In women with endometriosis anti-Müllerian hormone levels are decreased only in those with previous endometrioma surgery

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STUDY QUESTION: Are anti-Müllerian hormone (AMH) levels lower in women with endometriosis, notably those with endometriomas (OMAs) and deep infiltrating lesions, compared with controls without endometriosis?

SUMMARY ANSWER: Endometriosis and OMAs per se do not result in lower AMH levels. AMH levels are decreased in women with previous OMA surgery independently of the presence of current OMAs.

WHAT IS KNOWN ALREADY: The impact of endometriosis and OMAs per se on the ovarian reserve is controversial. Most previous studies have been conducted in infertile women. The strength of our study lies in the following points: (i) the selection of women undergoing surgery and not only according to the presence of infertility, (ii) the classification of women with endometriosis and controls based on strict surgical and histological criteria.

STUDY DESIGN, SIZE, DURATION: Cross-sectional study using data prospectively collected in all non-pregnant <42-year-old patients, who were surgically explored for a benign gynaecological condition at a university tertiary referral centre between 2004 and 2008. For each patient, a structured questionnaire was completed during a face-to-face interview conducted by the surgeon during the month preceding surgery. AMH levels were measured in serum samples drawn in the month preceding surgery, without regard to menstrual phase or hormonal therapy.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Operations were done on 1262 women between 2004 and 2008, of which 1133 signed the informed consent. Of the 566 women with a visual diagnosis of endometriosis, 411 had histologically proven endometriosis. Frozen serum samples for the AMH measurement were available in 313 of them. Out of the 554 women without visual endometriosis and without past endometriosis surgery, 413 had a frozen serum sample for the AMH measurement. Univariate analysis examined AMH levels according to baseline patient characteristics, the presence and type of endometriosis (superficial lesion, OMA, deep infiltrating lesion) and previous OMA surgery. Analysis of variance–covariance then examined the effects of co-variables on AMH levels. Finally, logistic regressions were conducted to examine the odds ratio (OR) of having AMH levels <1 ng/ml according to the same co-variables.

MAIN RESULTS AND THE ROLE OF CHANCE: The difference in AMH levels between women with endometriosis and controls did not reach significance (3.6 ± 3.1 versus 4.1 ± 3.4 ng/ml, P = 0.06). Analysis of variance–covariance demonstrated that AMH levels significantly decreased with age (P < 0.01) and in women with prior OMA surgery irrespective of whether OMAs were present or not at the time of study (P < 0.05). Logistic regression revealed that two major factors were related to AMH levels <1 ng/ml: (i) age (compared with <29 years; 30–34 years OR = 3.1, 95% CI: 1.5–6.4, P = 0.01; 35–39 years OR = 7.0, 95% CI: 3.5–14.1, P = 0.001; ≥40 years OR = 20.8, 95% CI: 9.1–47.4, P = 0.001) and (ii) prior OMA surgery (OR = 3.0, 95% CI: 1.4–6.41, P = 0.01).
Endometriosis and anti-Müllerian hormone

LIMITATIONS, REASONS FOR CAUTION: The selection of our study population was based on a surgical diagnosis. Women with an asymptomatic form of endometriosis are therefore not included in our study. We cannot exclude that infertile women with OMA(s) associated with a diminished ovarian reserve, as assessed during their infertility work-up, were less likely to be referred for surgery and might therefore be underrepresented.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings suggest that OMA(s) per se do not diminish the ovarian reserve reflected by AMH levels but that alterations seen in women with endometriosis are a deleterious consequence of OMA surgery. These findings should be taken into account in the decision to operate OMA(s) in women with a desire for future pregnancy.


Key words: endometriosis / endometrioma / surgery / AMH / MIS

Introduction

Endometriosis is a disease of unknown origin characterized by the growth of endometrium-like tissue—stroma and glands—outside of the uterine cavity. The process can affect all organs, but is primarily encountered in pelvic organs, including the ovaries. Practically, endometriosis bears two primary clinical consequences, pain (Fauconnier and Chapron, 2005) and infertility (de Ziegler et al., 2010a,b). The severity of endometriosis has been graded according to its territorial extension following the I–IV revised-American Fertility Society (r-AFS) score (Revised American Fertility Society classification of endometriosis, 1985) and/or the nature and degree of penetration of its lesions (Dubuisson and Chapron, 1994). The latter ranges from the least to the most severe lesion: (i) superficial peritoneal lesion (SUP), (ii) endometrioma (OMA) when endometriotic cysts form in the ovaries, (iii) deep infiltrating endometriosis (DIE) when lesions penetrate deep under the peritoneal surface and infiltrate the muscularis layer of surrounding organs including the vagina, bladder, intestine and/or ureter (Chapron et al., 2006).

