflects an inadequate study design. Variation in the surgical techniques can obviously affect the rate of adhesion formation (150). The recent European Guidelines (2012) indicated the good surgical strategy to reduce adhesion formation: to reduce pressure and duration of pneumoperitoneum in laparoscopic surgery, to reduce risk of infection, to perform diligent haemostasis and ensure diligent use of cautery, to use frequent irrigation and aspiration when needed and reduce the time of surgery (151).

THL was developed as a less invasive alternative to conventional laparoscopy. THL may be considered as a less traumatic and more suitable outpatient procedure than diagnostic laparoscopy . This approach was recently proposed as a first line procedure in the exploration of adenexal structures in infertile women (152). In a recent study we have evaluated the pain during office THL and we have shown that office THL is well tolerated by patients (153).

Some authors reported the feasibility of ovarian drilling with THL for anovulatory women with

PCOS to induce ovulation. [141, 154]. Fernandez et al. (141) suggest that THL with ovarian drilling using bipolar electrosurgery may be considered an alternative to conventional laparoscopy. In another study, Shibahra (155) investigated the postoperative endocrine alteration and clinical outcome of infertile women with PCOS undergone THL ovarian drilling, showing an improvement of the endocrine pattern.

In a recent study, Franz et al. (156) investigated the learning curve of diagnostic THL and operative THL ovarian drilling with bipolar electro surgery in patient with clomiphene citrate-resistance. These authors showed that the learning curve of THL is comparable or shorter than traditional laparoscopy and encourage to consider fertiloscopy as a first line in diagnostic work-up of infertile women and in clomiphene citrate-resistant PCOS for ovarian drilling. There are no data in literature on post-operative ovarian adhesion formation after THL ovarian drilling. The only studies available evaluates adhesion formation after LOD. This is the first study comparing post-operative ovarian adhesion formation after LOD and THL in patients with PCOS using THL follow-up. Our data showed that after 6 months significant differences exists between the two groups in favor of THL.

The exact mechanism through which THL seems to induce adhesion in a lower proportion of patients is difficult to explain. It may be hypothesized that the use of saline solution instilled into the

peritoneal cavity during THL, the shorter duration of the procedure and avoiding the creation of pneumoperitoneum may contribute to this reduction. While laparoscopic surgery has been considered to be less adhesiogenic in comparison with laparotomy, in particular for the development of *de novo* adhesions, (157) a meta-analysis revealed comparable results for open vs laparoscopic surgery (157). Factors influencing postoperative adhesion are the pelvis environment, and the pressure and duration of the pneumoperitoneum (151).

This study had some limitations. THL allows to visualize only the lower part of the pelvis and the posterior wall of the uterus, so the presence of anterior or cranial adhesions cannot be excluded. The not exact number of drilling points practiced in every patient but we have defined the number of ovarian puncture case by case depending on the size of the ovary .

Another possible limitation of the study may be the different types of electro surgery used in the two techniques: monopolar diathermy for LOD and bipolar diathermy for THL. On the other hand, we feel that this can be a significant bias for the primary outcome of this study, considering that the depth of the coagulation, as well as the total number of drills were similar between the two groups. Moreover, considering the studies published in literature (137, 158-160), where both mono- and bipolar electricity were used, there seems not to be differences in terms of adhesion formation between the two techniques.

Strengths of this study rely on its randomized design, with robust exclusion and inclusion criteria, and the use of a single operator for all procedures, eliminating one of the most important factor influencing surgical outcomes and an objective tool for the evaluation of adhesion formation (office THL).

In conclusion, this study seems to indicate that THL ovarian drilling may reduce the risk of ovarian adhesion formation and could be used as a safe and effective option to reduce ovarian adhesion formation especially in CC-resistant, anovulatory patients affected by PCOS. Prospective comparative studies including more patients should be conducted to confirm our preliminary results, but we are confident that this procedure may offered as a first-line therapy in treatment of clomiphene citrate-resistant infertile women with PCOS.

