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Effect of laparoscopic cystectomy for ovarian endometriomas on ovarian reserve, as measured by Anti-Müllerian hormone. A prospective cohort study.

Running title:

Cystectomy for endometriomas and Anti-Müllerian hormone.

Title page

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Effect of laparoscopic cystectomy for ovarian endometriomas on ovarian reserve, as measured by Anti-Müllerian hormone. A prospective cohort study.

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Abstract:

Title: Effect of laparoscopic cystectomy for ovarian endometriomas on ovarian reserve, as measured by Anti-Müllerian hormone. A prospective cohort study.

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Background: Cystectomy for ovarian endometriomas is commonly performed, however recent publications have shown a deleterious effect of cystectomy on ovarian reserve.

Objectives: To evaluate what effect laparoscopic cystectomy for ovarian endometriomas has on the ovarian reserve.

Methods: Prospective cohort study performing standard laparoscopic cystectomies for ovarian endometriomas. Primary outcome: assessment of the ovarian reserve using Anti-Müllerian hormone (AMH), done pre- and six months postoperatively. Secondary outcome: spontaneous pregnancy during follow up.

Results: We enrolled 59 participants. Twenty-five participants were lost to follow up and a further 3 were excluded from analysis. The average age was 33.1 years. Thirty-one participants completed the six months follow up. The mean preoperative and postoperative AMH value was 3.21ng/mL [95% CI: 2.24 – 4.18ng/mL, SD 2.64] and 1.48ng/mL [95% CI: 1.06 – 1.91ng/mL, SD 1.17] respectively: equating to a 53.89% decline, P=0.002. Twenty participants had unilateral cysts whilst the remaining 11 had bilateral endometriomas. The mean preoperative and postoperative AMH for unilateral cysts was 3.22ng/mL

[95% CI:1.93 – 4.51ng/mL, SD 2.76] and 1.82ng/mL [95% CI: 1.23 – 2.41ng/mL, SD 1.26] respectively, representing a 43.48% decrease, P=0.072. Of the 11 with bilateral endometriomas the mean preoperative AMH was 3.19ng/mL [95% CI: 1.49 – 4.89ng/mL, SD 2.54] with 0.88ng/mL [95% CI: 0.43 – 1.33ng/mL, SD 0.67] representing the postoperative AMH, equating to a 72.41% reduction, P=0.005.

Conclusion: Serum AMH is negatively affected by laparoscopic cystectomy for ovarian endometriomas, with a significant decline in ovarian reserve as measured six months postoperatively. A greater decline is seen in patients with bilateral endometriomas compared to unilateral disease.

Keywords: Anti-Müllerian hormone, AMH, cystectomy, endometrioma, endometriosis, ovarian reserve, three-step technique.

1) Introduction:

Endometriosis is defined by the presence of endometrial tissue outside the uterus, leading to a chronic inflammatory reaction. [1] It is an oestrogen dependent condition, affecting up to 10% of the general population. [2, 3] Ovarian endometriosis is the commonest site for endometriosis implants (54.9%), followed by the broad ligament, the vesicouterine space, the Pouch of Douglas and the uterosacral ligaments, in patients with endometriosis and infertility. [4] Endometriomas have no true capsule, instead a pseudocapsule. Therefore, damage to healthy ovarian tissue commonly occurs with accidental removal of healthy ovarian tissue, during cystectomy.

The formation of an ovarian endometrioma is postulated to form via the metaplasia theory. Ovarian epithelial inclusions invaginate into the ovarian cortex, undergoes metaplasia into endometrial tissue, forming an endometrioma. There is no real cleavage plane between the ovarian cortex and endometrial type stroma with follicles commonly found near endometrial stroma. The lining of the endometrioma cyst wall is commonly formed by fibrous and granulation tissue containing hemosiderin-laden macrophages. The histological diagnosis can often prove difficult in the absence of obvious endometrial stroma and or glands. [5]

This contrasts with Hughesdon and Brosens theory, who suggest that most endometriomas are formed by superficial endometrial implants on the surface of the ovarian peritoneum, originating from menstrual debris, becoming sealed off by adhesions and invaginating into the ovarian cortex. [6,7]

Multiple markers for ovarian reserve testing are available, including FSH, LH, Antral Follicular count, $Estradiol_2$, Inhibin B and Anti-Müllerian hormone (AMH). AMH has been shown to correlate well with the AFC in terms of ovarian reserve measurement, equaling AFC's predictive performance. [8]

AMH is produced by columnar granulosa cells of primary follicles, after these cells have differentiated from flattened pregranulosa cells of primordial follicles. [9] AMH belongs to the transforming growth factor ß family, of dimeric glycoproteins, involved in tissue growth and differentiation. [10] Ovarian

aging results in a gradual decline in the primordial follicular pool. It is not possible to directly measure this primordial pool; however, this pool can be indirectly assessed by assessing the pool of growing follicles. AMH is expressed from growing follicles and it can be viewed as an indirect marker of this primordial follicular pool. [9] AMH testing following cystectomy for ovarian endometriomas, allows the assessment of the impact of such surgery on the ovarian reserve.

