3 to 5 Years Later: Long-term Effects of Prophylactic Bilateral Salpingectomy on Ovarian Function

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1 **Full title**: **Three to five years later: long-term effects of prophylactic bilateral**

2 **salpingectomy on ovarian function.**

3 **Precis:** The addition of bilateral salpingectomy during hysterectomy is a safe

4 procedure and does not cause ovarian reserve impairment, even 3 to 5 years after

5 surgery.

6 **Authors**: Roberta Venturella[a], MD, Daniela Lico[a], MD, Massimo Borelli[b], Phd, Maria

7 G. Imbrogno[a], MD, Gabriele Cevenini[c], Phd, Errico Zupi[d], Phd, Fulvio Zullo[a], Phd,

8 and Michele Morelli[a], Phd.

9 [a]*Department of Obstetrics & Gynecology, "Magna Graecia" University - Catanzaro,*

10 *Italy.*

11 [b]*Department of Life Sciences, University of Trieste, Italy.*

12 [c]*Department of Medical Biotechnologies, University of Siena, Siena, Italy.*

13 [d]*Department of Biomedicine and Prevention, Obstetrics and Gynecological Clinic,*

14 *University of Rome "Tor Vergata," Rome, Italy.*

15 **Corresponding author:** Roberta Venturella, MD. mail: rovefa@libero.it Phone and

16 fax number: +390961883234. Postal address: Department of Obstetrics &

17 Gynaecology – "Magna Graecia" University - viale Europa, loc. Germaneto, 88100

18 Catanzaro.

21 **The authors have nothing to disclose.**

22

23 **Structured abstract**

24 **Study Objective:** Preliminary data on the effects of prophylactic bilateral

25 salpingectomy (PBS) show that postoperative ovarian function is preserved up to 3

26 months after surgery. The confirmation of PBS safety on ovarian function even many

27 years after surgery is essential to reassure the medical community that this new

28 strategy, recently proposed for the prevention of ovarian cancer, is at least able to

29 avoid the risk of premature surgical menopause. We investigated whether the

30 addition of PBS during total laparoscopic hysterectomy (TLH) causes long-term

31 effects on ovarian function.

32 **Design:** Observational study

33 **Design classification**: Canadian Task Force II-3

34 **Setting:** Department of Obstetrics & Gynecology, "Magna Graecia" University -

35 Catanzaro, Italy.

36 **Patients:** Seventy-nine patients who underwent TLH plus salpingectomy between

37 September 2010 and September 2012 at our Institution have been recalled to be

38 submitted to ovarian reserve evaluation in February 2015. Eight of 79 women

39 refused to participate in this follow-up study.

40 **Interventions:** The ovarian age of PBS patients has been determined through

41 OvAge®, a statistical model that combines Anti-Müllerian-Hormone (AMH), Follicle-

42 Stimulating-Hormone (FSH), 3D Antral Follicle Count (AFC), Vascular-Index (VI),

43 Flow-Index (FI) and Vascular-Flow-Index (VFI) values. The control group consisted

44 of a large population of 652 healthy women (with intact uterus and adnexa)

45 previously enrolled to build the OvAge® model. Comparisons between ovarian ages

46 of PBS patients and the control group have been assessed by (ANCOVA) linear

47 statistical modeling.

48 **Measurements and Main Results:** The main outcome measurement was the

49 differences in the behavior within OvAge/age relation between PBS and control

50 women. Descriptive statistics of those 71 enrolled PBS patients are the following:

51 age 49.61±2.15 years; OvAge 49.22±2.57 years; FSH 43.02±19.92 mU/mL; AMH

52 0.12±0.20 ng/mL; 3D AFC 1.91±1.28; VI 2.80±5.32%; FI 19.37±5.88; and VFI

53 0.56±1.12. ANCOVA analysis disclosed that PBS and control women do not exhibit

54 different behavior (p = 0.900) within OvAge/age relation.

55 **Conclusion**: According to our model, the addition of PBS to TLH in the late

56 reproductive years does not modify the ovarian age of treated women up to 3 to 5

57 years after surgery.