There are well-established relationships between the anatomical location and extension of endometriotic lesions and pain (Fauconnier et al., 2002). Conversely, however, the link between endometriosis and infertility is less well defined. Today, the prevailing vision is that infertility results from an array of factors rather than a single cause (de Ziegler et al., 2010a,b). For simplicity’s sake, the effects of endometriosis on fertility are grouped according to the territory where they are exerted on. Hence, infertility results from the consequences of endometriosis on: (i) the pelvic cavity, (ii) the ovary and (iii) the uterus (de Ziegler et al., 2010a,b). In the pelvic cavity, the inflammation related to endometriosis is thought to interfere with natural conception (de Ziegler et al., 2010a,b). Ovarian endometriosis is seen as capable of blunting the outcome of assisted reproductive technologies (ARTs; Catenacci and Falcone, 2008). Finally, the impact of endometriosis on the uterus—alteration of the eutopic endometrium—may hamper endometrial receptivity (May et al., 2011).

Prior reports consistently indicate that controlled ovarian stimulation (COS) responses are generally dampened in endometriosis, particularly when ovarian lesions—OMAs—are present (Geber et al., 2002; Dsemirol et al., 2006; Esinler et al., 2006; Matalliotakis et al., 2007). Al-Azemi et al. (2000) demonstrated that larger doses of gonadotrophins are needed to obtain less oocytes in the case of ovarian endometriosis. Remarkably, this phenomenon amplifies itself over successive ART attempts (Al-Azemi et al., 2000). Yet, not all investigators find that COS yields are decreased in the case of OMA(s) (Almog et al., 2011; Benaglia et al., 2011).

Anti-Müllerian Hormone (AMH) is produced by small antral and pre-antral ovarian follicles throughout reproductive life (Visser and Themmen, 2005). AMH levels are related to the number of antral follicles, which, having acquired FSH receptors, are recruitable through COS. Converging reports confirm that AMH levels predict the magnitude of COS responses (Nelson et al., 2009; Al-Azemi et al., 2011). Practically, AMH is the most readily accessible predictor of COS responses, because serum levels are unaffected by hormonal changes or treatments. Indeed, data concur to indicate that for all practical purposes, serum AMH levels remain relatively constant throughout the menstrual cycle (Helenkamp et al., 2006; Streuli et al., 2008) or during oral contraception (Somunokiran et al., 2007) or vaginally (Streuli et al., 2008). In a recent study, van den Berg et al. (2010) showed a slight elevation of AMH levels two cycles after discontinuation of oral contraception compared with AMH levels in the 7-day hormone-free interval and the first cycle after discontinuation suggesting a small influence of contraception on AMH levels. Serum AMH levels also remain stable when gonadotrophins are suppressed as, for example, with a GnRH agonist (GnRH-a) treatment (Mohamed et al., 2006). For all these reasons, AMH is the preferred quantitative marker (Broekmans et al., 2009) of ovarian follicles in endometriosis, as these patients commonly receive all kinds of hormonal treatments.

The adverse effect on OMA surgery on ovarian reserve parameters including AMH levels is now well recognized (Iwase et al., 2010; Hironaka et al., 2011; Hwu et al., 2011), whereas the impact of endometriosis and OMA(s) per se on the ovarian reserve is still subject to controversy. Few studies conducted in infertile women, in which endometriosis was not necessarily surgically confirmed, reported lower AMH levels in the case of severe endometriosis r-AFS stages III and IV (Sheib et al., 2009) and OMA(s) (Hwu et al., 2011).

The aim of the present study is to evaluate the AMH levels in women with surgically diagnosed and histologically proven endometriosis compared with controls and to assess the effects of OMA(s) per se and past OMA surgery on AMH levels.

Materials and Methods

The regional ethics committee (CCPBB: Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) approved the study protocol and all included patients signed a written informed consent form.

