Decreased anti-Müllerian hormone and altered ovarian follicular cohort in infertile patients with mild/minimal endometriosis

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Objective: To evaluate the ovarian reserve and follicular cohort of infertile patients with minimal/mild endometriosis.

Design: Prospective study.

Setting: University hospital.

Patient(s): Patients were divided into two groups: group I, minimal/mild endometriosis and group II, tubal obstruction. The following exclusion criteria were established: [1] patients with previous endocrine disorders; and [2] cases in which the cause for infertility was other than endometriosis (except for patients with tubal obstruction, in the control group).

Intervention(s): Serum FSH and anti-Müllerian hormone were measured on day 3. On the same day all patients were submitted to transvaginal ultrasound to evaluate the antral follicular count and the ovarian follicular cohort.

Main Outcome Measure(s): Serum FSH, anti-Müllerian hormone, and the follicular cohort with the respective antral follicular count.

Result(s): Serum FSH were not different between the groups. However, infertile patients with endometriosis have a decreased serum anti-Mullerian hormone (1.26 ± 0.7 ng/mL) compared to the control group (2.02 ± 0.72 ng/mL). The analysis of follicular cohort showed that the number of selectable follicles were similar, but the follicular diameter was different.

Conclusion(s): Minimal/mild endometriosis is associated with a decrease in the follicular ovarian reserve. In addition, the follicular cohort of these patients is more heterogeneous in comparison to the control group. (Fertil Steril® 2008;89:1064–8. ©2008 by American Society for Reproductive Medicine.)

Key Words: FSH, anti-Müllerian hormone HHHHhHH, selectable follicles, endometriosis, infertility

Endometriosis is a prevalent disease usually associated with subfertility (1–3). In addition, using assisted reproductive techniques (ART) infertile patients with endometriosis have a poorer prognosis compared to tubal obstruction (4).

To evaluate the ovarian follicular status, classically, early follicular phase serum FSH, inhibin B, and E_2 levels have been measured. However, the usefulness of those measurements and its clinical utility is limited (5, 6). In addition, the assessment of the number of antral follicles by ultrasonography may predict the number of retrieved oocytes after controlled ovarian hyperstimulation (COH). Furthermore, a recent meta-analysis concluded that antral follicle count might be considered the test of first choice in the assessment of ovarian reserve before IVF (7).

Anti-Müllerian hormone (AMH) is produced by small, early antral follicles and was strongly connected to the number of small antral follicles than FSH, E_2, and even inhibin B levels (8). In vivo and in vitro studies showed that AMH has an inhibitory effect on primordial follicle recruitment and it decreases the sensitivity of follicles for the FSH-dependent selection for dominance. Besides its functional role in the ovary, serum AMH level serves as an excellent candidate marker of ovarian reserve (9–11). Anti-Müllerian hormone assessment should be considered as a useful adjunct to serum FSH level and antral follicle count when estimating ovarian reserve (12) and AMH has also been shown to be a useful marker for ovarian aging (13).

In addition, AMH is a marker for ovarian reserve and, as previously demonstrated, a better predictor of the number of early antral follicles than FSH, inhibin B, E_2, and LH (9, 14). However, AMH is not the perfect marker and we are still searching for the best and accurate marker of ovarian response.

Although several investigators tried to investigate the hormonal status in endometriotic patients, only two studies specifically reported the status of ovarian reserve in infertile patients with endometriosis. However, those investigators merely evaluated the ovarian reserve using a retrospective design and during COH cycles (15, 16). They showed a decrease in inhibin B during COH in infertile patients with endometriosis and, an increased serum FSH level in patients with moderate/severe endometriosis during the early follicular phase.

Therefore, we do not have any convincing data with an adequate design regarding the follicular reserve status or ovarian cohort in this group of patient with minimal/mild endometriosis and infertility.

The aim of the present study is to present for the first time the evaluation of ovarian follicular cohort and its reserve in infertile patients with the milder forms of endometriosis, measuring serum FSH and AMH levels and the number of selectable follicles on cycle day 3.

MATERIALS AND METHODS
Design
We performed a prospective study.

Subjects
Patients were divided into two groups: group I (study group) patients with minimal or mild endometriosis as proven by laparoscopy (n = 17); group II, patients with tubal obstruction without endometriosis (controls) (n = 17).

