Resumption of Ovarian Function After Ovarian Biopsy/Scratch in Patients With Premature Ovarian Insufficiency

Xiruo Zhang, MD, PhD[1,2], Ting Han, MD, PhD[1,2],
Lei Yan, MD, PhD[1,2], Xue Jiao, MD, PhD[1,2],
Yingying Qin, MD, PhD[1,2], and Zi-Jiang Chen, MD, PhD[1,2,3,4]

Abstract

To evaluate the effect of ovarian biopsy and scratch on the resumption of ovarian function in women with premature ovarian insufficiency (POI), the follicle development and pregnancy outcome were retrospectively analyzed in women with POI after ovarian biopsy/scratch. Eighty patients with POI with secondary amenorrhea were accepted the laparoscopic surgery, and then hormone replacement treatment for 6 months was applied and in vitro fertilization and embryo transfer was suggested. No significant difference in clinical characteristics was observed before and after ovarian biopsy/scratch ($P > .05$). After the ovarian surgery, 11 (13.75%) patients presented with ovarian function resumption spontaneously or with human menopausal gonadotropin stimulation. Three metaphases II oocytes were retrieved in 10 patients. Two embryos were formed and freshly transferred followed by a healthy singleton delivery in 1 (1.25%) patient. Patients with follicle development had higher baseline estradiol compared to those without (78.20 [18.53-161.08] pg/mL vs 15.70 [8.40-43.80] pg/mL, $P < .01$). The technique of ovarian biopsy/scratch could promote follicle development in vivo, suggesting it could bring promising benefits for some women with POI. However, future improvement in efficiency and practice criteria is warranted.

Keywords

primary ovarian insufficiency (POI), ovarian biopsy, ovarian function, follicle development

Introduction

Premature ovarian insufficiency (POI), previously termed as premature ovarian failure, is cessation of ovarian function characterized by hypergonadotropic amenorrhea and hypoestrogenic syndrome before 40 years of age.[1] About 1% of women younger than 40 years old and 0.1% before 30 are affected. Premature ovarian insufficiency imposes a great challenge on women's reproductive and long-term health, such as infertility, amenorrhea, osteoporosis, and cardiovascular disease. Primary amenorrhea occurs in 15% of patients with POI and secondary amenorrhea (SA) accounts for 85%.[2] Women with POI with SA have a significantly shortened reproductive span of less than 10 years on average. They normally develop amenorrhea within 2 years once menstrual irregularity occurred, which indicates an extremely rapid decline of ovarian reserve in these patients. Most patients already had impaired or complete loss of fecundity when diagnosed. Hence, the treatment of POI is particularly tough. Currently, no optimal regimen exists to ameliorate ovarian function. The options to conceive, especially for genetically related offspring, are limited.[3,4] Typically, they end up with egg donation or adoption as an alternative way.[5]

Resumption of ovarian activity in patients with POI is hardly predictable, although $\sim 24\%$ of patients were reported to experience ovulation spontaneously after POI diagnosis.[6,7]

[1] Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan, China
[2] National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Key Laboratory for Reproductive Endocrinology of the Ministry of Education, and Shandong Provincial Key Laboratory of Reproductive Medicine, Jinan, China
[3] Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[4] Shanghai Key Laboratory of Assisted Reproduction and Reproductive Genetics, Shanghai, China