58

Main text

Introduction

Recent literature suggests that many high-grade serous carcinomas develop from the epithelium of the distal fallopian tube, and that serous tubal intraepithelial carcinoma (STIC) represents the putative precursor of these neoplasms (1). According to the new guidelines of the American Cancer Society and the American College of Obstetricians and Gynecologists (2,3), and considering the new theory on the pathogenesis and origin of these aggressive gynecologic cancers, prophylactic bilateral salpingectomy (PBS) has been suggested as a new preventive strategy for average-risk women, not carrying BRCA mutations, who completed their reproductive desire. The rationale of this approach is that, while eliminating the primary source of cancer, PBS in place of standard bilateral salpingo-oophorectomy could also reduce the risk of premature death due to cardiovascular disease seen in women subjected to salpingo-oophorectomy before the onset of natural menopause (4).

On the other hand, two recent publications about the effect of salpingectomy tubal surgery for hydrosalpinx prior to in vitro fertilization (IVF) (5,6) recommended laparoscopic salpingectomy or proximal tubal occlusion in cases of surgically irreparable hydrosalpinges to improve IVF pregnancy rates. Although meta-analytic data clearly demonstrate that salpingectomy increases pregnancy rate in women undergoing IVF (RR 2.24, 95% CI 1.27-3.95) (7), contradictory results are available on the comparison in terms of ovarian response to hyperstimulation during IVF between patients who did and did not undergo salpingectomy (8).

82   Preliminary data on the safety of PBS showed that postoperative ovarian

83 function is preserved at least 3 months after surgery (9-11), but to date no evidence

84 of the long-term effects of PBS are available in the literature. The confirmation of

85 PBS safety in ovarian function even many years after surgery is essential for

86 reassuring the medical community that the new proposed preventive strategy is at

87 least able to ward off the risk of premature surgical menopause. Adverse health

88 effects of premature surgical menopause include bone resorption; psychosexual,

89 cognitive, and cardiovascular dysfunction; and increased incidence of fatal and non-

90 fatal heart disease (4).

91   We previously evaluated the short-term effect of PBS on a population of 79

92 women subjected to total laparoscopic hysterectomy (TLH) with PBS between 2010

93 and 2012 (9). The goal of the current study was to evaluate the ovarian function of

94 these women up to 5 years after the primary surgery to evaluate, for the first time in

95 the literature, the long-term effect of PBS on ovarian function. For this purpose, we

96 used OvAge®, a validated generalized linear model (GzLM) that combines a

97 patient's biochemical and 3D ultrasonographic values and generates a number that

98 is an estimate of the woman's ovarian age (12).

99 **Materials and methods**

100 This was an observational study conducted at the University "Magna Graecia" of

101 Catanzaro, in the Department of Obstetrics and Gynecology, between February and

102 September 2015.

103   The procedures used in the study were in accordance with the guidelines of

104 the Helsinki Declaration. The study protocol was approved by the Ethical Committee

105 of the Department of Gynecology and Obstetrics, University "Magna Graecia" of

106 Catanzaro.

107   All patients who underwent TLH with PBS for abnormal uterine bleeding

108 related to benign pathology in our Department between September 2010 and

109 September 2012, and who had given their consent to participate in our retrospective

110 analysis in 2013 (9), were identified and contacted by two investigators (MGI and

111 DL).

112   All these women had undergone TLH and complete bilateral excision of the

113 fallopian tubes. Salpingectomy also had been performed at that time, according to

114 the standard technique, thus sparing the mesosalpinx. The tubes had been

115 coagulated and sectioned, beginning from the very distal fimbrial end, carefully

116 preserving the ovarian vascularization, and proceeding toward the uterine cornu.

117   Patients who experienced any of the following after the TLH plus PBS were

118 excluded from the study: acute or chronic pelvic inflammatory disorders; malignant

119 neoplasms; chemotherapy or radiotherapy; autoimmune diseases; chronic,

120 metabolic, endocrine, and systemic disorders; had ovarian surgeries; or received

121 estrogen-progestin therapy or metformin in the 2 months prior to enrollment (13).

