Impact of tubal ligation on ovarian reserve as measured by anti-Müllerian hormone levels: a prospective cohort study

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Abstract

Background: Tubal ligation (TL) is considered a very efficient contraceptive method. However, some patients complain of a variety of postoperative symptoms. The objective of this study was to investigate whether ovarian reserve may change after tubal ligation.

Study Design: This was a prospective cohort study of 80 fertile women who underwent TL. Ovarian reserve was evaluated by measuring pre- and postoperative (1 year after surgery) serum anti-Müllerian hormone (AMH) levels and transvaginal antral follicular count (AFC). Potential confounding factors were age, body mass index (BMI), smoking, surgical technique and prior contraceptive methods. The Wilcoxon test was used to compare pre- and postoperative (12 months) AMH and AFC, and simple and multiple linear regression were used to evaluate confounding factors.

Results: Fifty-two patients completed the study protocol. The median AMH level was 1.43 ng/mL (interquartile range 0.63–2.62) preoperatively and 1.30 ng/mL (interquartile range 0.53–2.85) after 12 months (p=.23). The mean AFC was 8.0 (interquartile range 5.0–14.0) and 11.0 (interquartile range 7.0–15.0) before and after TL, respectively (p=.12). These differences were not statistically significant. Use of a hormonal contraceptive method prior to TL was significantly associated with a postoperative increase in AMH.

Conclusions: This study suggests that ovarian reserve is not altered by TL.

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1. Introduction

Tubal ligation (TL) is one of the most widely employed contraceptive techniques worldwide, with an excellent safety record [1–3]. In Brazil, nearly 30% to 40% of women who have decided that their family is complete ask for this method of birth control [4]. Despite its efficacy, a non-negligible number of patients who undergo this procedure postoperatively complain of a variety of symptoms, including irregular cycles, increased or prolonged menstrual flow, spotting, dysmenorrhea and dyspareunia [5–7].

Since the first report of a so-called post-tubal ligation syndrome in 1951 by Williams et al. [8], many authors [9–22] have exhaustively tried to determine, in an objective manner, the existence of changes in ovarian reserve after tubal sterilization, which could characterize a real syndrome. Most of the tests and methods used to assess possible changes in ovarian reserve [serum levels of hormones, such as follicle-stimulating hormone (FSH) and estradiol; ultrasonographic parameters, such as ovarian volume; ovarian vessel Doppler; and determination of menstrual pattern by questionnaires] [23] are subjective or unable to demonstrate significant changes on a short-term basis, making them unreliable for characterization of this potential condition.
In the last decade, the literature on anti-Müllerian hormone (AMH) has presented promising data concerning its ability to determine ovarian reserve [24,25]. This hormone was recently considered the best existing predictor of ovarian reserve, as it can only be detected in serum from the menarche [26] until the onset of menopause [27], making it a reliable marker of the reproductive potential of a woman. Furthermore, it exhibits little inter- [28] and intra-cycle [29–31] variability and is able to detect subtle changes in ovarian reserve, as it decreases slowly as age advances [32]. Most studies also show that fluctuation of the antral follicle count (AFC), determined by transvaginal ultrasound, is consistent with the decrease in AMH levels as women approach menopause [33], making the combination of these two tests the best method currently available for evaluation of ovarian reserve and potential [24,32,34–39].

The aim of this study was to conduct an objective evaluation of the possible independent association between TL and decreased ovarian reserve in women who underwent TL by measurement of serum AMH prior to and 1 year after this procedure.

2. Materials and methods

2.1. Study design, setting and participants

This was a prospective cohort study of 80 women who requested tubal sterilization and had no contraindications for tubal ligation surgery, consecutively enrolled from May 2008 to February 2009. Patient follow-up was conducted until February 2010. The choice of this contraceptive method and of the surgical technique had already been made by patients and their physician at the time of recruitment. All women were recruited from the Family Planning Units of two university hospitals in Porto Alegre, Brazil (Hospital de Clínicas de Porto Alegre and Hospital Materno-Infantil Presidente Vargas).

2.2. Eligibility criteria

Women were eligible for inclusion if they were fertile (had at least one living child), between the ages of 20 and 40 years, requested TL spontaneously (independently from this study) and agreed to provide written informed consent for participation. The exclusion criteria were any of the following: history of ovarian or fallopian surgery, history of infertility, chemotherapy or radiation therapy, hemodialysis and refusal to provide written informed consent.

2.3. Study procedures

All patients had blood samples drawn prior to surgery (for baseline data) and after signing the informed consent form. Serum was stored at −80°C until the time of testing, at the end of the study, when all samples were tested for AMH together, to prevent potential variation in readouts. Measurements were obtained by enzyme-linked immunosorbent assay (ELISA) (Beckman Coulter, Genese Immunotech, France), as described elsewhere [40]. Also before TL, all patients underwent transvaginal ultrasonography for AFC. These tests were performed by two experienced sonographers at a private human reproduction clinic in Porto Alegre, Brazil, always using the same ultrasound equipment (Siemens Sonoline Adara™ with a 5-MHz transvaginal probe). AFC was defined as the number of all small follicles (between 2 and 10 mm) counted in both ovaries.

The choice of TL technique (laparoscopic bipolar coagulation or Pomeroy technique through a mini-laparotomy) was made by each patient’s physician, according to patient characteristics and to any contraindications. All surgical procedures were performed in each patient’s hospital of origin, independently of this study.

Six months after surgery, patients were called and asked whether they had experienced any unpleasant symptoms or needed any additional information. Twelve months after surgery, patients returned to the clinic where transvaginal ultrasounds were performed for repeat AFC (carried out by the same sonographers and using the same equipment) and blood collection for measurement of serum AMH levels. Samples were processed in the same manner as prior to surgery. After the end of the follow-up period, all serum samples were thawed at room temperature for AMH measurement.