Corresponding Author:
Yingying Qin, Shandong Provincial Hospital Affiliated to Shandong University, National Research Center for Reproductive Medicine, 157 Jingliu Weiwu Road, Jinan 250021, China.
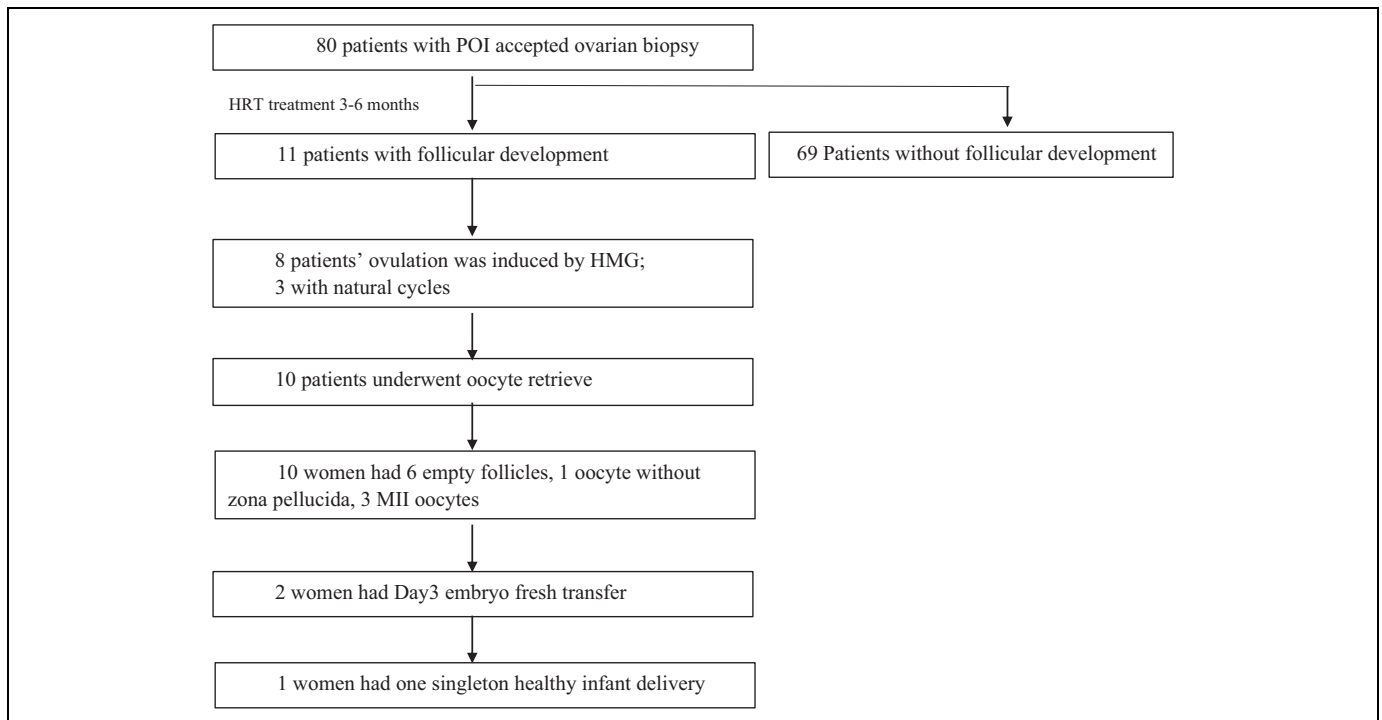Email: qinyingying1006@163.com

Figure 1. The flowchart for this study.

Currently, it depends on the evaluation of ovarian reserve, including endocrine hormone and ultrasound assessment. Antral follicle count (AFC) is of great importance to evaluate fecundity in patients with POI. However, the residual primordial follicle pool, follicles with diameter less than 2 mm, could not be detected by ultrasonography. Ovarian biopsy, as a diagnostically histologic method, can identify the residual follicles in ovaries. It could provide informative data about primordial follicles and help make decision of fertility treatment for patients with POI.[8,9] However, the application of ovarian biopsy is controversial.[10,11] Given its invasiveness, it is not routinely performed in clinic at present. Therefore, the priority of biopsy to identify the presence of ovarian follicles is still a matter of dispute.

Recently, a new promising approach—in vitro activation (IVA) has emerged for infertility treatment in patients with POI.[12-14] In vitro activation is based on an available primordial follicle pool. It activates dormant primordial follicles using a combination of mechanical signaling and biochemical factors. The mechanical disruption of ovarian cortex or ovarian fragmentation could disturb the Hippo signaling pathway and stimulate follicle growth. The hypothesis that local ovarian injury, such as ovarian biopsy or scratch, might exert a similar favorable effect in women with POI arouses our curiosity.

Previous studies on ovarian biopsy mainly focused on histologic diagnosis, and the reproductive outcome followed biopsy has not been reported. In the current study, we performed ovarian biopsy and scratch in 80 patients with POI with SA and followed up for 6 months to observe the ovarian activity and reproductive outcomes.

