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Original Article

The effects of vasopressin injection technique on ovarian reserve in laparoscopic cystectomy of bilateral ovarian endometrioma: a retrospective cohort study

Lingli Xin[1,2*], Mei Ye[2*], Lina Yang[3*], Ling Chen[2], Lirong Wang[2], Qingxiang Hou[2]

[1]Department of Graduate Administration, General Hospital of Chinese PLA, Beijing 100853, China; [2]Department of Obstetrics and Gynecology, PLA Rocket Force Characteristic Medical Center, Xinjiekou Outer Street 16#, Xicheng District, Beijing 100088, China; [3]Department of Personnel, PLA Rocket Force Characteristic Medical Center, Xinjiekou Outer Street 16#, Xicheng District, Beijing 100088, China. [*]Equal contributors.

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Abstract: Objective: To evaluate the effects of vasopressin injection technique (VIT) on ovarian reserve in laparoscopic cystectomy of bilateral ovarian endometrioma. Material and methods: A total of 122 patients with bilateral ovarian endometrioma undergoing laparoscopic cystectomy were assigned to control or the VIT group. Coagulation-event count and blood loss were recorded. Levels of serum anti-Müllerian hormone (AMH) were assessed at 1 day pre-operation, 1 month, 3 months, and 6 months post-operation. The follicles in the tissue sections were counted, and the maximum thickness of cyst wall was measured. Results: Coagulation-event count and blood loss in the VIT group were statistically less than those in control group (P<0.05). In both groups, AMH levels at pre-operation were the highest among different time-points (P<0.05); a remarkable decrease of AMH level was observed at 1 month post-operation, and the values were the lowest among different time-points (P<0.05). At 3 months post-operation, AMH levels gradually increased to approximately 60% of those at pre-operation (P<0.05); at 6 months post-operation, AMH levels were significantly higher than those at 3 months post-operation in VIT group (P<0.05). AMH levels in VIT group were significantly higher than those in control group at each time-point post-operation (P<0.05). The cyst walls in control group were statistically thicker than those in VIT group (P<0.05). Consistent with results of cyst wall thickness, numbers of total follicle loss, primordial and primary follicles in VIT group were less than those of control group (P<0.05). Conclusion: VIT effectively protected ovarian reserve in laparoscopic cystectomy of bilateral ovarian endometrioma.

Keywords: Laparoscope, cystectomy, endometriosis, ovarian reserve

Introduction

Ovarian endometrioma, accounting for 17-44% of the endometriosis, is a common gynecological disease, and leads to chronic pelvic pain and infertility in women of reproductive age [1, 2]. Currently in clinical practice, laparoscopic cystectomy, drainage and ablation are the main treatments for ovarian [3]. Previous study showed that the laparoscopic cystectomy could significantly lower the recurrence rates and symptoms in patients with ovarian endometrioma than drainage or ablation [4]. During laparoscopic cystectomy, stripping and coagulation are routinely used [4, 5]. Thus, ovarian reserve is inevitably damaged in the procedures due to loss of normal ovarian tissue and thermal injury to ovarian follicles by excessive coagulation, especially in patients with bilateral ovarian endometrioma [6-9].

Saeki Ai *et al.* reported a practical surgical procedure that helped to isolate the cyst wall from normal ovarian tissue by injecting diluted vasopressin into ovarian endometrioma excisions [10]. Besides, the procedure decreased leakage and bleeding during tissue separation, which minimized the use of coagulation. Thus, they suggested that this procedure could be used to protect ovarian reserve. Qiong-Zhen *et al.* showed that vasopressin injection technique (VIT) significantly reduced the number of coagulation events and loss of ovarian tissues [11]. Whereas, Ghafarnejad M. *et al.* found no