The Cochrane review by Hart et al. 2008 concluded that cystectomy for endometriomas measuring more than three centimeters is favored over drainage alone. [11] However recent evidence suggests a significant decline in ovarian reserve in such patients. In a systematic review and meta-analysis done by Raffi F et al. evaluating the impact of surgery for ovarian endometrioma on ovarian reserve, AMH fell by 38% after surgery. In the sub analysis, bilateral disease was associated with a greater decline in AMH. [12] This is corroborated by a systematic review by Somigliana E et al. where nine of 11 studies reported a significant reduction in serum AMH following surgery. [13]

We hypothesize that by using experienced endometriosis surgeons, we can minimize accidental damage to the surrounding healthy ovarian tissue, during laparoscopic cystectomies for ovarian endometriomas, thereby preserving the ovarian reserve.

1) Materials and Methods:

The study was approved by the Health Research Ethics Committee 1 (HREC1) under the auspices of Stellenbosch University, ethics number: S13/11/230, which abides by the declaration of Helsinki. Participants were recruited from Aevitas Fertility Clinic, Western Cape, South Africa. This was a prospective cohort study.

Primary outcome was the assessment of ovarian reserve, by AMH, done pre- and six months postoperatively, in participants undergoing standard laparoscopic cystectomy for ovarian endometriomas. Secondary outcome: spontaneous pregnancies within the 6 months postoperative follow up. Inclusion

criteria were: 18 to 45 years of age, sonographic evidence of an endometrioma and participants who agreed to partake in the study signed informed consent. Exclusion criteria were: pregnancy, clinical or sonographic concern of malignancy or post-menopausal at enrollment.

We elected to use AMH to determine the ovarian reserve as it is easily obtained, avoids the need for further ultrasonography, nor is it operator dependent. We were not constrained by where participants were in their menstrual cycle at the time of surgery, when the ovarian reserve was initially evaluated.

AMH was measured preoperatively and repeated six months following surgery. AMH assays used were the AMH GenII ELISA (Beckman Coulter), the automated Elecsys (Roche) and Access (Beckman Coulter). Most of the samples were analyzed using the Elecsys assay. AMH was quantified as ng/mL with a lower detection limit of 0.16ng/mL (manually attained). The automated assays resulted in an increased sensitivity and resulted in a lower detection limit of 0.10ng/mL.

Surgery was performed by experienced sub specialists in the field of endometriosis surgery with one surgical team performing all the surgeries. General anaesthesia preceded Verres needle insufflation and port placement. Bipolar electrocautery and insertion of ovarian sutures to obtain haemostasis was avoided. Argon plasma coagulation (APC) was used instead. Firstly, anatomy was restored, drainage of the chocolate coloured fluid followed, thereafter the cleavage plane between the ovary and the pseudocapsule was identified. Starting furthest away from the cyst hilus, traction and counter traction was applied using two atraumatic graspers to the cyst and the ovary, performing the cystectomy. If bleeding was encountered or the cyst hilus was reached, the cystectomy was stopped. The cyst was then excised and the hilus was vaporized using APC, minimizing injury to the underlying healthy ovarian tissue.

For participants with endometriomas measuring ≥6cm, we offered the option of the three-step technique as described by Donnez J et al. instead of standard cystectomy. [5] An initial laparoscopic drainage procedure was followed by a three-month course of a GnRH agonist, followed by cystectomy as described above, during a second procedure.

Data was captured via an Excel datasheet, anonymized and analyzed using StataSE version 15.1 of 2017. As the continuous data was not normally distributed, it was analyzed descriptively and graphically. Descriptive statistics include means (with 95% confidence interval), standard deviations, minimums and maximums. Box plots (excluding outliers) indicate interquartile ranges in relation to the median. For comparing mean pre- and post-operative AMH levels, we performed the Mann-Whitney U and the Students T test, and a non-parametric equality-of-medians test.