Materials and Methods

Study Participants

Eighty women with POI were recruited in the Center for Reproductive Medicine, Shandong University, Jinan, China, from January 2012 to October 2016. Informed consent was obtained from all participants. The study was approved by the Institutional Review Board of Reproductive Medicine, Shandong University. The recruitment criteria included age ≤38 years, SA >6 months, and basal follicle-stimulating hormone (FSH) >40 IU/L. Patients with chromosomal abnormality, endometriosis, thyroid gland dysfunction, previous ovarian surgeries, and pelvic radio/chemotherapy were excluded.

As shown in the flowchart (Figure 1), 80 patients with POI accepted ovarian biopsy and scratch by laparoscopy. The patients were prescribed hormone replacement treatment (HRT, Femoston, 2 mg; Abbott Biologicals B.V., Veerweg, the Netherlands) for 6 months after the laparoscopy. During HRT, when FSH <20 IU/L or visible follicles by ultrasound were detected, in vitro fertilization and embryo transfer (IVF-ET) was suggested.

Hormone Assessment and Pelvic Ultrasonography

Blood was sampled on day 2 to 4 of menstrual cycle or randomly (for women not menstruating frequently). The level of FSH, LH, estradiol, prolactin, and total testosterone was measured by chemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). The intra- and interassay coefficients of variation were <10%. Gynecological examination and transvaginal ultrasonography were routinely conducted. The ovarian texture and AFC were recorded. Antral

follicle count is defined as the number of follicles 2 to 10 mm at early follicular phase.

Ovarian Biopsy and Scratch

Ovarian biopsy/scratch was performed under intravenous general anesthesia through laparoscopy by the same experienced surgeon. The laparoscopic transverse incision was made through the umbilicus and 2 adjuvant 5-mm trocars were inserted under the monitor vision. A grasper and scissor were inserted respectively for immobilization and biopsy/scratch operation. Small pieces of ovarian cortex (approximately 5 mm × 5 mm) distant from the hilum were sampled from the left ovary. Suture was used for hemostasis. The cortical layer of the right ovary (about 2-4 mm thickness) was scratched for 3 superficial lesions separately. Hemostasis was not used in the right ovary. The biopsied tissues were fixed in 4% paraformaldehyde and embedded in paraffin. After being serially sectioned (5 mm), 3 to 4 (1 out of every 5) sections were stained with hematoxylin–eosin and examined.

In Vitro Fertilization and Embryo Transfer Procedures

Eleven patients received IVF-ET treatment, among which 72.73% (8/11) accepted ovarian stimulated with human menopausal gonadotropin (225-375 IU/d; Lizhu Ltd, Guangdong, China) from cycle day 3 until the day with dominant follicle exceeding 18 mm, and the other 3 patients (3/11, 27.27%) were suggested with natural protocol. Patients were monitored with hormonal level and ultrasound examination. When the dominant follicle(s) reached 18 mm in diameter, ovulation was triggered with urinary human chorionic gonadotropin injection (8000-10 000 IU; Lizhu Ltd, Guangdong, China) and oocytes were retrieved 36 hours later.

The oocytes were inseminated in vitro. Fresh embryos in good quality were transferred on day 3 and the remaining were cryopreserved on day 5. Oral progesterone (Duphaston, Abbott Biologicals B.V., Veerweg, the Netherlands) at a dose of 40 mg was administrated for luteal-phase support until 12 weeks after conception.

Outcome Evaluation

The primary outcome was the resumption of ovarian function characterized by decreased FSH, increased estradiol (E_2), and follicle development by ultrasound. The secondary outcomes are pregnancy outcomes of IVF-ET.

Statistical Analysis

Data were analyzed by Statistical Package for Social Sciences version 22 (SPSS Inc, Chicago, Illinois). Mean (standard deviation [SD]) was used for normally distributed data, otherwise median (ranges) was used. Comparisons were analyzed using independent samples *t* test or Kruskal-Wallis test for continuous data, χ^2 or Fisher tests for categorical data. Paired samples test was used between preoperative and postoperative

Table 1. The Clinical Characteristics of Patients With POI Before Ovarian Biopsy/scratch.