2.4. Variables and outcomes of interest

The primary outcome of interest was change from baseline serum AMH (ng/mL), defined as a 20% decrease in AMH values at 1-year follow-up. The secondary outcome measure was any change in AFC in the same period. Age, body mass index (BMI), smoking, surgical technique and prior contraceptive methods were considered potential confounders (independent variables).

For statistical analysis, patients were stratified by age into three groups (25–30, 31–35 and >35 years), as no patients were <25 years old. BMI was calculated as weight in kilograms divided by height in meters squared. Patients were considered nonsmokers if they had remained completely smoke-free for at least 1 year prior to inclusion in the study. All others were considered smokers (active smoking), regardless of number of cigarettes smoked, including those who quit smoking less than 1 year before recruitment. Surgical technique was a dichotomous variable (laparoscopic bipolar cauterization or Pomeroy technique through a mini-laparotomy). Contraceptive methods used before TL were divided into hormonal methods (combined oral contraceptive pill, hormonal implant and injectable contraceptive) and nonhormonal methods (condom, copper IUD and no contraception).

2.5. Sample size calculation

Sample size was calculated on the basis of the method of van Rooij et al. [41]. To detect a change in serum AMH of
≥20% from baseline at 12-month measurement, considering a significance level of \( p < 0.05 \) and 80% statistical power, the minimum sample size was calculated as 40 subjects.

### 2.6. Statistical analysis

The Wilcoxon test was used to compare pre- and postoperative (12-month follow-up) AMH levels and AFC. The differences between post- and preoperative AMH levels and AFC were calculated as the difference between 12-month and baseline measurements, and are hereafter referred to as AMH delta and AFC delta, respectively.

The relationship between AMH delta and AFC delta was evaluated through the Spearman correlation coefficient, as AMH delta exhibited a non-normal distribution.

The relationship between AMH delta and AFC delta and its possible predictors (use of hormonal contraceptives, smoking habit, surgical technique, age and BMI) was evaluated through simple and multiple linear regression techniques. A \( p \) value < 0.05 was considered statistically significant.

### 2.7. Ethical aspects

This study was conducted in accordance with the Brazilian national principles and policy for human subject research (National Health Council Resolution 196/96) and was approved by the ethics committees of both hospitals involved. All patients were informed of the study protocol before surgery and provided written informed consent for participation.

### 3. Results

From May 2008 to February 2009, 80 patients who fulfilled the eligibility criteria for this study and who provided written informed consent were recruited. Twenty-eight patients were excluded during the follow-up period: 18 due to loss (phone numbers and/or addresses provided were no longer available); 5 due to incomplete data (patients who did not have a blood sample collected or ultrasound performed), 3 who moved away from the city, state or country; and 2 who withdrew their consent. Fifty-two patients completed the protocol and had their data analyzed.

Patient characteristics are detailed in Table 1. Baseline AMH ranged from 0.05 to 9.2 ng/mL (median 1.43 ng/mL, interquartile range 0.63–2.62), and 12-month levels, from 0.05 to 9.8 ng/mL (median 1.30 ng/mL, interquartile range 0.53–2.85). Median baseline AFC was 8.0 (interquartile range 5.0–14.0), and the median 12-month count was 11.0 (interquartile range 7.0–15.0). The changes between baseline and 12-month AMH and AFC (AMH delta and AFC delta) were not statistically significant (\( p > 0.05 \)).

Analysis of predictors of AMH and AFC delta is shown in Table 2. The only variable associated with AMH delta during the 12-month follow-up period, both on univariate and on multivariate analysis, was use of hormone contraceptives, which was significantly associated with increased AMH levels at 12-month follow-up. No variables showed a consistent association with AFC delta (Table 2). BMI was associated with AFC delta on comparison between the BMI 25–30 and BMI <25 strata, but this association did not persist on analysis of BMI >30 vs. <25 and should therefore be interpreted with caution.

### 4. Discussion

This study evaluated the possibility of changes in ovarian reserve after TL by measurement of serum AMH levels and, secondarily, quantification of ovarian AFC. Women who underwent TL showed no significant changes in median serum AMH and AFC 1 year after the procedure as compared to baseline values. Furthermore, with the exception of hormonal contraceptive methods, none of the potential influencing factors analyzed (age, BMI, smoking or surgical technique) showed any independent association with AMH delta or AFC delta. This suggests that, at least during a 1-year follow-up period, TL does not alter ovarian reserve.

These results are consistent with those of previous studies [10–13,16,18] that showed no significant variation in ovarian reserve after TL using even longer follow-up. However, these studies used now-outdated and inefficient methods for assessment of ovarian reserve on a short-term basis, such as estradiol and FSH levels, ovarian Doppler and menstrual questionnaires. Nonetheless, studies that found evidence of significant ovarian reserve changes after TL [6,42,43] used the same methods, which makes it impossible to distinguish whether their findings are a reliable reflection of changes in ovarian reserve. Conversely,
Small and temporary fluctuations in AMH levels occur routinely in young and, especially, fertile women, but also because minor fluctuations in AMH levels are irrelevant, not only because it was not statistically significant, though slight, increases in median AMH values observed at 1-year follow-up, no control group), this was, to the best of our knowledge, the first Brazilian study on ovarian reserve changes after TL to use AMH levels and AFC quantification. Furthermore, the ovarian reserve tests used in this study are currently considered the best parameters for evaluation of minor changes in ovarian reserve. Previous trials evaluating the influence of hormonal contraception on AMH levels were carried out on samples smaller than those used in this study.

5. Conclusion

The results of this current 12-month follow-up cohort study suggest that TL does not induce any significant decrease in ovarian reserve.
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References