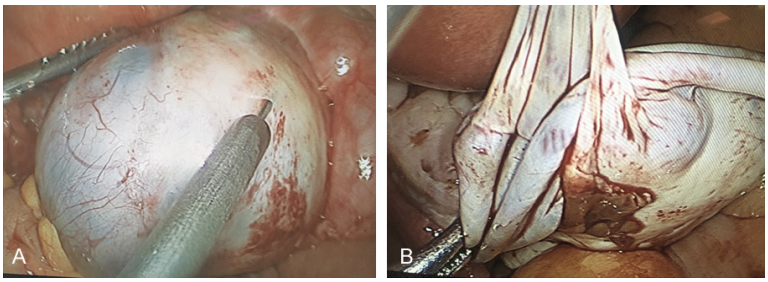

Figure 1. A: The injection of vasopressin in ovarian endometrioma cystectomy; B: The boundary between cyst wall and ovarian cortex is clear after VIT.

Table 1. Clinical parameters in patients of two groups

Variable	Control group (n=61)	VIT group (n=61)
Age (year)	30.3 (8.3)	33.1 (9.2)
BMI (Kg/m^2)	23.0 (3.6)	21.7 (4.2)
Mean diameter of endometrioma (mm)	54.2 (10.4)	56.1 (9.5)
r-ASRM staging		
III	22 (36.1%)	20 (32.8%)
IV	39 (63.9%)	41 (67.2%)

The data of r-ASRM staging was given as n (%), the other data was given as mean (SD). Student's t-test was used in comparison between two groups.

significant difference in the number of coagulation events and postoperative antral follicles between vasopressin injection group and control group [12]. Therefore, the effects of VIT on preserving ovarian reserve in laparoscopic cystectomy of ovarian endometrioma are still controversial.

Anti-Mullerian Hormone (AMH) is a member of the transforming growth factor-β family and secreted by primary, preantral, antral follicles [13]. AMH levels correlate with the number of growing follicles, and do not change significantly during menstrual cycle [14, 15]. Therefore, AMH has been used to predict the decline of ovarian function, and is the preferred biomarker of ovarian reserve [16, 17]. In this study, we analyzed the effects of VIT on preserving ovarian reserve in laparoscopic cystectomy of bilateral ovarian endometrioma. The AMH levels were used to assess the ovarian reserve.

Material and methods

The retrospective cohort of patients who experienced laparoscopic bilateral ovarian endometrioma cystectomy in the Department of Obstetrics and Gynecology in the PLA Rocket Force Characteristic Medical Center from April 2017 to May 2020 were analyzed. Eligible criteria were as follows: 1. patients diagnosed with bilateral ovarian endometrioma; 2. aged 20-45 years old; 3. planned to undergo laparoscopic cystectomy; 4. diameter of the bigger cyst was over 5 cm and was confirmed by transvaginal ultrasound; 5. menstruation was regular; 6. no history of surgery or drug treatment of endometriosis. 6-month of follow-up was performed in all patients. This study was approved by the Ethical Review Committee of The PLA Rocket Force Characteristic Medical Center (Beijing, China) (KY2021039). All patients have signed the informed consent before laparoscopic surgery.

Patients were divided into two groups, namely control group and VIT group. In the control group, no injection was conducted; in the VIT group, 10 mL diluted vasopressin (concentration: 0.06 iu/mL, 6 units diluted to 100 mL) was injected into the space between ovarian cortex and cyst capsule of each cyst.

All the surgeries were performed by the same surgeon under general anesthesia. The procedure was similar to the method reported by Qiong-Zhen *et al.* [11]. In the VIT group, diluted vasopressin (Harvest, Shanghai) was injected into the space between ovarian cortex and cyst capsule. Then, the ovarian cortex was cut off by scissors and the cyst capsule was exposed. In the control group, the cyst capsule was exposed directly using a scissors. The other procedures were the same for the both groups. After identification of the cleavage plane, two grasping forceps were used to isolate normal ovarian tissue and the cyst capsule in the opposite direction until completely removal of the cyst wall. Bipolar forceps were used to stop bleeding, and the number of coagulation events was counted. No suture was performed in all patients (**Figure 1**). The blood loss was estimated by combining the volume of blood collected