All patients were undergoing routine exploration and laparoscopy for investigation of infertility and as a part of this study; the procedure was always performed by the same investigator. Endometriosis staging was performed according to the classification of the American Society for Reproductive Medicine (1985). We also excluded endometriosis in patents from group II (control). All patients and partners had regular menses, normal sperm analysis, both ovaries, absence of ovarian tumor (endometrioma), serum TSH and PRL into normal range, and body mass index (BMI) <27 kg/m^2.

The following exclusion criteria were established: [1] patients with previous endocrine disorders; [2] cases in which the cause for infertility was other than endometriosis (except for patients with tubal obstruction, in the control group); and [3] ovarian endometriosis.

Measurements
We collected blood samples on cycle day 3 to measure serum FSH and AMH. On the same day all patients underwent transvaginal ultrasound to determine the ovarian follicular cohort,

measuring number and diameter of all selectable follicles by the same investigator (E.A.). The plasma was centrifuged at 2,500 rpm and frozen at −80°C for later analysis. Patients were informed about the procedures and gave signed, informed consent form. The research project was approved by the Ethics Committee and registered at the Graduate Research Group of the hospital (Institutional Review Board [IRB] equivalent).

Serum analysis was performed at the RIA laboratory of Hospital de Clínicas of Porto Alegre, Brazil, with specific kits.

Serum FSH levels was determined using chemiluminescence detection (Systems Elecsys, Roche Diagnostics GmbH, Mannheim, Germany) and serum AMH were determined using an ultrasensitive ELISA (ACTIVE; DSL, Webster TX). The coefficients of variability (CV) for AMH were: functional sensitivity, 0.2 ng/mL, intra-assay CV, 4%, and interassay CV, 8%, and for FSH, functional sensitivity, 0.1 mIU/mL, intra-assay CV, 3%, and interassay CV, 5%.

Statistics
The two-tailed Student's t-test was used to analyze continuous data, whereas the χ^2 test or Fisher's exact test were used for categorical data. A P value $<.05$ was considered statistically significant.

The power calculation before this study protocol required the inclusion of 30 patients for a $\beta = 80\%$, considering AMH and the mean follicular diameter as the primary end points and taking into consideration the SD = 0.75 and to detect a difference of 0.80 ng/mL between groups.

RESULTS
Clinical characteristics of both groups were similar. The median and 95% confidence interval (CI) for age in the study group were 29.5 years (20–37 years) and 30.5 years (24–37 years) for the control group, $P>.05$. In addition, BMI (22.2 ± 2 kg/m^2 and 22 ± 2.4 kg/m^2, $P = .472$) were also similar between groups.

The groups were not different regarding serum FSH levels on day 3, in addition all studied subjects presented with serum FSH levels below 12 IU/mL. The serum FSH level (mean ± SD) was 5.2 ± 1.8 IU/mL for patients with endometriosis and 4.9 ± 1 IU/mL for those patients with tubal obstruction ($P>.05$).

The number and diameter of all selectable follicles were shown in Table 1. The number of selectable follicles was similar (12 ± 1.3 and 11 ± 1.6; $P=.732$), but the mean follicular diameter was different (5.7 ± 2.4 mm and 4.6 ± 1.5 mm; $P=.001$). Furthermore, using Levene's test to compare its SD, the groups were different, showing a heterogeneous follicular cohort ($P = .001$).

Figure 1 shows that infertile patients with endometriosis had a decreased serum AMH level (1.26 ± 0.7 ng/mL) compared to the control group (2.02 ± 0.72 ng/mL), $P=.004$.

Number of selectable follicles and follicular diameter: *t*-test.

	Group I endometriosis (n = 17)	Group II tubal obstruction (n = 17)	*P* value
Number of selectable follicles	12 ± 1.3	11 ± 1.6	.732
Follicular diameter (mm)	5.7 ± 2.4[a]	4.6 ± 1.5[a]	.001

Note: values are expressed as mean and SEM.
[a] Levene's test for SD comparison: *P*= .001.

Lemos. Follicular cohort, AMH, and endometriosis. Fertil Steril 2008.