2) **Results:**

Between June 2014 and November 2015, a total of 59 participants were enrolled. The average age was 33.1 years. (Table 1) Three participants were excluded from analysis: two never underwent surgery and a further participant did not have an endometrioma at surgery. Furthermore 25 participants were lost to follow up after surgery. Therefore, 31 participants completed follow. (Figure 1) All participants underwent standard laparoscopic cystectomy apart from 2 who underwent the three-step technique. Both had bilateral ovarian endometriomas with a cyst of at least 6cm.

The mean preoperative and postoperative AMH value was 3.21ng/mL [95% CI: 2.24 – 4.18ng/mL, SD 2.64] and 1.48ng/mL [95% CI: 1.06 – 1.91ng/mL, SD 1.17] respectively: equating to a 53.89% decline, P=0.002. (Figure 2) Twenty participants had unilateral cysts whilst the remaining 11 had bilateral endometriomas. The mean preoperative and postoperative AMH for unilateral cysts was 3.22ng/mL [95% CI:1.93 – 4.51ng/mL, SD 2.76] and 1.82ng/mL [95% CI: 1.23 – 2.41ng/mL, SD 1.26] respectively, representing a 43.48% decrease, P=0.072. Of the 11 with bilateral endometriomas the mean preoperative AMH was 3.19ng/mL [95% CI: 1.49 – 4.89ng/mL, SD 2.54] with 0.88ng/mL [95% CI: 0.43 – 1.33ng/mL, SD 0.67] representing the postoperative AMH, equating to a 72.41% reduction, P=0.005. (Figure 3)

There was one pregnancy during follow up, unfortunately no postoperative AMH is available for this participant.

3) **Discussion:**

AMH does not fluctuate significantly during the menstrual cycle and is dependent on the follicular pool. The pool of growing follicles is consistently renewed from the primordial pool during the menstrual cycle, with the AMH level remaining constant. [14] This allowed for ovarian reserve testing via AMH, no matter where the participant was in her menstrual cycle.

As stated in the methods section, during the study period there was a change in the AMH assay from the manual Generation II (Beckman Coulter) ELISA to a fully automated Access (Beckman Coulter) and Elecsys (Roche) AMH assay. Pearson K et al. evaluated whether the Access assay performed equivalently to the Gen II ELISA by comparing 142 patient samples. Passing-Bablok regression analysis showed good correlation between the 2 methods. [15] However Nelson SM et al. showed considerably lower AMH values with the automated Access assay compared to Gen II, P<.0001, comparing the various assays in 83 women. Similarly, AMH results were found to be lower when comparing the Elecsys assay to the Gen II assay. Median AMH using the Gen II ELISA assay: 3.21 (95% CI: 2.25 – 4.34) compared to 2.83 (95% CI: 1.90 – 3.61) Access automated assay, 22% less. Median AMH using the Elecsys assay was 2.44 (95% CI: 1.78 – 3.01). To standardize the AMH results done via various assays in our cohort, we used the Passing-Bablock regression equation as described by Nelson SM et al. [16]

We elected to study the AMH 6 months postoperatively due to previously published evidence of partial recovery in the levels the longer one moves away from surgery. This was corroborated by Vignali M et al. in 22 participants who underwent laparoscopic cystectomy for ovarian endometriomas looking at the AMH at 1, 3, 6 and 12 months postoperatively. Comparing baseline AMH of (3.98± 3.27ng/mL) to 1 month (1.67 ± 1.56ng/mL), 3 months (2.01 ± 1.70ng/mL), 6 months (2.43 ± 2.39ng/mL), 12 months (4.01 ± 3.39ng/mL) postoperative AMH, we notice that there is a dramatic decrease in AMH 3 months after surgery. Thereafter, there is a gradual increase up to 12 months postoperatively, where there was no statistical difference between the pre and postoperative AMH levels at 12 months, P >0.05. [17]

The study by Donnez J et al. described the approach to surgical management of the large ovarian endometriomas. They showed that using a GnRH agonist after initial drainage for 12 weeks provoked up to a 50% reduction in the size of the endometrioma compared to baseline. It was noted that the surrounding ovarian cortex became progressively thicker after initial drainage and a GnRH agonist for 12 weeks. [5]

Does a GnRH agonist effect AMH, and would this alter the results obtained after such administration? Thirty-three participants given a GnRH agonist in the midluteal phase who had serial AMH assessments in the early follicular phase, the midluteal phase as well as on day 7,14 and 30 after administration of the GnRH agonist. There was a significant decline in AMH with the trough found on day 7 after administration. AMH then rose to above initial midluteal levels by day 14 and 30. One can conclude that AMH might not be a reliable marker within 4 weeks of receiving a GnRH agonist in the assessment of ovarian reserve. [18] However in 22 participants with endometriosis receiving a GnRH agonist for down regulation prior to in vitro fertilization. AMH levels done on the day of receiving the GnRH agonist compared to AMH levels at 12 weeks after administration, showed no difference. Median AMH initially was 1.12 (0.60 – 1.93) and 1.15 (0.62 – 1.70) at 12 weeks after administration. [19] Of our cohort, 2 participants received a GnRH agonist with postoperative AMH testing done 9 months thereafter, negating any effect that the GnRH agonist had on the postoperative AMH level by this time.