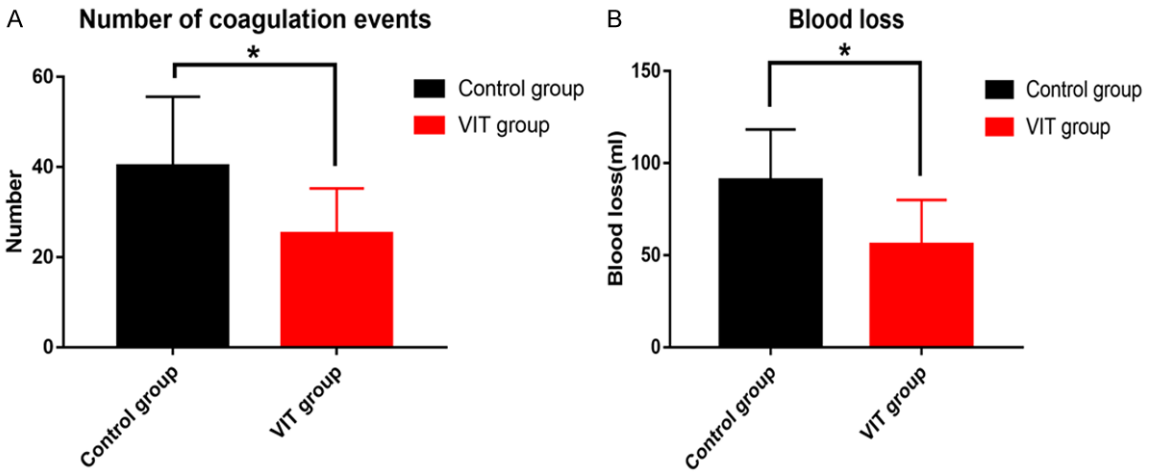

Figure 2. A: The number of coagulation-event in VIT group is significantly smaller than that in control group. B: The volume of blood loss in VIT group is remarkably less than that in control group. *: P<0.05, comparing with control group.

Table 2. The number of coagulation events and blood loss in two groups

	Control group	VIT group
number of coagulation events (n)	40.2 (15.4)	25.5 (10.1)*
Blood loss (mL)	90.7 (27.6)	55.6 (24.5)*

All data were given as mean (SD). Student's t-test was used in comparison between two groups. *: P<0.05, comparing with control group.

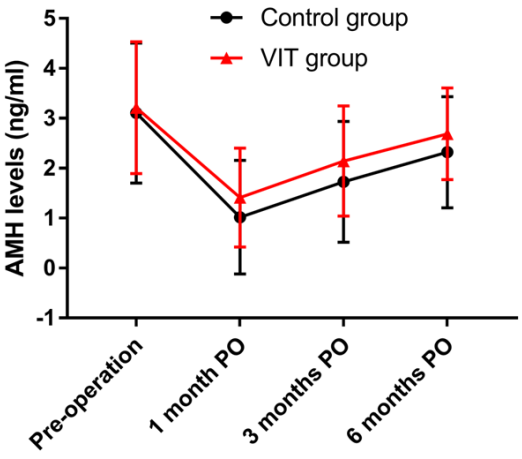

Figure 3. AMH levels at different time-points in two groups. PO: post-operation. In both groups, AMH levels at pre-operation were the highest and declined to the lowest value at 1 month PO; at 3 months PO, AMH levels increased to approximate 60% of those at pre-operation; at the 6 months PO, AMH levels were significantly higher than those at 3 months post-operation in VIT group. AMH levels were comparable between the two groups at pre-operation. AMH levels in VIT group were significantly higher than those in control group at each time-point PO.

within the suction canister and the weight of gauze used during surgery. The stage of the endometriosis was determined referring to the revised classification of the American Society of Reproductive Medicine (r-ASRM) [18].

After examining the entire cyst wall thoroughly, a 1×1 cm^2 specimen of cyst wall was selected and made into paraffin sections for histological evaluation. The maximum thickness of the cyst wall was measured and all stages of follicles in specimens were counted under microscope (BX-50, Olympus, Tokyo, Japan).