Furthermore, the per follicle AMH secretion (Fig. 2) shows that infertile patients with minimal/mild endometriosis also had a decrease in AMH secretion.
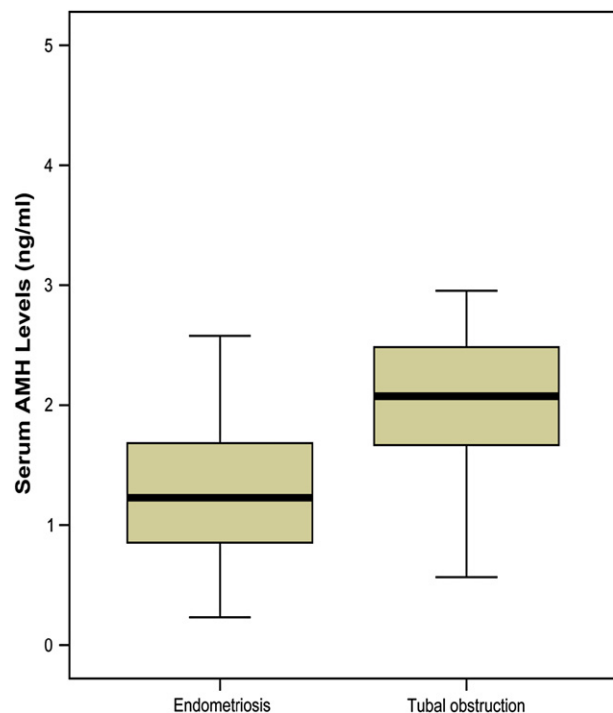
To verify that our results were not obtained only by chance, we analyzed the power calculation using our data. The power was 90% for serum AMH levels and 95% for ovarian cohort evaluation.

DISCUSSION

This study demonstrated that infertile patients with minimal and mild endometriosis had lower serum AMH levels on day 3. In addition, these patients presented with a more heterogeneous follicular cohort and its mean follicular diameter was larger than those of infertile patients with tubal obstruction.
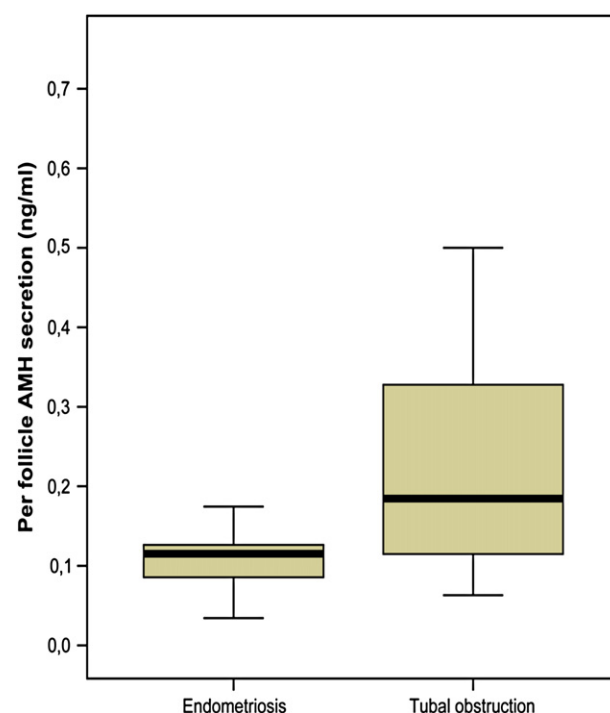
Serum anti-Müllerian hormone (AMH) (in nanograms per milliliter) comparison between infertile patients with minimal/mild endometriosis and infertile patients with tubal obstruction (without endometriosis). The box represents the interquartile range that contains the 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. *P*= .004, Student's *t*-test.

Lemos. Follicular cohort, AMH, and endometriosis. Fertil Steril 2008.

Per follicle anti-Müllerian hormone (AMH) (in nanograms per milliliter) secretion comparison between infertile patients with minimal/mild endometriosis and infertile patients with tubal obstruction (without endometriosis). The box represents the interquartile range that contains the 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. *P*= .001, Student's *t*-test.

Lemos. Follicular cohort, AMH, and endometriosis. Fertil Steril 2008.

Our research verified that the number of selectable follicles was not modified by the presence of endometriosis.