4. OUTCOME

Lastly, we evaluated the positive effects of THLOD on the ovulation rate

4.1 III STUDY: Operative transvaginal hydrolaparoscopy improve ovulation rate after clomiphene failure in polycystic ovary syndrome

Laparoscopic ovarian drilling (LOD) is considered by the ESHRE/ASRM PCOS consensus workshop, the second-line intervention in the CC-resistant PCOS patients [130, 136]. Some authors

underlined the fact that LOD can significantly increase the rate of ovulation and conception in the women with anovulatory infertility due to PCOS, without the risks of multiple pregnancy or ovarian hyperstimulation syndrome induced by pharmacological treatments (152, 161).

Several studies assessed THL to be a successful technique for ovarian drilling (162, 163). Some authors have assessed the ovulation and the conception rate in CC-resistant PCOS patients who undergone LOD (161, 163). The aim of our study was to assess the ovulation rate, with mid-luteal progesterone level and ultrasound follicles monitoring, in PCOS CC-resistant patients undergone THL ovarian drilling (THLOD)

4.2 MATERIALS AND METHODS

A prospective observational study was performed in PCOS, infertile, CC-resistant patients who underwent THLOD between December 2009 and July 2016. The patients are defined as CC-resistant if the ovulation fails after receiving a dosage of 150 mg per day for five days beginning on the third day of menstrual cycle, for at least six consecutive cycles (136, 164). All participants met the Rotterdam consensus criteria for the diagnosis of PCOS (165). Inclusion criteria were: desire for pregnancy; patent fallopian tubes, confirmed by hysterosalpingography or hysteroscopic diagnosis;

normal semen analysis parameters of the patients' spouses according to the criteria of the World Health Organization. Exclusion criteria were age 518 and 440 years; evidence of premature ovarian failure, previous ovarian surgery; endocrine anomalies other than PCOS (congenital adrenal hyperplasia, Cushing's syndrome, previous administration of androgens and androgensecreting tumor), use of drugs affecting ovarian function (e.g. gonadotropin-releasing hormone (GnRH) analogs, danazol, estroprogestin, etc.) within the previous 6 months, pregnancy, fixed retroverted uterus; lateral displacement of the cervix; suspected pelvic tumor; vaginal infections; abnormalities at vaginal examination and transvaginal ultrasound; psychiatric conditions impairing the ability of the patient to cooperate, obliteration of the pouch of Douglas or inability to perform the vaginal exam or any other contraindication to THL. Patients who met the study criteria and agreed to participate in the study gave their written informed consent, according to the institutional review board (IRB) of the study institution. Before the surgical procedure, all patients underwent clinical examination, routine preoperative biochemical testing and a transvaginal pelvic ultrasound examination. Each participant after surgical treatment underwent blood sampling on days 20–24 of the cycle to measure serum Pg levels. Progesterone level 43 ng/mL was considered as ovulation (166 - 168). The endometrial thickness and follicle size were monitored on days 10, 12 and 14 of the cycle and the subsequent surveillance time point was adjusted according to the individual situation until ovulation. The

presence of ovulation was found only in those cycles in which the follicle reached at least 16 mm in diameter. Ovulation frequency and the highest mean follicular diameters during the monitoring were recorded. Both tests were repeated each month for 6 months after surgery. The serum hCG concentration was measured in case of amenorrhea longer than 45 days. Biochemical pregnancy was considered when hCG was 42.5 mIU/ml in the absence of menstruation, and clinical pregnancy was defined by a fetal heart beat monitored by ultrasound at 6 weeks of gestation. Twenty patients with PCOS were not included in the study: 15 who refused to undergo THLOD and five who did not meet the inclusion criteria. The remaining 123 patients who satisfied the inclusion criteria underwent THL ovarian drilling. All serum samples were stored at 80 C until assay. Assays for LH, FSH and Pg were performed by automated micro particle enzyme-immunoassay (Abbott Axsym analyzer, Abbott Diagnostic). The normal laboratory ranges for serum LH, FSH, LH:FSH on day 3 of the cycle in women of reproductive age were, respectively, 1–18 mlU/ml, 1.0–8.8 mlU/ml and 1. The normal laboratory ranges for serum Pg on luteal phase were 3.28– 38.63 ng/mL. A single surgeon who had already performed over 50 procedures prior to this study performed surgical procedures. 123 patients underwent ovarian drilling with THL. THL was performed with the patients in gynecologic position, under spinal anesthesia. Using a Verres needle, 300 ml of normal saline solution was instilled into the peritoneal cavity through the posterior vaginal fornix. A specially designed needle dilating trocar