	POI Patients (N = 80)
Age (years)	29.36 (3.41)
BMI (kg/m^2)	22.80 (3.65)
Age at menarche (years)	14.73 (2.11)
Age at abnormal menses (years)	20.59 (4.99)
Age at amenorrhea (years)	22.71 (5.01)
Duration of amenorrhea (years)	6.97 (4.45)
FSH (IU/L)	52.02 (26.40)
LH (IU/L)	26.07 (12.60)
E_2 (pg/mL)	18.85 (9.72, 54.48)
Prevalence of pregnancy (% [n/N])	11.25 (9/80)
Frequency of AFC in ultrasound (% [n/N])	43.75 (35/80)

Abbreviations: AFC, antral follicle count; BMI, body mass index; E_2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; POI, premature ovarian insufficiency.

endocrine data. The *P* value < .05 was considered to be statistically significant.

Results

Baseline Characteristics

Among the 80 women with SA, the mean age at recruitment was 29.36 ± 3.41 years. Amenorrhea has been lasting 6.97 ± 4.45 years on average. Nine (11.25%, 9/80) women had a history of spontaneous pregnancy. The baseline characteristics are summarized in Table 1.

Ovarian Biopsy With Laparoscopy

All patients presented with small or steak ovaries with hard texture under laparoscope. Most ovaries showed atrophy or fibrosis, with connective tissues completely devoid of follicles or mixed with atretic follicles or corpora luteum rarely. The presence of follicles, either resting or growing, was identified in 12 (15.0%, 12/80) patients according to the histological assessment. The representative figure of the ovarian histology in women with POI is shown in Figure 2.