Minimal and mild endometriosis was related to subfertility due to several mechanisms, including some hormonal and ovarian dysfunctions. Those patients present with altered LH and PRL secretions, reduced ability of the preovulatory follicle, oocyte dysfunction, and altered luteal function (4, 17). Other researchers showed that endometriosis could affect the reproductive outcome resulting in low quality embryos, with reduced implantation rates (18). The heterogeneous follicular cohort demonstrated in this study reinforces these hypotheses, that endometriosis could be linked to an altered ovarian milieu and, consequently, follicular development.

However, we really could not conclude, based in our data, that AMH plays some role in the pathogenesis of endometriosis. We investigated the association between endometriosis and an altered ovarian environment. Before any controlled stimulation protocol, we recommend investigating the ovarian reserve of all infertile patients with endometriosis to properly assess the reproductive potential.

We could evaluate the ovarian follicular reserve using the assessment of the number of antral follicles by ultrasonography, the antral follicle count, which is associated with several reproductive outcomes, including pregnancy rates (PR) (11, 14). Therefore, AMH may represent a more independent and reliable marker of early antral follicle count than inhibin B, E_2, and FSH on cycle day 3 (8). In addition, plasma AMH assessments were superior to FSH in identifying women with reduced ovarian reserve (13, 19).

The control and dynamic of AMH secretion was not completely understood. Serum AMH levels decline gradually during multiple follicular maturation, reflecting the reduction in the number of small antral follicles due to COH.

In addition, AMH is preferentially and constantly secreted by small antral follicles during COH or during the regular menstrual cycle. These observations support the hypothesis that differentiation of granulosa cells (GC) during follicular growth is likely to alter its AMH expression (20).

The fact that serum AMH levels were different, but that the number of antral follicles was the same between the groups was probably explained by the fact that, as stated by several investigators, serum AMH levels are more sensitive than other tests to evaluate the ovarian reserve.

In addition, if we assume that small follicles secrete more AMH and it was well demonstrated that follicles with a diameter >8 mm could not secrete AMH (11), the follicular discrepancy demonstrated in patients with endometriosis might explain the decreased AMH secretion in this group of patients.

Recently, serum baseline AMH levels were associated with oocytes quality in stimulated cycles (21). It was shown that AMH seems to be superior to FSH in predicting both oocytes quality and number after COH. Anti-Müllerian hormone may serve also as a follicular ovarian regulator, mainly during the initial stages of follicular development (22). It has an inhibitory effect on early follicular growth and development in vitro. This could partially explain its in vivo action during COH.

However, serum AMH levels did not show a significant fluctuation during a spontaneous menstrual cycle (23). We can conclude that the effect of this hormone is mainly restricted to the ovarian follicular milieu and its mechanism needs to be clarified.

Although several investigators studied the hormonal environment in infertile patients with endometriosis, only two articles directly investigated the role of ovarian reserve in endometriotic patients (15, 16). However, differently from our study, those investigators included infertile patients with all stages of endometriosis. In addition, they assessed the ovarian reserve measuring FSH or using a retrospective design only to compare inhibin B concentration in patients submitted to IVF. We included only patients with minimal/mild endometriosis or tubal obstruction to exclude other possible infertility etiology (bias) in the group of subjects.

Although serum AMH levels and all other tests to predict or evaluate ovarian reserve have limited value (6), our results, regarding the ovarian reserve in infertile patients with minimal/mild endometriosis, were absolutely consistent and could explain the subfertility in this group of patients.

Clinically, we did not have a cutoff to properly distinguish poor responders using the AMH as an ovarian marker. In addition, using our data, some patients with endometriosis had serum AMH levels similar to the control group. This occurred because as expected and already demonstrated by other investigators (6), the accuracy of AMH to investigate the ovarian reserve is limited and not all infertile patients with endometriosis will be a poor responder.

Our data, concerning follicular cohort, indicated that we need more studies to investigate the luteal–follicular transition in this group of patients to explain and recognize all involved physiopathological pathways.

In conclusion, patients with minimal/mild endometriosis present a decreased serum AMH level. In addition, the follicular cohort in those patients was heterogeneous compared to infertile patients with tubal obstruction. This finding may be associated with poorer results in terms of COH. Our data is the first direct evidence showing an important role of endometriosis in the follicular status and ovarian reserve, which could explain the subfertility in this group of patients.

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