Since January 2004, we collected clinical and biological data prospectively in all non-pregnant <42-year-old patients, who were surgically
explored, by operative laparoscopy or laparotomy, for benign gynecological conditions at our institution. Serum samples were collected in the month prior to the surgical intervention and stored frozen for future analysis. Excluded from this population were women with cancer and those who did not consent to the study. For each patient, data were collected in face-to-face interviews conducted by the surgeon during the month preceding surgery. For this, we use a previously published structured questionnaire (Chapron et al., 2010). Briefly, for all patients, we collected general information such as age, gravidity, parity, height, weight, body mass index (BMI), age of menarche, existence and duration of infertility, pelvic pain, lifestyle habits and use of hormonal treatments.

The diagnosis of endometriosis was based on surgical exploration and histological confirmation. Patients visually diagnosed with endometriosis but without histological confirmation were excluded from the study (Chapron et al., 2010). When present, the extent of endometriosis was staged surgically according to the r-AFS classification (Revised American Fertility Society classification of endometriosis, 1985). Based on histological findings, endometriotic lesions were also classified into three groups: SUP, OMA and DIE. As these three types of endometriotic lesions are frequently associated (Somigliana et al., 2007), endometriotic lesions were classified according to the most severe finding. Endometriotic lesions are usually ranked from the least severe to the most severe in SUP, OMA and DIE (Chapron et al., 2009).

We decided to measure AMH levels in frozen serum samples of women included in our study between January 2004 and December 2008. As mentioned above, serum samples were obtained in the month before surgery and serum aliquots were frozen for subsequent analysis. As described in Fig. 1 (flowchart), 1262 non-pregnant women <42 years were surgically explored for benign conditions at our institution during that time period. Of these, 129 refused to participate and 1133 signed the informed consent and had a surgical procedure and diagnosis. We excluded 155 women who had a visual suspicion of endometriosis during the surgical procedure but without subsequent histological confirmation. Of the 411 women with surgically diagnosed and histologically confirmed endometriosis, frozen serum aliquots were available for AMH measurements in 316 of them. Three women with scar endometriosis and no other endometriotic lesion were excluded leaving 313 women in the study group.

After exclusion of 13 women with a past history of endometriosis surgery, 554 women had neither current nor past endometriosis. Serum aliquots for AMH measurements were available in 413 women (control group).

AMH levels were measured by a commercial enzyme-linked immunosorbent assay kit according to the manufacturer’s instructions [Diagnostic Laboratories systems (DSL), Webster, TX, USA]. The limit of detection of the kit is 0.006 ng/ml, the intra-assay coefficient of variation between 2.4 and 4.6% and the coefficient of variation between 4.8 and 8.0% as described in the directions of use. AMH measurements were all performed in the same laboratory (Dr G. Bijaoui, Laboratoire Port Royal, 75005 Paris, France). Measurements were run in duplicate and mean levels were determined.

**Statistical analysis**

All statistical data were collected in a computerized database. Statistical analysis was performed using SPSS 19.0 for Macintosh and SAS 9.1.3.

**Figure 1** Patient inclusion flowchart.
Continuous data are presented as mean and standard deviation. Student’s t-test and ANOVA were carried out when appropriate. The χ² test was used for categorical data with usual correction for small samples when appropriate. We conducted two types of multivariate analysis: (i) variance–covariance analysis (generalized linear model) to explain the variations of AMH levels according to co-variables and (ii) logistic regressions to explain the risk of having AMH levels <1 ng/ml according to the same co-variables. In each analysis, we first included variables a priori and then excluded variables of lesser interest a posteriori in order to give more weight to the model.

We calculated, using the PS software (PS, Power and sample size calculations), that a sample size of more than 300 subjects in each group would allow us to show a difference in AMH levels between subjects and controls of 0.8 ng/ml with a power of 80% and a type I error of 0.05. A