Resumption of Ovarian Function and Pregnancy Outcomes of IVF-ET

All patients were monitored 6 months by hormone measurement and ultrasound examination after ovarian biopsy/scratch. Eleven patients (11/80, 13.75%) manifested features of ovarian resumption, such as decreased FSH level below 20 IU/L or follicular development by ultrasound. No significant differences in clinical characteristics were observed before and after ovarian biopsy/scratch (Table 2). Patients with follicle development had higher baseline E_2 compared to those without (78.20 [18.53-161.08] pg/mL vs 15.70 [8.40-43.80] pg/mL, *P* < .01; Table 3). Age at menarche/irregularity/amenorrhea was comparable between patients with and without follicle
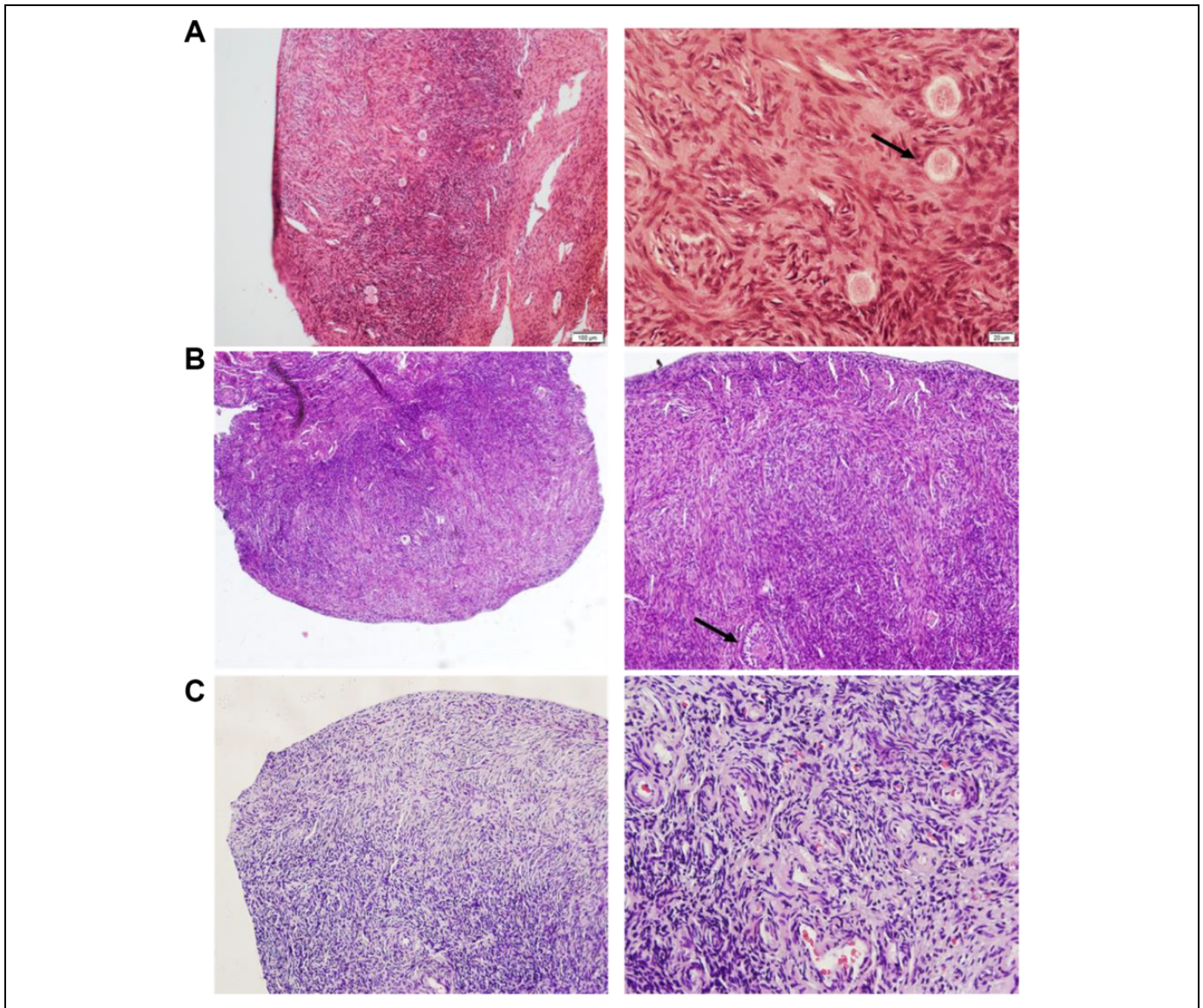
Figure 2. Representative figures of the ovarian histology in women with POI (left figure: ×40 original magnification, right figure: ×100 original magnification). A, Primary follicle. B, Secondary follicle. C, Fibrotic ovarian tissue deprived of follicles. POI indicates premature ovarian insufficiency.

Table 2. The Ovarian Reserve in Patients With POI Before and After Ovarian Biopsy.

Characteristics	Prebiopsy	Postbiopsy	P Value
FSH (IU/L)	51.21 (25.57)	47.52 (29.85)	.26
LH (IU/L)	25.73 (12.50)	27.35 (17.02)	.37
E_2 (pg/mL)	18.85 (9.72, 54.48)	24.80 (11.65, 65.02)	.81
Frequency of follicles in ultrasound, % (n/N)	43.75 (35/80)	38.75 (31/80)	.52

Abbreviations: E_2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; POI, premature ovarian insufficiency.

development. Fourteen dominant follicles were observed in 11 patients. Six patients had growing follicles in the left biopsied ovary (54.55%, 6/11), 4 in the right scratched ovary (36.36%, 4/11), and the 11th patient had bilateral follicle growth (9.09%, 1/11). Oocyte retrieval was canceled in 1 patient for sharp decline of E_2 level. Among the 10 patients receiving oocyte retrieval, 3 metaphases II oocytes were retrieved, 1 oocyte without zona pellucid, and 6 was empty oocyte. Among the 3 mature oocytes, 2 embryos were formed on day 3 and freshly transferred resulting in a singleton healthy infant delivery in 1 patient (1.25%, 1/80; Table 4). The other patient did not achieve biochemical pregnancy.

Discussion

Infertility is an important issue for women with POI since spontaneous pregnancy is a rare event. Oocyte donation or adoption to date remains the first-line fertility treatment. New approaches to ameliorate ovarian function and conceive their

Table 3. The Clinical Characteristics of Patients With and Without Follicles in Biopsy.