system with a total diameter of 3.9 mm was placed 10–20 mm below the insertion of the posterior vaginal wall to the cervix. A 2.7 mm diameter semirigid endoscope with a 3.5 mm sheath was used with an optical angle of 30 to visualize the pelvic cavity (Karl Storz, GmbH & Co., Tuttlingen, Germany). The posterior aspect of the uterus was examined first, followed by the ovary and fallopian tube on each side. A bipolar electrosurgical probe (Versapoint- Gynecare Inc., menlo Park, CA) was introduced through the auxiliary channel of the sheath. By using this probe, the ovarian cortex was drilled in 3–6 points per ovary, using a power setting of 110–130 W. All subjects received antibiotic prophylaxis immediately before the procedure (cefazolin 2 g intravenously). Data analysis was performed using the SPSS 20.0 software package (SPSS Inc. Chicago, IL). Data were evaluated for distribution by Shapiro–Wilks'test. Data were shown as mean ± standard deviation or as percentage.

4.3 Results

One hundred and seventeen patients completed the study protocol, since six patients were lost to follow-up. Two patients decided to postpone pregnancy and started contraceptive therapy. Other four patients decided to start a PMA program. Patient's mean age at enrollment was 29.5 years ± 3.9 (SD). Hormonal profile at baseline was LH: 8.85 ± 1.39 mlU/ml; FSH: 5.35 ± 0.71 mlU/mL; LH:FSH ratio 1.68 ± 0.35. Mean ovarian volume at baseline was 11.78 ± 1.61 cm3 . Ovulation rate at the follow up was 64.1% one month after treatment, 79.5% after three months and 82.9% after 6 months (Figure 3).

82 patients conceived during follow-up period. Pregnancy rate was 70.1% (Figure 3). Hormonal and clinical data after TVHLOD at ultrasound examination at 6 months follow up are shown in Table 3. Mean major follicular diameter during ovulation monitoring was 16.37 mm.

Table 3. Hormonal and clinical data at ultrasound examination during 6 months follow up.

	Study group post TVH-OD (N ¼ 117)
Mean follicular diameter (mm)	16.37 ± 4.27
Endometrial thickness (mm)	7.08 ± 2.25
Pg levels (ng/mL)	17.39 ± 10.65

Data are shown as mean ± standard deviation.

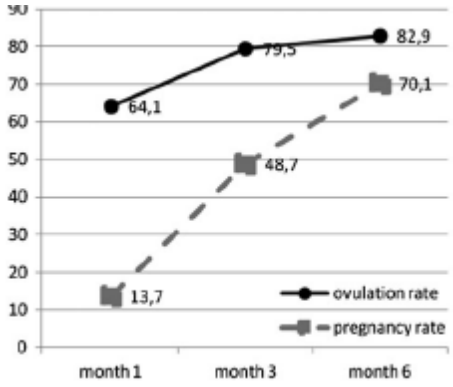


FIGURE 3: Ovulation and pregnancy rate during follow up period. Data are shown as cumulative percentage from baseline to 6 months