We know that the bigger the endometrioma the bigger the reduction in AMH postoperatively after cystectomy. [20] This could be related to a larger percentage of healthy ovarian tissue being removed at cystectomy, a greater postoperative local inflammatory response damaging the remain ovary or a greater negative affect on blood supply to the remaining ovary. We therefor offered participants with large endometriomas the option of the three-step technique to minimize damage to the remaining healthy ovary.

We found a deleterious effect of cystectomy for endometrioma on ovarian reserve using experienced surgeons and sound surgical techniques. Muzii et al. showed that the level of surgical expertise of surgeons, performing laparoscopic excision of endometriomas, has an inversely proportional relationship regarding experience and accidental removal of healthy ovarian tissue. However, even in the presence of

experienced surgeons there is a significant percentage (>50%) of accidental injury to healthy ovarian tissue as confirmed by histological examination of the excised cyst following surgery. [21] Furthermore, the presence of normal ovarian tissue was found in 9 out of 19 women who underwent ovarian cystectomy for ovarian endometrioma, which translated to a larger percentage decline in AMH in a study by Kitajima et al. [22]

Tsolakidis et al. compared laparoscopic cystectomy to the three-step technique evaluating serum AMH prior to surgery and six months postoperatively. A decline from 3.9ng/mL to 2.9ng/mL in the cystectomy group compared to 4.5ng/mL to 3.9ng/mL in the three-step technique group was found, P=0.026. [23] Average cyst diameter in the Tsolakidis study was equal between the two groups, whereas in our study the participants undergoing the three-step technique had larger endometriomas. The size of the endometrioma does matter, translating to a greater decline in AMH the larger the cyst. This has been corroborated by Kashi et al. who showed that in those with unilateral cysts of at least 50mm, preoperative AMH was lower compared to smaller cysts, P=0.027. These patients had a greater decline in AMH postoperatively, P=0.028. Bilateral cysts also showed a greater deleterious effect in terms of AMH decline, P=0.046. [20]

A theory to negate surgery related reduction in AMH is to avoid electrocautery all together. Shao et al. avoided electrocautery, instead used 3-0 Polyglactin sutures for ovarian reconstruction and haemostasis. They however failed to show an AMH sparing effect using this technique. [24]

Lee et al. found that despite a partial recovery in AMH at three months following cystectomy the levels remained well below preoperative levels, P=0.002. [25] Another study by Chang et al. found that AMH recovered partially up to three months postoperatively. [26]

Our study has corroborated the deleterious effect that ovarian cystectomy, for endometriomas, has on ovarian reserve. The Cochrane review of 2008 states that laparoscopic cystectomy is superior to drainage alone. [11] This was prior to the growing body of evidence that has subsequently accumulated regarding

ovarian reserve and AMH testing postoperatively. It is imperative that we incorporate this new knowledge into the future management of such patients.

Study weakness: our large loss to follow up. Despite a substantial loss to follow up we have a cohort of 31 participants. Reasons cited for the high dropout rate were: busy participant lifestyles, fear of a low AMH result and lack of funding for the AMH testing.

4) **Conclusion:**

Laparoscopic cystectomy for ovarian endometriomas negatively effects the ovarian reserve with a statistically significant reduction in AMH as measured six months following surgery. We found a greater decline in participants with bilateral endometriomas compared to unilateral disease. We plan a follow up study looking at serum AMH in our cohort at 12 months postoperatively. Further studies should evaluate the AMH at 12 months postoperatively to show whether there is a complete recovery in AMH or not. Furthermore, future studies should look at employing the three-step technique for all participants, irrespective of the size of the endometrioma, to potentially minimize AMH decline postoperatively.

Conflict of interests:

We have no conflict of interests to declare.

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Legend to figures:

Table 1: Participant demographics

Figure 1: Participant flow chart

Figure 2. Box plot, median preoperative and postoperative AMH

Figure 3: Box plot, median preoperative and postoperative AMH for bilateral versus unilateral ovarian endometriomas.

Supplementary material:

Graphical abstract