122 Women with and without menopausal symptoms were analyzed.

123   Anti-Müllerian-Hormone (AMH), Follicle-Stimulating-Hormone (FSH), Estradiol

124 (E2), 3D Antral Follicle Count (AFC), Vascular-Index (VI), Flow-Index (FI) and

125 Vascular-Flow-Index (VFI) were measured in all women. In ovulating women, ovarian

126 reserve had been evaluated when early follicular phase was confirmed by the

127 absence at ultrasound of a dominant follicle >10 mm in any of the ovaries in

128 conjunction with the presence of serum E2 level <60 pg/mL and P<1 ng/mL. Given

129 the poor reliability of the FSH values in the presence of E2 levels >60 pg/mL, data

130 from women with basal E2 levels greater than this cut-off were excluded from

131 analysis, and both patients and women with follicles >10 mm in any of the ovaries or

132 with the evidence of corpus luteum were asked to come back 10 to 30 days later,

133 according to their previous menstrual histories or to the dimension of the pre-

134 ovulatory follicle.

135   A single experienced investigator (DL) performed all of the ultrasound scans

136 using a Voluson-i (GE Healthcare Ultrasound, Zipf, Austria) and a 5–9-MHz

137 transvaginal volume transducer, which has 3D ultrasound scanning modes. AFC and

138 VI were measured using a 3D ultrasound dataset, with a sonography-based

139 automated volume count and virtual organ computer-aided analysis imaging program

140 (VOCALTM) (SonoAVC^{TM} and VOCALTM, GE Healthcare Ultrasound, Zipf, Austria),

141 as previously described (12).

142   The intra-observer reliability was expressed as the mean intra-class

143 correlation coefficient (ICC) with a 95% CI. The mean ICCs (95% CI) for the 3D

144 scanning of the VI, FI, and VFI were 0.9792 (0.9654, 0.9869), 0.8871 (0.7139,

145 0.9719), and 0.9929 (0.9748, 0.9967), respectively. The mean ICCs for data

146 acquisition of VI, FI, and VFI were 0.9823 (0.9412, 0.9934), 0.9869 (0.9619, 0.9934),

147 and 0.9825 (0.9513, 0.9977), respectively.

148   On the same day of the ultrasonography, blood samples obtained by

149 venipuncture were centrifuged within 30 minutes of collection for 10 min at 3500 rpm

150 and 4℃. Aliquots of each serum sample were frozen at -80℃ and stored for

151 subsequent assays of AMH, FSH, and E2. To measure serum AMH levels, an AMH-

152 Gen II ELISA assay kit (Beckman Coulter) was used. The lowest detection limit of

153   AMH is 0.08 ng/mL, and the intra- and inter-assay coefficients of variation were

154   <3.4% and 4.0%, respectively. An ECLIA method was used to measure the levels of

155   serum FSH and E2 by a COBAS e411 auto-analyzer (Roche Diagnostics). The

156   lowest detection limit for FSH was 0.1 IU/L, and the intra- and inter-assay

157   coefficients of variation were <2.6% and 3.5%, respectively. The lowest detection

158   limit for E2 was 18.4 pmol/L, with intra- and inter-assay coefficients of variation of 2%

159   and 3%, respectively.

160   To determine the long-term effect of surgery on each patient's ovarian reserve,

161   we used OvAge®, a mathematical formula that takes a patient's biochemical and

162   ultrasonographic values as input and generates an easy-to-interpret number, also

163   called OvAge, which is an estimate of a woman's ovarian age, according to the linear

164   relation as follows: $OvAge = 48.05 - 3.14*AHM + 0.07*FSH - 0.77*AFC - 0.11*FI +$

165   $0.25*VI + 0.1*AMH*AFC + 0.02*FSH*AFC$ (12). For obtaining this formula, we

166   previously recruited 652 healthy women, aged 18 to 55, with a history of

167   spontaneous conception(s); intact ovaries, fallopian tubes, and uterus; and regular

168   menses with a mean interval of 21 to 35 days to serve as control subjects. Exclusion

169   criteria for the selection of the these subjects, enrolled as training population for the

170   OvAge model, had been: estrogen or progestin use or breastfeeding in the 2 months

171   before enrollment; pregnancy; history of female infertility; endometriosis; presence of

172   ovarian follicles measuring more than 10 mm at study entry ultrasonography and

173   other cystic masses of the ovary; history of autoimmune disease' polycystic ovary

174   syndrome (PCOS); ovarian surgery; gynecologic malignancy; previous radiation or

175   chemotherapy; known chronic, endocrine, systemic, and metabolic disease including

176   diabetes mellitus; hyperandrogenism; hyperprolactinemia and thyroid diseases;

177 hypogonadotropic hypogonadism; and a history of use of a drug that can cause

178 menstrual irregularity.

