

Postoperative AMH reduction is not associated with reduced fecundity two years following ovarian cyst surgery

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In a prospective study, we investigated the impact of anti-Mullerian hormone (AMH) changes following ovarian cyst surgery on the probability to achieve pregnancy and live birth. Women of reproductive age ($n=60$) were included before surgery for benign ovarian cysts. Serum AMH concentrations were determined pre- and postoperative at 6 and 24 months. Information regarding pregnancy wish and attempts to conceive were obtained by a questionnaire. At the time of inclusion, 45/60 women reported desire of children. At six months, the levels of AMH decreased significantly in the whole group and further reduction was observed at two years (from 2,7 mg/L to 2,0 mg/L to 1,1 mg/L, respectively, $p<0,008$), with a percentage reduction of 42,9%. At two-year follow-up, 36 women reported to have attempted to conceive and 18 achieved pregnancy (50%), with a live birth rate of 33%. The percentage change in AMH at two years did not differ significantly between the women who conceived versus those who did not ($p=0.117$). Data reported herein demonstrate that the AMH reduction following ovarian cyst surgery is maintained two years after surgery; however, the postoperative AMH decrease that follows ovarian cyst surgery might not reduce the chances to achieve pregnancy.

Key words: AMH, cyst enucleation, live birth rate, ovarian reserve, ovarian cyst surgery, pregnancy outcome.

Previous data have indicated that serum concentrations of anti-Mullerian hormone (AMH) decrease after ovarian surgery, even when applying careful cyst enucleation techniques without macroscopic evidence of removal of ovarian tissue [1–7]. Hence, concerns have been raised regarding the need for surgery of certain ovarian cysts in young women, mainly due to the fear of reducing the women's ovarian reserve as a consequence of the surgical procedure and thereby reducing fertility. In a recent systematic review, nine out of the 11 studies that were included demonstrated a significant decline of AMH following surgery for endometriomas but also after surgery of other ovarian cyst types [3]. At present, no study has prospectively investigated the long-term impact of AMH changes in fertility outcomes.

Pregnancy rates between 4% and 22% had been reported after surgery for endometriomas [8]. Additionally, a cumulative pregnancy rate up to 40% five years following surgery for endometriomas was reported in a RCT study [9].

The objective: was to investigate the impact of ovarian cyst surgery on ovarian reserve, as determined by postoperative changes in serum AMH and on reproductive outcome of women that attempted to achieving pregnancy within a two-year postoperative follow-up period.

PATIENTS AND METHODS

Women of fertile age (18–44 years) were included in this prospective cohort study between 14 March 2011 to 26 March 2012. At the time of inclusion, the women were scheduled for the surgery of

ovarian cysts at the Department of Obstetrics and Gynecology, Södersjukhuset, Stockholm, Sweden. Indications for cyst surgery included pain, a growing cyst, and fear of cancer. The complete cohort included 112 women of which the following were excluded due to pregnancy at inclusion ($N=12$), unilateral oophorectomy ($N=3$), requirement of repeated surgery for new ovarian cysts during follow-up ($N=4$), no attendance at follow-up ($N=10$) or malignancy ($N=6$).

Hence, the original study population entailed 77 women. Follow-up visits were scheduled at six and 24 months post-operatively. Data regarding the six-month follow-up of this cohort have been reported previously [6]. The outcome of the 60 women who completed a two-year follow-up is reported herein. The Regional Ethical Review Board Committee in Stockholm, Sweden approved the study (no 2011/107-31-4; 2013-532-34).

The women underwent ovarian cyst surgery by either laparoscopy ($N=55$) or laparotomy ($N=5$) depending on the cyst size. The surgical technique has previously been described in detail [6]. Adhesions and/or endometriosis in the peritoneum were always removed, and adhesion prevention was systematically used (Hyalobarrier®, Anika Therapeutics S.r.l, Padova, Italy).