<table>
<thead>
<tr>
<th>Table I Patient characteristics.</th>
<th>Study group (endometriosis; n = 313)</th>
<th>Control group (n = 413)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.9 ± 5.0</td>
<td>32.1 ± 5.9</td>
<td>0.56a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0 ± 3.7</td>
<td>23.4 ± 4.0</td>
<td>0.001a</td>
</tr>
<tr>
<td>Smoking status [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>93 (29.8)</td>
<td>112 (27.1)</td>
<td>0.08b</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>44 (14.1)</td>
<td>40 (9.7)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>175 (56.1)</td>
<td>261 (63.1)</td>
<td></td>
</tr>
<tr>
<td>Gravidity [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>201 (64.2)</td>
<td>225 (54.6)</td>
<td>0.001b</td>
</tr>
<tr>
<td>1</td>
<td>68 (21.7)</td>
<td>81 (19.7)</td>
<td></td>
</tr>
<tr>
<td>2 and more</td>
<td>44 (14.1)</td>
<td>106 (25.7)</td>
<td></td>
</tr>
<tr>
<td>Parity [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>251 (80.2)</td>
<td>306 (74.3)</td>
<td>0.01b</td>
</tr>
<tr>
<td>1</td>
<td>44 (14.1)</td>
<td>54 (13.1)</td>
<td></td>
</tr>
<tr>
<td>2 and more</td>
<td>18 (5.7)</td>
<td>52 (12.6)</td>
<td></td>
</tr>
<tr>
<td>Infertility [n (%)]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No infertility</td>
<td>186 (59.8)</td>
<td>271 (65.8)</td>
<td>0.001b</td>
</tr>
<tr>
<td>Primary</td>
<td>86 (27.2)</td>
<td>61 (14.8)</td>
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</tr>
<tr>
<td>Secondary</td>
<td>39 (12.5)</td>
<td>80 (19.4)</td>
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</tr>
<tr>
<td>Hormonal treatment [n (%)]</td>
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<td></td>
</tr>
<tr>
<td>OC pill</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>30 (9.6)</td>
<td>120 (29.1)</td>
<td>0.001b</td>
</tr>
<tr>
<td>Ex-user</td>
<td>224 (71.8)</td>
<td>189 (45.9)</td>
<td></td>
</tr>
<tr>
<td>Current user</td>
<td>58 (18.6)</td>
<td>103 (25.0)</td>
<td></td>
</tr>
<tr>
<td>GnRH-a (current use)</td>
<td>99 (31.6)</td>
<td>49 (11.9)</td>
<td>0.001b</td>
</tr>
<tr>
<td>Progestin (current use)</td>
<td>57 (18.2)</td>
<td>56 (13.6)</td>
<td>0.09b</td>
</tr>
<tr>
<td>Prior endometriosis surgery [n (%)]</td>
<td>143 (45.6)</td>
<td>0 (0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Prior OMA surgery [n (%)]</td>
<td>57 (18.2)</td>
<td>0 (0.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Unilateral</td>
<td>45 (14.2)</td>
<td>12 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

N/A, not applicable.

*aStudent’s t-test.

*bχ² test.

difference: 0.45 (95% CI −0.02 to 0.96) ng/ml, \( P = 0.06 \) and an age-related decline. In the control group, there were no differences in AMH levels according to the surgical indication: benign ovarian cysts, 4.1 ± 3.5 ng/ml; tubal infertility, 4.5 ± 4.1 ng/ml; leiomyomas, 3.8 ± 2.9 ng/ml; chronic pelvic pain, 4.7 ± 4.1 ng/ml; other benign condition, 3.8 ± 2.9 ng/ml (\( P = 0.36 \)).

In the univariate analysis, AMH concentrations were slightly lower in the case of DIE [controls (n = 413) 4.1 ± 3.4, SUP (n = 35) 4.5 ± 3.6, OMA (n = 95) 3.8 ± 2.9, DIE (n = 183) 3.4 ± 3.0 ng/ml] compared with controls although not quite reaching statistical significance.

**Results**

The patients’ distribution according to their worst endometriotic lesion was as follows: SUP (35 patients, 11.1%), OMA (95 patients, 30.4%) and DIE (183 patients, 58.3%). Of the 183 classified as DIE, 65 (35.5%) had an associated OMA.

Women in the endometriosis group with OMA and/or DIE were operated because of severe pain symptoms and endometriotic lesions identified by transvaginal ultrasound and/or magnetic resonance imaging before the surgical intervention. In the 35 women with SUP, endometriosis was diagnosed during the surgical intervention. Fourteen of them suffered from infertility and dysmenorrhea, 12 suffered only from dysmenorrhea and 4 were infertile without associated dysmenorrhea. Six women with superficial endometriosis suffered neither from infertility nor from dysmenorrhea. In these women, SUP was discovered during a surgical intervention performed for leiomyomas in four women and benign cysts in two women.

In the 413 women of the control group, 160 women were diagnosed with leiomyomas, 99 with benign ovarian cysts, 62 with tubal infertility, 64 with chronic pelvic pain without endometriosis and 42 other benign conditions (certain women had more than one surgical diagnosis).