Serum samples were obtained at 1 day before operation, 1 month, 3 months, and 6 months after operation. All serum samples were stored at -80°C until tested. The serum AMH concentration was measured by enzyme-linked immunosorbent assay (ELISA) using an AMH ELISA kit (Catalog No. CEA228Hu, Cloud-Clone Corp. Wuhan, China). The inter-assay CV was less than 10% and the intra-assay CV was less than 12%. The detection limit is 185.2-15000 pg/ml.

The data of r-ASRM staging was expressed as n (%), the other data were expressed as mean (standard deviation). The analysis of variance (ANOVA) was used in intra-group comparison at different time-points. Student's t-test was used in comparison between the two groups. The

Table 3. Serum AMH levels (ng/mL) at pre-operation and post-operation in two groups

Group	n	Pre-operation	1 month PO	3 months PO	6 months PO
Control group	61	3.105 (1.401)	1.018 (1.137)#	1.723 (1.209)#,&	2.320 (1.246)#,&
VIT group	61	3.213 (1.321)	1.412 (0.998)*,#	2.143 (1.102)*,#,&	2.689 (0.918)*,#,&,$

All data were given as mean (SD). PO: post-operation. The ANOVA was used in intra-group comparison. *: P<0.05, comparing with control group. #: P<0.05, comparing with pre-operation. &: P<0.05, comparing with 1 month PO. $: P<0.05, comparing with 3 months PO.

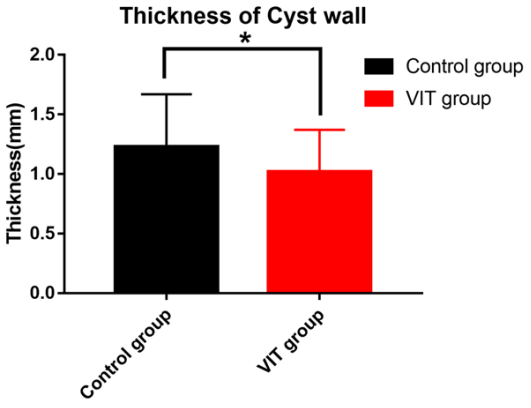

Figure 4. The cyst wall in control group was statistically thicker than that in VIT group. *: P<0.05, comparing with control group.

two-sided *P* value less than 0.05 was considered to be significant. All data were analyzed with the SPSS version 20 (IBM Corp., Armonk, NY, USA) and PRISM version 7.0 (GraphPad Software, La Jolla, CA, USA).

Results

A total of 125 patients were enrolled. Three patients were excluded for unilateral ovarian endometrioma confirmed by surgery and pathology. Of the 122 remaining patients, 61 were in the control group, and 61 in the VIT group. No intraoperative and postoperative complications were observed. No premature ovarian failure was reported in the 6 months follow-up in all patients. There was no significant difference in age, BMI, mean diameter of endometrioma and r-ASRM staging between the two groups (**Table 1**).

The number of coagulation events and the volume of blood loss in VIT group were significantly less than those in control group (P<0.05) (**Figure 2**; **Table 2**).

A similar trend of AMH changes was observed in both groups both pre-operation and post-operation. AMH levels at pre-operation were the highest among different time-points (P<0.05); a remarkable decrease of AMH level was found at 1 month post-operation, and the value was the minimum among the time-points (P<0.05); at 3 months post-operation, AMH levels gradually increased to approximate 60% of those at pre-operation (P<0.05); at the 6 months post-operation, AMH levels were significantly higher than those at 3 months post-operation in the VIT group (P<0.05). The AMH levels at every time-point were compared between VIT group and control group. AMH levels were comparable between the two groups at pre-operation (P=0.662). Notably, AMH levels in VIT group were significantly higher than those in control group at all the three time-points post-operation (P<0.05) (**Figure 3**; **Table 3**).