179   In the original OvAge study, in women enrolled as healthy fertile controls, the

180 model showed a high level of fit between chronological age and predicted OvAge,

181 meaning that in the absence of risk factors known to be detrimental for ovarian

182 function, chronological age and predicted OvAge are equivalent. Conversely, in

183 patients with known causes of impaired ovarian function, a significant difference

184 between these two parameters was shown, indicating that the formula produced was

185 able to recognize pathological deviation from physiologic gonadal activity (12).

186   For the purpose of the current study we assumed that, if the addition of PBS

187 to TLH did not have any detrimental effect on ovarian function, considering our

188 previous and current exclusion criteria, the predicted OvAge of our 79 patients would

189 be similar to their chronological age. Specifically, if the addition of PBS to TLH does

190 not cause detrimental effects to ovarian function worse than those reported 3 months

191 after surgery (9), differences in the behavior within OvAge/age relation between PBS

192 and control women would not be significant.

193   To summarize data, means and standard deviations or absolute frequencies

194 and proportion were addressed to continuous or count covariates, accordingly. To

195 assess differences between OvAge® and age within PBS patients versus the healthy

196 control group, ANCOVA linear modeling was used (14). In all instances, a 0.05

197 significance level was assumed, and the calculations have been performed by R

198 (15).

199 **Results**

200 From February to September 2015, 79 women were called and asked to participate

201 in this long-term follow-up study. Eight of 79 (10.1%) women refused to participate,

202 leaving 71 patients for the study group. The patients were given the OvAge test, and

203 the results were analyzed. Descriptive statistics of those 71 enrolled women are

204 shown in Table 1.

205   To confirm the hypothesis that the addition of PBS to TLH did not have any

206 detrimental effects on ovarian function, so that the predicted OvAge of our patients

207 would be similar to their chronological age, we proceeded in the following way. We

208 considered the age of a woman to be a continuous response with respect to OvAge

209 and group (i.e., PBS treated or control) covariates. We addressed several statistical

210 models, as explained in Crawley (14); in particular, we focused on three models: an

211 ANCOVA model with interaction between covariates, an ANCOVA model without

212 interaction, and a simpler linear model (i.e., a regression line) over OvAge without

213 group information. Proceeding by a top-down strategy in model selection according

214 to a deviance analysis (14), we disclosed that all models are equivalent in a

215 statistical sense. This finding allows us to retain, as a minimal adequate model, the

216 simpler model as shown in Table 2.

217   The simpler model, the minimally adequate one, exhibits the significant role of

218 the OvAge in estimating women ages and, implicitly, neglects any role of the PBS

219 treatment/control covariate, with a very similar residual standard error (sigma = 4.208

220 on 729 degrees of freedom), but with an excellent multiple R-squared (0.9885). A

221 deviance analysis confirms the equivalence (p = 0.986) of two models, confirming

222 the hypothesis that the addition of PBS to TLH did not have any detrimental effect on

223 ovarian function (Fig. 1).

224 **Discussion**

225 By means of OvAge linear model, in the current study we demonstrated that in our

226 population the addition of PBS to TLH in the late reproductive years did not have

227 negative effects on ovarian function, not only a few months after surgery, but also 3

228 to 5 years later. In our patients, ovarian age (or OvAge) was found to be similar to

229 their chronological age, since linear models implemented for the statistical analysis

230 showed that there is no difference between treated and untreated groups. Although

231 limited to a well-selected population of women undergoing PBS with TLH in their late

232 reproductive years, this is the first published study reporting information about the

233 long-term effect of salpingectomy performed for cancer prophylaxis.

234 This finding can be supported by the observation that ovarian blood supply is

235 guaranteed both by infundibolopelvic vessels and by the ovarian branch of the

236 uterine artery, which anastomose with each other at the tubal level (16). At the time

237 of bilateral salpingectomy, the whole infundibolopelvic blood volume, which was

238 previously distributed between tubes and ovaries, becomes fully available to the

239 ovaries, ensuring adequate gonadal vascularization and maintained ovarian steroid

240 hormone synthesis (16).