As shown in Table I, the mean age was not different between the study and the control group; the distribution, however, was different with more women in the extremes (<25 and >35 years) in the study group. Women in the study group had a slightly lower BMI, lower gravidity and parity and were more often infertile. The smoking status was not different between groups. Women in the study group were less likely to be current OC pill users and were more often under hormonal treatments such as GnRH-a or progestin.

In the univariate analysis, there was an inverse correlation between AMH and age (\( r = −0.33, P < 0.001 \)), BMI (\( r = −0.11, P < 0.001 \)), gravidity (\( P = 0.01 \)) and parity (\( P = 0.01 \)). AMH levels were lower in ex-smokers (\( P = 0.02 \)) and women with secondary infertility (\( P < 0.05 \)). After adjustment for age, none of these associations persisted (BMI \( P = 0.70, \) gravidity \( P = 0.63, \) parity \( P = 0.70, \) smoking \( P = 0.28, \) secondary infertility \( P = 0.93 \)). AMH levels were also not related to the type of hormonal treatment used (OC pill \( P = 0.65, \) GnRH-a \( P = 0.95, \) progestin \( P = 0.78 \)).

AMH levels were not significantly different between women in the study group and the control group, showing similar distribution of values [study group: 3.6 ± 3.1, control group 4.1 ± 3.4 ng/ml, mean
The difference in global ANOVA was also non-significant ($P = 0.07$). AMH levels were significantly lower in r-AFS stage IV [r-AFS I ($n = 44$), $4.6 \pm 3.4$; II ($n = 56$), $3.6 \pm 3.0$; III ($n = 86$), $3.7 \pm 2.5$; IV ($n = 127$), $3.2 \pm 3.2$ ng/ml] compared with controls ($P = 0.03$). The difference in the global ANOVA was at the limit of significance ($P = 0.05$).

Out of the 313 women with endometriosis, 142 had had prior endometriosis surgery (45.4%) of which 59 (18.8%) had an OMA operation. AMH levels were reduced only in the 59 women whose endometriosis surgery included the removal of OMA, when compared with women without OMA surgery ($2.3 \pm 2.1$ versus $3.9 \pm 3.5$ ng/ml, $P < 0.01$). In women without prior surgery, there were no differences in AMH levels according to endometriosis staging: SUP ($4.5 \pm 3.6$ ng/ml), OMA ($4.0 \pm 3.0$ ng/ml) and DIE ($3.8 \pm 3.2$ ng/ml) ($P = 0.51$; Fig. 2).

AMH levels in study and control women are depicted in Table II. In women with endometriosis, data were sorted according to lesion staging (SUP, OMA and DIE), extension (r-AFS scores I–IV) and past history of OMAs. We then analysed AMH levels according to the association of lesion type, r-AFS score, presence of OMA and previous surgery of OMA (Table II). In all groups combined, AMH levels were lower in women with prior OMA surgery compared with women without OMA surgery ($2.3 \pm 2.1$ versus $3.9 \pm 3.5$ ng/ml, $P < 0.01$). In women without prior surgery, there were no differences in AMH levels according to endometriosis staging: SUP ($4.5 \pm 3.6$ ng/ml), OMA ($4.0 \pm 3.0$ ng/ml) and DIE ($3.8 \pm 3.2$ ng/ml) ($P = 0.51$; Fig. 2).

AMH levels were not reduced in women with current OMAs without past OMA surgery when compared with OMA-free cases (Figs 2 and 3).

In women with current OMAs, AMH levels were comparable in the case of unilateral ($n = 117$) or bilateral ($n = 43$) OMAs ($3.5 \pm 2.8$ versus $4.1 \pm 3.2$ ng/ml, $P = 0.51$). AMH levels were also not different in unilateral OMAs of $< 3$ and $\geq 3$ cm ($3.7 \pm 2.8$ versus $3.8 \pm 2.9$ ng/ml, $P = 0.76$).

In women with prior OMA surgery, AMH levels were not different in the case of unilateral ($n = 45$) or bilateral ($n = 12$) OMA surgery ($2.4 \pm 2.2$ versus $2.2 \pm 2.0$ ng/ml, $P = 0.69$).