To evaluate the loss of ovarian tissue, the cyst wall thickness of specimens was measured and the number of follicles was counted. The cyst wall in control group was statistically thicker than that in VIT group (P<0.05). The total follicle number in the VIT group was significantly smaller than that in control group (P<0.05). The number of follicles at each developmental stage was further counted and compared, there were significant differences in the number of primordial and primary follicles (P<0.05), but no difference in the number of secondary follicles (P=0.622) between the VIT group and control group (**Figures 4**, **5**; **Table 4**).

Discussion

Studies have shown that loss of ovarian tissue, secondary inflammation, and vascular injury secondary to coagulation contributed to the decrease of ovarian reserve in patients who received laparoscopic bilateral ovarian endometrioma cystectomy [6, 19-24]. Since the ovarian reserve reflects the reproductive capacity, it is essential to preserve ovarian reserve during laparoscopic cystectomy especially in patients with bilateral ovarian endometrioma.

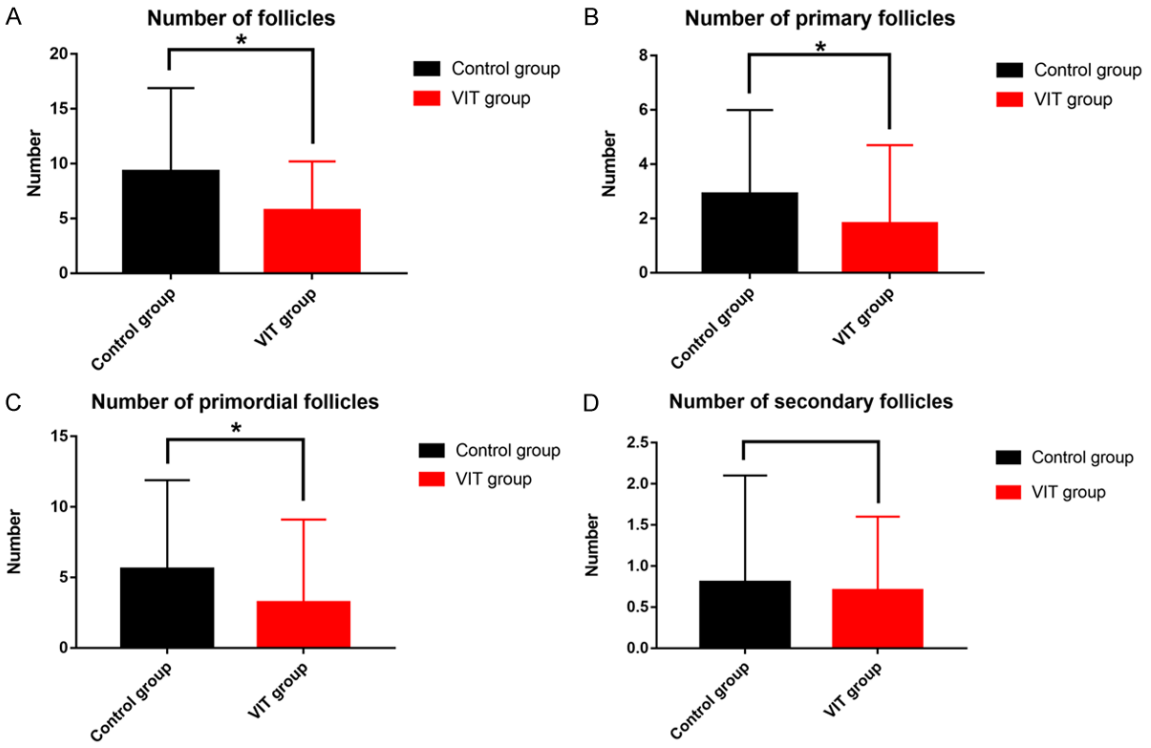

Figure 5. A: The number of follicles in VIT group was significantly less than that in control group (P=0.0019). B: The number of primary follicles in VIT group was significantly less than that in control group (P=0.0318). C: The number of primordial follicles in VIT group was significantly less than that in control group (P=0.0452). D: There was no significant difference in the number of secondary follicles between two groups. *: P<0.05, comparing with control group.