4.4 Discussion

The aim of this study was to evaluate the feasibility and the efficacy of THL ovarian drilling in terms of ovulation rate and pregnancy rate in PCOS CC-resistant patients. From our study, these techniques have shown to be a feasible and efficacy option for ovarian drilling which allows an improvement of ovulation rate, evaluated by mid-luteal progesterone level and ultrasound monitoring, in CC-resistant PCOS patients. To the best of our knowledge, this is the first study that evaluates ovulation rate after THL ovarian drilling (THLOD) in those patients, by measurement of serum progesterone levels and US monitoring. Previously, studies have reported the effects of LOD on ovulation rate, demonstrating a value between 60 and 80% and evaluating ovulation rate by ovarian ultrasound and by measurement of progesterone on 21 day [16–18]. Zahiri Sorouri et al. evaluated the ovulation rate in CC-resistant PCOS patients who undergone LOD using serum progesterone levels. The authors showed an ovulation rate after LOD between 60% and 64.4% six mount follow up, on day 21 by measurement of progesterone, considering the level of Pg43 ng/mL as sign of ovulation (168). Our study has shown an ovulation rate of 80%, six months after THLOD treatment in CC-resistant PCOS and without hormonal stimulation, comparable with LOD. Many authors have reported not only high ovulation

rates (80%), but also high pregnancy rates (60–70%) following LOD (169 - 173). We have shown a pregnancy rate of 70% in patients undergone THLOD, comparable to these data. This data are in agreement with the work by Fernandez et al. (141) that have shown a cumulative pregnancy rate of 70% after 6 months, and Gordts et al. (152) that reported a pregnancy rate of 85% after the procedure. It has been also shown that THLOD improves endocrine pattern in PCOS patients; in particular, THL is responsible for decreasing serum LH levels as well as LH/FSH ratio, without inducing a modification of serum FSH levels (174). In addition, a similar trend was reported in the reduction of serum AMH levels after THL, as observed after LOD. This finding may indicate that the mechanism of action of THLOD is similar to those occurring after LOD (174). Moreover, the high pregnancy rate and ovulation rate of THL comparable with LOD add an additional benefit in the use of these techniques especially in patients that require a tubal patency evaluation with the advantages that it can be performed without general anesthesia. To the best of our knowledge, no studies have assessed the effect of THLOD on ovulation rate using both the measurement of Pg levels and US monitoring. We have demonstrated that Pg levels in post-luteal phase after THLOD were 17 ng/mL. THL has been developed as a less invasive alternative to conventional laparoscopy in infertile PCOS patients (175). Several authors have proposed THL as the first-line procedure in the exploration of the infertile patients because it allows a direct access and an accurate visualization of ovaries, Fallopian tubes and

ovarian fossa (176, 177), with the opportunity of minimally invasive operative procedures (178, 179). This study shows further advantages of THLOD compared to LOD in the treatment of PCOS CC-resistant patients: the reduced risk of postoperative adhesion formation, the low morbidity of the procedure, the safety and the advantage of transvaginal access in obese patients, the possibility of not having to perform it with general anesthesia, and also the advantages in term of cost, comfort and length of hospital stay compared to LOD. Moreover, in a recent study we have assessed that THLOD is better tolerated by patients than LOD (180). The limitation of this study is the relatively small number of patients undergoing operative THLOD; nevertheless, THL is an invasive procedure and, as such, cannot be imposed to patients unless clear indications to the procedure. Moreover, a possible selection bias may be present because the design is not randomized. On the other hand, the limited number of CCresistant PCOS patients who agree to undergo a surgical procedure makes it very difficult to set up such a study.The strengths of this study rely on its robust inclusion and exclusion criteria, the use of a single operator for all procedures, a long follow-up period of 6 months and the double evaluation of women undergone to THLOD both with USG monitoring and with Pg serum levels. In conclusion, our study shows that THLOD improve ovulation and pregnancy rate in women with CC-resistant PCOS; based on our data and previous studies, we believe that THLOD should mean an option in the management of anovulatory CC-PCOS patients and should be offered as

second-line therapy at all women who fail the medical methods of ovulation induction. Prospective, comparative studies including more patients should be undertaken to confirm our results.

5. Conclusions

In conclusion, our studies show the new approach to the conservative treatment of uterine malignant lesions and our commitment will be to continue on this line of research to investigate further conservative approaches and to improve the follow-up of these issues.

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