241 In the current study, for the first time in the literature, a long-term evaluation of

242 the effect of salpingectomy on ovarian age has been carried out on a cohort of

243 patients who underwent standard TLH with PBS 3 to 5 years earlier. This was

244 possible by using both a new algorithm that combines hormonal and three-

245 dimensional ultrasonographic parameters and a very large population of healthy

246 women as the control group (12). This sample of 652 healthy fertile women,

247 previously enrolled to generate the OvAge algorithm, is to date the larger dataset

248 available for comparing treated and untreated women in terms of ovarian function. It

249 represents the nomogram of reference for the variable "ovarian age," having been

250 built on a population of women deeply screened for all those factors currently known

251 to be able to affect ovarian function. The assumption on which this study is based is

252 that, if PBS does not cause significant detrimental effects on hormonal and

253 ultrasonographic ovarian parameters, the ovarian age of the treated patients should

254 not deviate significantly (i.e., as to raise at least one OvAge unit/year) from that of

255 women who have never been submitted to this kind of surgery. This assumption was

256 verified by our results; despite a mean 47% decrease in AMH levels from baseline

257 through 3 to 5 years for the 71 PBS women, their OvAge was comparable with that

258 of the control population, meaning that a similar change probably occurs in untreated

259 women in course of their life.

260   Up to now, the effect of PBS on ovarian function was evaluated at only 3

261 months postoperatively, and the obtained trend was encouraging in all the studies. In

262 2007, even before the diffusion of the new theory about the tubal origin of most high-

263 grade serous cancers, Sezik et al.(17) measured hormonal markers of ovarian

264 function (FSH,LH, and estradiol) to evaluate ovarian reserve of women treated by

265 hysterectomy, and they found no difference among women who underwent

266 salpingectomy versus those who did not. In 2013, we published a retrospective

267 analysis in which the ovarian reserve was not reduced in patients in whom PBS was

268 added to TLH performed for benign uterine pathologies (9). In our study, ovarian

269 reserve was evaluated by AMH, FSH, AFC, mean ovarian diameters, and peak

270 systolic velocity. In the same year, Findley et al. (10), in a pilot randomized controlled

271 trial, used AMH to measure ovarian reserve and concluded that salpingectomy at the

272 time of laparoscopic hysterectomy with ovarian preservation had no short-term

273 deleterious effects on ovarian function.

274   In 2015, at our institution, women undergoing laparoscopic surgery for uterine

275 myoma or tubal surgical sterilization were randomly subjected to standard

276 salpingectomy or wide mesosalpinx excision (11). Ovarian function was evaluated by

277 the measurement prior to and 3 months after surgery of AMH, FSH, three-

278 dimensional indexes, and OvAge for each patient. We found no significant difference

279 between groups, and we speculated that even when the surgical excision includes

280 the removal of the entire mesosalpinx, salpingectomy does not damage the ovarian

281 reserve (11).

282   Recently, a systematic review and meta-analysis of studies comparing the

283 pregnancy outcomes of patients diagnosed with hydrosalpinx treated with

284 salpingectomy versus those treated with proximal tubal occlusion prior to IVF

285 showed comparable responses to controlled ovarian hyperstimulation and pregnancy

286 outcomes between the groups (18). This is an additional demonstration that

287 salpingectomy does not worsen the reproductive prognosis of patients who choose

288 excisional surgery. On the other hand, results on ovarian response to medical

289 induction of superovulation in patients treated by salpingectomy are conflicting (19-

290 21), but authors agree that bilateral salpingectomy has been proved to be a safe

291 procedure at least in terms of surgical outcome and short-term postoperative

292 complications (22). Moreover, three different meta-analyses also reported increased

293 ongoing and clinical pregnancy rates with salpingectomy versus no intervention and

294 no difference in the clinical pregnancy rates between salpingectomy and tubal

295 occlusion (7,23,24).