Table 4. Thickness of cyst wall and number of follicle loss in two groups

	Control group	VIT group
thickness of cyst wall (mm)	1.23 (0.44)	1.02 (0.14)*
number of follicles (n)	9.3 (7.6)	5.7 (4.5)*
Number of primordial follicles (n)	5.6 (6.3)	3.2 (5.9)*
Number of primary follicles (n)	2.9(3.1)	1.8 (2.9)*
Number of secondary follicles (n)	0.8 (1.3)	0.7 (0.9)

All data were given as mean (SD). Student's t-test was used in comparison between two groups. *: P<0.05, comparing with control group.

Vasopressin is effective and safe for controlling regional blood flow, and has been frequently used as hemostatic agents during myomectomy [25, 26]. In laparoscopic cystectomy of ovarian endometrioma, Qiong-Zhen *et al.* and Ghafarnejad M *et al.* reported no complications during and after surgery in patients receiving vasopressin injection [11, 12]. Consistent with previous studies, no intraoperative or postoperative complications was found in patients of the VIT group in our study. These results confirmed the safety of local vasopressin application in laparoscopic cystectomy.

Previous studies suggested that the protective effects of VIT on ovarian reserve in laparoscopic cystectomy are still controversial [10-12]. AMH levels have been used to assess functional ovarian reserve in autoimmune disease and post cancer treatment [15]. In present study, we used AMH levels to assess functional ovarian reserve in patients treated with VIT during laparoscopic cystectomy. As reported previously [27], we found that AMH levels declined remarkably at 1 month post-operation in both groups, suggesting that cystectomy inevitably damaged the functional ovarian reserve. Furthermore, our results showed that AMH levels at 1 month, 3 months, 6 months post-operation in the VIT group were statistically higher than those in control group. Meanwhile, we also found significantly less number of coagulation events and volume of blood loss in VIT group; however, we observed significantly thicker cyst wall and larger number of follicles in control group. These findings implied that VIT injection reduced the damage

to ovarian reserve in laparoscopic cystectomy of bilateral ovarian endometrioma. Since the loss of normal ovarian tissues and damage of ovarian tissues contribute to decrease in ovarian reserve [6], we speculated that VIT injection reduced the damage to functional ovarian reserve for two reasons: firstly, the "water bag" of vasopressin between ovarian cortex and cyst wall made the boundary easily to be separated, which reduced loss of normal ovarian tissues; secondly, local vasoconstriction by vasopressin led to less use of coagulation as reported previously [10, 11], which reduced the damage to ovrian tissue. Thus, the VIT physically reduced loss of normal ovarian tissue and preserved ovarian reserve.

In our study, AMH levels had a trend of increase after 1 month post-operation, and restored to approximate 60% of preoperative levels at 3 months post-operation. In line with us, Chang *et al.* reported that AMH levels recovered to 65% of preoperative levels at 3 months after surgery [28]. Moreover, Vignali *et al.* found that decline of AMH was temporary, and it could completely restore to the preoperative level at 12 months after surgery [29]. Longer follow-up is required to observe recovery of AMH in future study.

There are some limitations in this study. First, this study analyzed data of one single center, so selection bias of patients is inevitable. Second, longer follow-up is needed to determine the extent to restore AMH levels, cyst recurrence, and effects of vasopressin injection on fertility.

In conclusion, our study showed that VIT effectively and safely protected ovarian reserve through reducing ovarian tissues loss in laparoscopic cystectomy of bilateral ovarian endometrioma. Further more prospective studies with longer follow-up and data of multiple centers on clinical practice and underlying mechanisms are needed.

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Lingli Xin and Qingxiang Hou, Department of Obstetrics and Gynecology, PLA Rocket Force Characteristic Medical Center, Xinjiekou Outer Street 16#, Xicheng District, Beijing 100088, China. Tel: +86-10-66343383; Fax: +86-10-66343092; E-mail: cindy0920@163.com (LLX); houqx73@163.com (QXH)

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