In a variance–covariance analysis (generalized linear models), we analysed AMH levels according to the following co-variables: age, BMI, smoking status, infertility and endometriosis (type of endometriosis, r-AFS stage and prior OMA surgery). As stated before, AMH levels significantly decreased with increasing age ($P < 0.001$), and in all women with a prior OMA surgery irrespective of the presence or the absence of OMAs [comparison with controls without endometriosis: (i) prior OMA surgery, current OMA, $P = 0.04$; (ii) prior OMA surgery, DIE without associated OMA, $P = 0.01$; (iii) prior OMA surgery, DIE with current associated OMA, $P = 0.01$]. AMH levels were decreased in overweight and obese women (BMI $> 25$ kg/m$^2$) ($P = 0.01$), but not in relation to infertility and smoking status. AMH levels were not decreased in women with current OMA or DIE who did not have prior OMA surgery [comparison with controls without endometriosis: (i) current OMA, no prior OMA surgery, $P = 0.56$; (ii) current DIE, no prior OMA surgery, $P = 0.17$; (iii) current DIE with OMA, no prior OMA surgery, $P = 0.14$].

We conducted logistic regressions to determine the co-factors related to AMH levels below 1 ng/ml ($n = 131$, 18.5%). As described in Table III, the two major factors predicting AMH levels below 1 ng/ml were increasing age and prior OMA surgery [odds ratio (OR) = 3, 95% Cl: 1.4–6.41, $P = 0.01$].

**Discussion**

We observed that AMH levels are not diminished in women with endometriosis, including cases with current uni- or bilateral OMAs unless they had had previous OMA surgery. In women with endometriosis, the ORs of having AMH levels $< 1$ ng/ml were significantly increased with advancing age—as expected—but also in the case of a prior OMA surgery—uni- or bilateral.

The strength of our study lies in the following points: (i) selection of women undergoing surgery and not only according to the presence of infertility, (ii) the distinction of women with endometriosis and controls based on strict surgical and histological criteria, (iii) relying on a surgical classification, (iv) using clinical data prospectively collected by questionnaire prior to surgery on various epidemiological variables, (v) having controls who were all surgically explored for excluding asymptomatic endometriosis, (vi) a large number of severe forms of endometriosis (DIE and r-AFS stages III and IV), (vii) a large number of women who had a previous endometriosis or OMA surgery and (viii) another strength of our study is the fact that AMH levels were determined in serum aliquots only after the presurgical consultation. AMH levels did, therefore, not influence the surgeon’s decision to operate.

The primary limitations of our study are the following. (i) Our database only included the information of whether women had undergone previous endometriosis, OMA or uterine surgery. Information about
previous cystectomies for non-endometriotic cysts is therefore missing in both controls and endometriosis patients. (ii) Our database provided information about prior endometriosis and OMA surgery, but not the date at which the surgery was conducted, neither the size of the OMAs that were removed. (iii) There were only 35 women in the group with SUP. The small sample size precluded determining whether the slightly higher AMH levels found in these women truly differed from findings made in controls and women with OMA or DIE. (iv) Our study included women referred to our surgery department; we can therefore not exclude that infertile women with OMAs associated with a diminished ovarian reserve, as assessed during their infertility work-up, were referred less frequently to surgery and might therefore be underrepresented. Against a potential referral bias is the fact that, in our population, AMH levels were not different in women with OMAs whether they were infertile (42%) or not (P = 0.9).

We recognize that there is no ideal control group for studying AMH levels in endometriosis. Our control group consisted of women operated for benign gynaecological conditions. This may lead to biases stemming from the fact that certain benign gynaecological conditions, such as tubal infertility or ovarian cysts, might be associated with altered ovarian reserve. Speaking against that possibility is the fact that AMH levels were not lower in any of the surgical subgroups of this control population. Our endometriosis population constituted women who were all scheduled to undergo surgery may differ from
One of the women who are asymptomatic or not needing surgery. One can assume, however, that women needing surgery for pain and/or infertility tend to have more severe forms of the disease, a recruitment bias for lower AMH levels if anything. Thus, we do not see that the characteristics of our study and control populations could account for our finding that AMH levels are not or at most very slightly reduced in endometriosis.

Our findings of unaltered AMH levels in all stages of endometriosis, including uni- or bilateral OMA, when compared with disease-free controls are unexpected. Indeed, patients with OMAs have been described as responding less well to COS in ART than age-matched controls (Al-Azemi et al., 2000; Matalliotakis et al., 2007). Our findings are particularly compelling in the case of bilateral OMA, considering the recent report of poor ART outcome in this case (Somigliana et al., 2008). Taken together, our results raise questions as to whether AMH levels carry the same predictive value in the case of endometriosis as in other ART patients (Broekmans et al., 2009).

The originality of our study lies in the fact that AMH values were prospectively collected prior to surgery. We were therefore able to evaluate the differential impact of prior OMA surgery and current endometriosis, diagnosed by laparoscopy and confirmed histologically, on AMH levels.