296   The strength of the current study is the long-term evaluation, for the first time

297   in the literature, of a well-screened population of women subjected to PBS in the

298   absence of tubal pathologies, even when in their late reproductive years. All the

299   currently known confounding factors that can interfere on ovarian reserve were listed

300   among the exclusion criteria for both the first (9) and the current study. Reliable and

301   definitive information on the safety of PBS is critically important for the entire medical

302   community, considering that the idea that salpingectomy should be proposed as a

303   preventive strategy for low-risk women who undergo gynecologic surgery is gaining

304   increasing popularity. Moreover, although risk-reducing salpingo-oophorectomy

305   (RRSO) at around age 40 years is currently recommended to women who carry the

306   BRCA1/2 mutation, an alternative preventive strategy has been put forward for high-

307   risk women: early PBS and delayed oophorectomy (RRO). Although preventive

308   RRSO decreases the ovarian cancer risk by 80% to 96%, based on its short-term

309   and long-term morbidity, which potentially affect quality of life, and on recent insights

310   into the fallopian tube as the possible site of origin of serous ovarian carcinomas,

311   early PBS and delayed RRO could be the best solution.

312   Recently, a multi-center non-randomized trial has started enrollment, and

313   participants will choose between standard RRSO at age 35 to 40 years (BRCA1) or

314   40 to 45 years (BRCA2) and the alternative strategy of PBS on completion of

315   childbearing and RRO at age 40 to 45 years (BRCA1) or 45 to 50 years (BRCA2).

316   The aim of the study is to measure menopause-related quality of life, but also

317   ovarian/breast cancer incidence, surgery-related morbidity, histopathology,

318   cardiovascular risk factors and diseases, and cost-effectiveness (25).

319   The increasing interest in PBS as a preventive strategy for both low- and high-

320   risk women and the related crucial importance of long-term follow-up data are

321 confirmed by many web-based surveys of health professionals' acceptability/attitude

322 toward the new proposal (26-29).

323   A limitation of the study is the relatively small sample size of women

324 evaluated. However, given the homogeneous distribution of results among this

325 population, and considering that this is currently the largest sample of women

326 studied in the literature, the results presented deserve attention. Another limitation of

327 the current study is that the control population is not the same used in the original

328 study (9), that being composed of women treated by standard TLH (with adnexal

329 preservation) between September 2008 and September 2010, matched for uterine

330 weight. Unfortunately, none of these women had undergone an OvAge test at that

331 time, and the gap of average age of at least 2 years makes it impossible to

332 accurately compare that group with the PBS population. Age at menopause of these

333 women will be an interesting outcome for forthcoming analysis, despite the need of

334 consensus for a correct definition for women without a uterus. In this context, further

335 research on the validation of the OvAge model to accurately predict the time to

336 menopause is already in progress.

337   Two other possible limits of our study are: first, the range of time

338 postoperatively at which the subjects have being evaluated creates a variable in

339 comparison of the data. However, according to our previous experience (30), when

340 there is evidence of impaired ovarian reserve caused by vascular damage, the

341 modification of biochemical and ultrasonographic parameters is already evident at 3

342 months follow-up, and it remains constant at 1 and 2 years. In the same way,

343 reassuring parameters at 3 months do not worsen at the next re-evaluation, allowing

344 us to speculate that the results of the current study are poorly affected by the range

345 of evaluation times.

346   Furthermore, at surgery and at the time of follow-up, our patients already had

347   evidence of diminished ovarian reserve, given their mean age of 45.97±2.36 and

348   49.61±2.15, respectively. Although it would be better to assess the impact of surgery

349   on younger women, in which ovaries have greater potential for functional loss,

350   unfortunately any assessment of the impact of prophylactic salpingectomy on

351   ovarian reserve is not ethically conducible in patients who have not yet fulfilled their

352   reproductive desire. That is why, to date, in all studies conducted in patients

353   undergoing salpingectomy for cancer prophylaxis, in the absence of tubal pathology

354   and not for reproductive intent, the average age of the population has always been

355   more than 37 years (10,11,17). Ideally, the same rigorous approach used for women

356   subjected to PBS should be applied on all future studies aimed at evaluating the

357   effect of this kind of surgery in women undergoing IVF, to extend the assessable

358   population and to definitively conclude the mid- and long-term safety of laparoscopic

359   salpingectomy.

360   In conclusion, according to our results, the addition of PBH during TLH in the

361   late reproductive years does not cause ovarian function impairment up to 3 to 5

362   years after surgery.