Serum AMH concentrations were determined before surgery at six and 24 months (\pm two weeks) following surgery. The AMH determinations were performed at the Department of Clinical Chemistry, Karolinska University Hospital using an enzyme-linked immunosorbent assay (ELISA) kit (ACTIVE AMH gen II ELISA, Beckman-Coulter Inc. Webster, NY). All blood samples were centrifuged within four hours. Serum samples were stored ($-20\text{ }^{\circ}\text{C}$) for up to 24 h and if not analyzed directly, stored in a freezer at $-70\text{ }^{\circ}\text{C}$. Baseline and six-month AMH samples were analyzed sequentially. The samples from the 24-month AMH follow-up visit were frozen and analyzed in four batches. The intra-assay and interassay coefficients of variation were 5.4% and 5.6%, respectively.

The AMH levels at six months could be categorized into three groups; Group A included women with AMH levels $<1.0\text{ mg/L}$, group B included women with AMH $1.0\text{--}3.5\text{ mg/L}$ and group C with AMH levels $>3.5\text{ mg/L}$ (range $3.7\text{--}11\text{ mg/L}$) at six months postsurgery.

The women answered a questionnaire on reproductive issues including pregnancy wish, fertility treatments, reproductive outcomes and fertility distress at both the preoperative visit and at the two-year postoperative follow-up. The study-specific items in the baseline and two-year follow-up questionnaires have been validated and used in previous studies [6, 10, 11].

STATISTICAL ANALYSIS

Baseline characteristics are presented as absolute and relative frequencies with either means and standard deviations (SDs) or medians with ranges, as appropriate. Independent t -test, Chi-square test/Fischer's exact test or Pearson Chi-square test were used for comparisons.

Clinical characteristics of study participants at baseline.
Three out of four women reported in the baseline questionnaire a desire of children

	All women, N=60	Desire for children at time of inclusion, N=45	No desire for children at time of inclusion, N=15	p values
	N %	N %	N %	
Age at surgery mean (SD)	31.8 (6.3)	30.4 (5.9)	35.9 (5.9)	0.003*
BMI (kg/m ²)	25.3 (4.4)	24.9 (4.6)	26.2 (3.5)	0.319*
<i>Previous pregnancies 0,376 [1]</i>				
Yes	29 (48.3)	20 (44.4)	9 (60)	
No	31 (51.7)	25 (55.6)	6 (40)	
<i>Previous children 0.202 [1]</i>				
Yes	19 (31.7)	12 (26.7)	7 (46.7)	
No	41 (68.3)	33 (73.3)	8 (53.3)	
<i>Previous infertility 0.719 [1]</i>				
Yes	13 (21.7)	9 (20)	4 (26.7)	
No	47 (78.3)	36 (80)	11 (73.3)	
<i>Previous infertility treatment 0.580 [1]</i>				
Yes	4 (30.1)	2 (22.2)	2 (50)	
No	9 (69.2)	7 (77.8)	2 (50)	
<i>Histopathology of cysts 0.343 [1]</i>				
Endometriotic cyst	20 (33.3)	17 (37.8)	3 (20)	
Nonendometriotic cyst	40 (66.6)	28 (62.2)	12 (80)	
<i>Type of surgery 0.463 [2]</i>				
Cyst enucleation	50 (83.3)	39 (86.7)	11 (73.3)	
Cystectomy with removal of ovarian tissue	3 (5.0)	2 (4.4)	1 (6.7)	
Fenestration or puncture of cyst	7 (11.7)	4 (8.9)	3 (20)	

*Independent t-test. [1] Chi-square test/Fischer's exact test. [2] Pearson Chi-square test.