Our results of lower AMH levels in women who were operated for OMAs are in agreement with other studies reporting on the adverse effect of OMA surgery on the response to COS (Al-Azemi et al., 2000; Matalliotakis et al., 2007; Catenacci and Falcone, 2008) and on the ovarian reserve (Nargund et al., 1996; Geber et al., 2002; Ho et al., 2002; Somigliana et al., 2003). The fact that OMA surgery negatively affects ovarian reserve, especially in the case of bilateral surgery, is now well recognized (Iwase et al., 2010; Hirokawa et al., 2011; Hwu et al., 2011). The lack of real plane of cleavage between the endometrium-like stroma of the endometriotic cysts and the ovarian tissue can lead to the removal of adjacent healthy ovarian tissue, which may harm ovarian reserve (Hachisuga and Kawarabayashi, 2002). AMH levels are significantly decreased post-operatively after cystectomy for OMA as shown by measurements before and after surgery (Iwase et al., 2010; Hirokawa et al., 2011; Hwu et al., 2011). Studying a much smaller cohort of patients, Hirokawa et al. (2011) report a decrease in AMH levels measured 1-month post-operatively of 24.7 ± 32.5% (n = 20) in unilateral cystectomy and 62.8 ± 29.6% (n = 18) in bilateral cystectomy. Neither the pre-operative AMH levels nor the decline in AMH levels were correlated to the size of the OMA. Chang et al. (2010) demonstrated that AMH levels are most reduced 1 week after cystectomy with a subsequent partial recovery to ~65% of the preoperative level in the months after surgery. Long-term values are, however, not provided.

The impact of endometriosis and OMAs per se on the ovarian reserve is subject to controversy with only few reports. Our results, in women who were not selected according to their fertility and in whom the diagnosis of OMA was surgically and histologically confirmed, suggest that OMAs per se do not diminish AMH levels significantly. In contrast, Sheibl et al. (2009) first reported that AMH levels were lower in infertile women with severe endometriosis (r-ANS stages III and IV) compared with controls with male factor infertility. Women with minimal/mild endometriosis had AMH levels comparable with controls. These study results are limited by the fact that controls were not investigated for the absence of endometriosis and that endometriosis lesions were not classified according to their type (OMA and DIE). Moreover, there is no information about previous endometriosis surgery. In a more recent report, Hwu et al. (2011) compared AMH levels in a control population of infertile women without OMAs with those of patients with unoperated OMAs > 3 cm and to those of patients with a history of laparoscopic cystectomy. In women with unoperated OMAs, the diagnosis was established by ultrasound. Their results show a decrease in AMH levels in women with unoperated OMAs >3 cm in every age category and an even greater decrease in women with a prior cystectomy. Their study design significantly differs from ours in several aspects: (i) all their subjects were infertile; (ii) the diagnosis of OMA was based on ultrasound evaluation without surgical and histological confirmation; (iii) only women with OMAs >3 cm were included, whereas women with smaller OMAs were excluded and (iv) there was no evaluation of associated superficial or deep infiltrating endometriotic lesions. Moreover, this study has a retrospective design and women were divided into groups according to the presence or the absence of OMAs and according to whether OMAs have been operated or not. There is no information about the reason that led to the decision of performing OMA surgery or not. Since women recruited were infertile, low AMH levels could potentially have played a role in the decision not to operate OMAs. This could be a hypothesis explaining their results of diminished AMH levels in women with current unoperated OMAs.

Surprisingly in our population, current smokers, but not ex-smokers, had higher AMH levels [OR 0.51 (95% CI: 0.30–0.88)] of having AMH levels below 1 ng/ml compared with never smokers. These results are in contradiction with all previous studies on AMH and smoking that report either reduced or unchanged AMH levels in current smokers (Freour et al., 2008; Dafopoulos et al., 2010; Waylen et al., 2010; Freour et al., 2011). Plante et al. (2010) studied AMH levels in women in their late reproductive years (age 38–50 years) according to their smoking status and found decreased AMH levels in active smokers but not in former smokers suggesting a possible direct effect of smoking on the depletion of antral but not primordial follicles. Freour et al. (2008) conducted two studies in a population of fertile women and demonstrated the negative impact of active smoking on determinants of the ovarian reserve such as AMH levels and antral follicular counts and on ART outcome (Freour et al., 2012) with poorer responses to COS and lower clinical pregnancy rates. These findings were corroborated by a report on diminished follicular fluid AMH levels in smokers undergoing ART (Fuentes et al., 2012). Differences in AMH levels between active smokers and non-smokers might be more apparent in these studies that were conducted in specific subgroups. Dafopoulos et al. (2010) found no relationship between AMH levels and the smoking status in younger women with a normal reproductive history (mean age: smokers 30.1 ± 7.4 years, non-smokers 33 ± 10 years). The population of our study consisted of women undergoing surgery in the context of a benign gynaecological condition. Most women were not infertile and the mean age was 32 years. The detrimental effect of smoking on AMH levels might therefore not be apparent in this population. It is also possible that infertile women with low AMH levels—possibly related to active smoking—would have undergone rapid ART rather than surgery and may therefore be
underrepresented in our study. Such a selection bias could explain finding higher AMH levels in current smokers.