363

364   **Table legends**

365   Table 1. **Descriptive statistics of enrolled women**

366   Table 2. **Linear models applied**. Two models are summarized. In model 1, an

367   ANCOVA with interaction (maximal) model is applied. A simpler model, the minimally

368   adequate one, neglects any role of the treatment/control covariate, with a very

369   similar residual standard error, confirming the hypothesis that the addition of PBS to

370   TLH had no detrimental effect on ovarian function.

371

372   **Figure Legend**

373   **Figure 1: Two-dimensional plot for regression analysis.** The OvAge control

374   group (gray and white bullets) and the TLH plus PBS treated group (black and dark

375   gray diamonds). In the panel, three regression lines have been drawn: a 'two-

376   parameters' thin dashed line, with intercept and slope specific for the control group; a

377   'two-parameters' thin continuous line, with intercept and slope specific for the treated

378   group; and, a 'one-parameter' solid dashed line, with common slope (and null

379   intercept) for both groups. All three regression lines do not differ in a statistical

380   sense; consequently, the 'one-parameter common line' is the minimal adequate

381   model to retain as a valid description of both groups.

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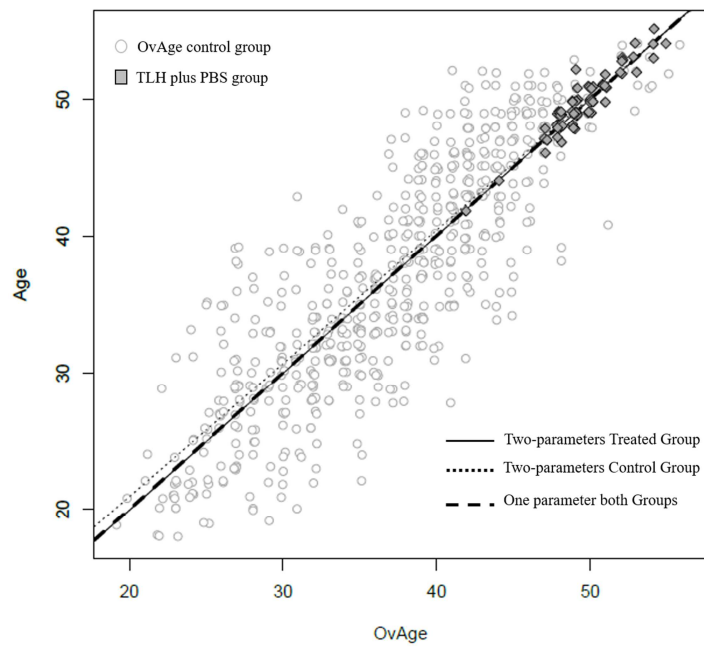
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Table 1. Descriptive statistics of enrolled women

Parameters	Mean values±SD
Age at surgery (years)	45.85 ± 2.40
Age at follow-up (years)	49.61 ± 2.15
OvAge at follow-up (years)	49.34 ± 2.12
FSH at follow-up (mU/mL)	43.02 ± 19.92
AMH at follow-up (ng/mL)	0.12 ± 0.20
3D AFC at follow-up (n.)	1.91 ± 1.28
VI at follow-up (%)	2.80 ± 5.32
FI at follow-up (1-100)	19.37 ± 5.88
VFI at follow-up (1-100)	0.56 ± 1.12

Table 2. **Linear models applied**. Two models are summarized. In model 1, an Ancova with interaction (maximal) model is approached. A simpler model, the minimal adequate one, neglect any role of the Treatment/control covariate, with a very similar residual standard error, confirming the hypothesis that the addition of PBS to TLH did not have any detrimental effect on ovarian function.

2

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Maximal model			
	Estimate	Standard Error	p-value
Intercept	0.1005	0.8474	.906
OvAge	1.0028	0.0225	< .001
Treatment	1.5632	11.2572	.890
OvAge : Treatment	0.0286	0.2282	.900
Minimal adequate model			
	Estimate	Standard Error	p-value
OvAge	1.0008	0.0040	< .001

Precis:

The addition of bilateral salpingectomy during hysterectomy is a safe procedure and does not cause ovarian reserve impairment, even three to five years after surgery.