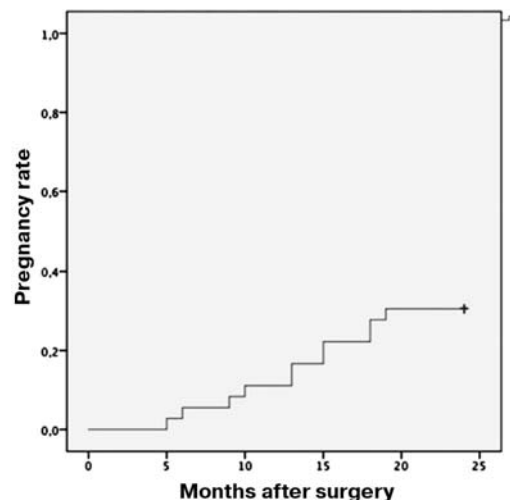
Percentage change over time was calculated as ((AMH baseline – AMH 2 years)/AMH baseline) × 100. As Shapiro-Wilk's tests indicated non-normality of distribution of AMH levels, nonparametric tests, including Wilcoxon's signed-rank test and Mann-Whitney U-test, were used for analysis. One-way analysis of variance (ANOVA) test, the Kruskal-Wallis test and Pearson's Chi-squared test were used to compare subgroups based on desire for children at baseline, achievement of pregnancy and absolute AMH concentration at the six-month follow-up visit. All statistical analyses were performed using PASW Statistics 22 software (SPSS Inc., Chicago, IL). Statistical significance was set at a two-tailed p values of 0.05 or less.

Survival analysis of the probability to achieve pregnancy leading to a live birth after ovarian cyst surgery. For these analysis only women with desire of children and who attempted pregnancy were considered (N=36). Vertical axis: cumulative pregnancy rate; horizontal axis: months after surgery.

RESULTS OF RESEARCH AND DISCUSSION

Table 1 summarizes clinical characteristics of the study participants. At study inclusion, the mean age was 31.8 years (range 18–43 years) and most of the women (45/60) expressed a desire to have one or more future children. Serum AMH concentrations decreased significantly in the whole group from 2.7 mg/L (range 0.2–16.9 mg/L) to 2.0 mg/L (0.2–11.0) at six months and were further reduced to 1.1 mg/L (0.2–20.7) (p=0.001 for both) with a median 43% reduction from baseline levels. There was a significant negative linear correlation between baseline AMH concentration and age (r=-0.3, p=0.002). One-third of the cysts were endometriotic, and the remainder nonendometriotic (Table 1); however, the degree of AMH decline after surgery was similar and did not differ significantly between patients with endometriotic and non-endometriotic cysts.

During the two-year study period, 36 women attempted to con-



ceive, 18 achieved pregnancy (50%) and 12 had a live birth (33%) (Figure). Among the women who attempted to conceive, 34 completed all AMH determinations at two years (Table 2). Two women who became pregnant did not complete all AMH determinations. Out of the 34 women with complete AMH data, significant AMH reductions were observed both in the group of women who became pregnant (n=16) and those who did not conceive (n=18), from 3.3 mg/L to 1.0 mg/L (p=0.057), and from 3.2 mg/L to 2.0 mg/L (p=0.003), respectively. However, the reduction in AMH over time was similar and did not differ significantly between these two groups (p=0.112).

Reproductive behavior, pregnancy rate or live births among women who attempted to conceive were similar and did not differ significantly between the three AMH category groups (Table 2). In

Table 2

Women who reported a desire for children at baseline and completed all three AMH determinations (N=42)

	Group A Low AMH <1.0 mg/L at 6 months, N=7	Group B Normal AMH 1.0-3.5 mg/L at 6 months, N=27	Group C High AMH >3.5 mg/L at 6 months, N=8	p values
Age (years) Mean (SD)	35.3 (5.0)	29.2 (5.4)	29.3 (7.1)	0.045*
AMH mg/L Median (min-max) baseline	0.3 (0.2-3.0)	2.9 (0.7-7.2)	5.9 (3.3-16.9)	<0.001 [1]
6 months	0.4 (0.2-0.9)	2.1 (1.0-3.4)	7.3 (3.7-11.0)	<0.001 [1]
2 years	0.2 (0.2-0.7)	1.3 (0.3-5.4)	6.0 (1.7-20.7)	<0.001 [1]
Worry about fertility (Scale 1-10) presurgery	5.0 (1.0-8.5)	6.3 (0-10.0)	5.0 (0-8.0)	0.530 [2]
Worry about fertility at 2 years	2 (0-10)	6.0 (0-10.0)	2.5 (0-6.0)	0.121 [2]
Women with desire of future children at two-year follow-up	3	21	5	0.194 [2]
Infertility at two-year follow-up	2	8	0	0.213 [2]
Women who attempted to conceive and had complete	4	23	7	0.211 [2]
AMH data N=34				
Pregnancies among women who attempted to conceive (17/34)	2 (50%)	11 (48%)	4 (57%)	0.911 [3]
Live birth rate among women who attempted to conceive (11/34)	0	8 (35%)	3 (43%)	0.312 [3]
The women were informed about their AMH levels and changes at the 6-months follow-up visit and categorized into three groups according to their AMH (Groups A, B, C). during the two-year study period, 36 women attempted to conceive, 18 achieved pregnancy and 12 had a live birth. However, only 34 of these women completed all three AMH determinations and are included in the table.				