Our study shows that AMH levels are diminished in women with previous OMA surgery but not in women with current OMA who have never had OMA surgery. As endometriosis causes infertility, a sizable fraction of young women with OMAs will eventually require ART to conceive. Surgery for OMA may thus affect ovarian response to COS to the point of compromising ART outcome, even if embryo implantation rates are not affected following ovarian suppression (Surrey et al., 2002; de Ziegler et al., 2010a,b). As surgery does not improve the ART outcome, it should be avoided whenever ovarian reserve is compromised and/or ART immediately necessary (Garcia-Velasco et al., 2004). The fact that OMAs are not associated with diminished AMH levels is intriguing, considering the reports of reduced responses to COS in these cases (Al-Azemi et al., 2000; Geber et al., 2002; Esinler et al., 2006; Matalliotakis et al., 2007).

We therefore suspect that AMH levels may not predict COS response in the case of ovarian endometriosis due to some local ovarian effect of the disease. Gonzalez-Fernandez et al. (2011) recently showed post-receptor alterations in the FSH receptor-signalling pathway in granulosa cells of women with endometriosis, comforting the hypothesis of a local effect that could potentially hamper the response to COS.

The assessment of the ovarian reserve should be part of the preoperative assessment in young women planning to have children, especially in the case of a previous ovarian surgery. In the case of reduced ovarian reserve, the benefit of OMA surgery should be assessed carefully and discussed with the patient. OMA surgery has been shown to reduce ovarian reserve even in the hands of experienced surgeons (Biacchiardi et al., 2011). Taking into account our results, women, with asymptomatic OMAs or endometriosis-related pain responsive to medical therapy, should in principle not be operated in order to spare the ovarian reserve. In the future, preoperative fertility preserving measures (Elizur et al., 2009) such as oocyte or embryo cryopreservation (Tao and Del Valle, 2008) could be offered in as part of the treatment strategy to young women who require extensive ovarian surgery for severe endometriosis-related pain.

Conclusion

Our study shows that AMH levels are diminished in women with previous OMA surgery but not in those currently having OMA but no past OMA surgery. Our finding of unaltered AMH levels in women with OMA is unexpected because these women are known to respond poorly to COS. Hence, we are led to suspect that normal AMH levels may not predict COS response in the case of ovarian endometriosis because of some local ovarian effect of the disease. Our findings therefore call for studying the relationship between AMH levels and COS responses when OMAs are present.

In our view, young women desiring to conceive should be operated for OMAs only in the case of severe pelvic pain (Chapron et al., 2012) or doubts about the benign nature of their cyst.

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Authors’ roles

I.S., D.Z. and C.C. conceived and designed the study. All the authors analysed and interpreted the data. I.S. and J.M. supervised and reviewed all the statistical analysis. C.C. and P.S. contributed to data collection and performed the surgical procedures. G.B. contributed to the data collection and performed all AMH measurements. I.S., D.Z., J.M. and C.C. contributed to writing the manuscript. All the authors approve the final version of the manuscript.

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Conflict of interest

None.

References


Chapron C, Souza C, de Ziegler D, Lafay-Pillet MC, Ngo C, Bijouzi G, Goffinet F, Borghese B. Smoking habits of 411 women with


Matalliotakis IM, Cakmak M, Mahutte N, Fragouli Y, Arci A, Sakkas D. Women with advanced-stage endometriosis and previous surgery respond less well to gonadotropin stimulation, but have similar IVF implantation and delivery rates compared with women with tubal factor infertility. *Fertil Steril* 2007; 88:1568–1572.