*One-way ANOVA. The statistically significant difference was between Group A and Group B.

[1] Kruskal-Wallis test. The statistically significant difference was between Group A and the other two groups. [2] Pearson Chi-square test.

[3] Pearson Chi-square test. The p values are counted for total pregnancies (both spontaneous and ART) and life birthrates (excluded: live birth after oocyte donation).

the women with a low AMH (<1 mg/L) prior to surgery, two spontaneous pregnancies occurred but none of those resulted in live births and the only live birth was achieved after oocyte donation.

This is the first prospective study that reports ovarian reserve, pregnancy rate and live birth rates of women followed for a two-year period after ovarian surgery. Data presented here indicate acceptable fecundity rates among the women who attempted to conceive, with a live birth rate of 33% during the follow up period of two years. As such, it could be inferred that the chance of pregnancy does not seem to be reduced, as ovarian surgery primarily impacts the number of residual ovarian follicles and not the quality of the oocytes they contain.

Ovarian surgery for benign indications is common. The current global trend aims towards fertility sparing surgery by cyst enucleation in young women and the frequency of oophorectomies has decreased [12, 13]. However, fertility-sparing surgery for ovarian cysts has been associated with a significant reduction of female ovarian reserve as estimated by changes in serum AMH concentration [1, 3, 4]. During the two-year follow-up of the present study, AMH concentrations were markedly reduced in the study group by nearly half compared to baseline levels.

A progressive decline of AMH over time in fertile healthy women has earlier been established [14–16]. Bentzen et al reported a 5.6% yearly decline of AMH [15] and La Marca et al reported a median decline of 0.16 mg/L per year [16]. The 43% median decline of serum AMH concentrations found in our cohort (from 2.7 mg/L to 1.1 mg/L) is larger than that previously described in association with aging and without ovarian surgery. In similar prospective studies, such a sustained AMH reduction up to 12 months post-surgery has been demonstrated [1, 3, 17], but to our knowledge, no extended follow-up of has been presented. Our data confirm a significant reduction at six months after ovarian cyst surgery and demonstrates that AMH continues to further decline at 24 months.

Studies following patients after conservative ovarian cystectomy have indicated that fertility is preserved with acceptable reproductive outcomes [8, 9, 18, 19]. In a meta-analysis of 14 studies, the pregnancy chance after laparoscopic surgery for endometriomas varied from 30% to 67%, with a calculated weighted mean of 50% during

a follow-up time of 36 months [20]. Our study confirms these results with a pregnancy rate of 50% in women who attempted to conceive during a follow-up time of 24 months. As regards to pregnancy rates after surgery for benign nonendometriotic cysts, such data are still lacking. Importantly, there were no significant differences in pregnancy rates between women with endometriotic and nonendometriotic cysts. Age is the best validated single predictive factor for achieving pregnancy. The women with AMH in the lowest percentile (<1.0 mg/L) were older in the present cohort and there were no live births among those women with the only exception of the woman that underwent *in vitro* fertilization (IVF) with donor oocytes.

A limitation of our study is the lack of knowledge of additional infertility factors, such as a male factor in the infertile couples. Another reason for infertility might be the risk of adhesions at surgery. At our clinic, it is standard procedure to use antiadhesion barriers prevention of postsurgical adhesions in all women of fertile age [21]. In a Scottish study, no difference was seen in readmissions for adhesions after gynecological laparotomies or laparoscopies [22].

In the present study, women were not recruited at an infertility clinic, which provides external validity for young women presenting with ovarian cysts and facing ovarian surgery. It could be inferred the possibility of selection bias as some women did not complete the two-year follow-up. However, analysis of this group (N=17) showed no significant differences in age, BMI, AMH levels or wish for children when compared to the women who completed the study follow-up, which lends support to the notion that the women who completed the study follow-up are representative of the entire study cohort.

CONCLUSIONS

Data reported herein indicate that the AMH decline that occurs after conservative ovarian cyst surgery is prolonged up to two years after surgery. However, our study does not support the notion that AMH should be routinely measured after conservative ovarian cyst surgery in women with normal to high AMH levels, as the data do not provide proof of a substantial effect on female fertility due to the decrease in AMH following ovarian cyst surgery.

Послеоперационное снижение АМГ не связано со снижением фертильности в течение двух лет после операции по поводу кист яичников
Tekla Lind, Claudia Lampic, Jan I. Olofsson, Kenny A. Rodriguez-Wallberg

В проспективном исследовании мы исследовали влияние изменений концентрации антимюллерова гормона (АМГ) после операций по поводу кист яичников на вероятность наступления беременности и рождения живого ребенка. Женщины репродуктивного возраста (N=60) были включены в исследование до оперативного лечения доброкачественных кист яичников. Концентрации АМГ в сыворотке крови определяли до и после операции через 6 и 24 мес. Информацию о желании и попытках забеременеть получали с помощью опросника. В момент включения 45/60 женщин сообщили о желании забеременеть. На шестом месяце наблюдения уровни АМГ значительно снизились во всей группе, дальнейшее снижение наблюдалось на протяжении двух лет (от 2,7 мг/л до 2,0 мг/л и до 1,1 мг/л соответственно; $p < 0,008$) при процентном уменьшении 42,9%. В период двухлетнего наблюдения 36 женщин сообщили, что пытались зачать, и 18 из них достигли беременности (50%) с рождением живого ребенка у 33%. Процентное изменение в концентрации АМГ за два года существенно не отличалось между женщинами, которые забеременели, и теми, кто не забеременел ($p = 0,117$). Данные, представленные в настоящем исследовании, показывают, что снижение уровня АМГ после операций по поводу кист яичников сохраняется через два года после операции; однако это послеоперационное снижение уровня АМГ не уменьшает шансов женщины забеременеть.

Ключевые слова: АМГ, цистэктомия, частота рождения живых детей, овариальный резерв, хирургическое лечение кист яичников, исход беременности.

Післяопераційне зниження АМГ не пов'язане зі зниженням фертильності протягом двох років після операції з приводу кист яєчників
Tekla Lind, Claudia Lampic, Jan I. Iofsson, Kenny A. Rodriguez-Wallberg

У проспективному дослідженні ми досліджували вплив змін концентрації антимюллерова гормону (АМГ) після операцій з приводу кист яєчників на ймовірність настання вагітності і народження живої дитини. Жінки репродуктивного віку (N=60) були включені в дослідження до оперативного лікування доброякісних кист яєчників. Концентрації АМГ у сироватці крові визначали до і після операції через 6 і 24 міс. Інформацію про бажання і спроби завагітніти отримували за допомогою опитувальника. У момент включення 45/60 жінок повідомили про бажання завагітніти. На шостому місяці спостереження рівні АМГ значно знизилися у всій групі, подальше зниження спостерігали протягом двох років (від 2,7 мг/л до 2,0 мг/л і до 1,1 мг/л відповідно; $p < 0,008$) за відсоткового зменшення 42,9%. У період дворічного спостереження 36 жінок повідомили, що намагалися завагітніти, і 18 з них завагітніли (50%) з народженням живої дитини у 33%. Відсоткова зміна у концентрації АМГ за два роки істотно не відрізнялася між жінками, які завагітніли, і тими, хто не завагітнів ($p = 0,117$). Дані, представлені у цьому дослідженні, свідчать, що зниження рівня АМГ після операцій з приводу кист яєчників зберігається через два роки після операції; однак це післяопераційне зниження рівня АМГ не зменшує шансів жінки завагітніти.

Ключові слова: АМГ, цистектомія, частота народження живих дітей, овариальний резерв, хірургічне лікування кист яєчників, результат вагітності.

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