Characteristics	With Follicle Growth (N = 11)	Without Follicle Growth (N = 69)	P Value
Age (years)	28.91 (3.70)	29.43 (3.39)	.64
BMI (kg/m^2)	20.17 (2.45)	23.22 (3.65)	.009
Age at menarche (years)	14.63 (1.29)	14.74 (2.22)	.88
Age at abnormal menses (years)	20.45 (6.49)	20.61 (4.77)	.93
Age at amenorrhea (years)	22.55 (6.25)	22.73 (4.83)	.91
Duration of amenorrhea (years)	6.45 (6.23)	7.06 (4.15)	.68
FSH[a] (IU/L)	39.99 (29.55)	53.99 (25.55)	.1
LH[a] (IU/L)	20.99 (10.93)	26.90 (12.73)	.15
E$_2$[a] (pg/mL)	78.20 (18.53-161.08)	15.70 (8.40-43.80)	.005
Prevalence of pregnancy (% [n/N])	18.18 (2/11)	10.14 (7/69)	.603

Abbreviations: BMI, body mass index; E$_2$, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.
[a]FSH, LH, and E$_2$ were baseline values before ovarian biopsy/scratch.

own baby have always been challenging. Nonetheless, POI is not merely an early menopause, nor is it permanent. Previous studies found ∼24% of women with POI had resumption of ovarian function, ∼4% resulted in baby births.[15,16] These data indicated residual follicles available in atrophic ovaries with potential of development and even fertilization. Therefore, strategies to enable ovarian resumption predictable and follicle activation manipulable are promising for POI treatment.

Recently, IVA approach has been proposed and live births have been achieved in patients with POI. Phosphatase and tensin homolog (PTEN) enzyme inhibitors and phosphatidylinositol-3 kinase activators could activate AKT pathway and activate the dormant follicles. Ovarian fragmentation could lead to ovarian primary follicle growth by interfering with Hippo signaling pathway.[14] Residual follicles in patients with POI could be activated to develop for egg retrieval by combination of mechanical and chemical stimulation. Suzuki et al[17] reported that 9 patients (24%, 9/37) had follicle growth and 2 patients finally ended in 2 healthy baby deliveries (5%, 2/37) by IVF-ET. Subsequently, one healthy baby (7.14%, 1/14) was delivered in Chinese patients with POI.[18]

In the current study, 13.75% of patients presented with follicular development after biopsy/scratch, and a healthy baby was born by IVF-ET (1.25%). It suggested that only mechanical stimulation by biopsy/scratch could be a potentially effective method for follicle activation. In addition to the mechanical disruption by biopsy/scratch, aseptic inflammation induced by the surgery might also have a beneficial effect. It could stimulate production and secretion of different cytokines and growth factors, which might improve the immune microenvironment of the ovary and promote follicle growth.

Compared to the previous IVA results, lower efficacy of follicle activation and live birth rate was observed in the present study. The discrepancy could be explained by different methods used for activation. Scratch or only a small piece of ovarian cortex tissue was sliced in this study, whereas whole ovary was removed and cut into strips in previous reports. The mild injury instead of complete fragmentation might not disturb the Hippo signaling efficiently. Moreover, free of chemical agents—AKT stimulator, only mechanical damage was implemented through ovarian biopsy and scratch. Additionally, in IVA approach, after ovarian fragmentation, ovarian cortical strips with residual follicles in histology would undergo subsequent Akt stimulation and transplantation. The efficacy of local mechanical-induced activation approach was constrained because the stage and the number of follicles remaining in individual ovaries varied. For patients without residual follicles, it was impossible to respond to biopsy or scratch. Based on histological assessment, 85.0% (68/80) of patients in this study did not show available follicles, whereas 50.0% (7/14) and 45.9% (17/37) of patients had no residual follicles in the previous 2 IVA treatment,[12,17-18] respectively. A higher proportion of patients without remaining follicles in this study may account for the lower activation efficacy.

Of note, the advantages of our study are noteworthy. First of all, compared to 2 surgeries in previous IVA technique, only one laparoscopic surgery was utilized in this study. It minimized the surgical risks. Furthermore, less damage of ovary was induced by biopsy/scratch compared to bilateral ovariectomy. Last but not least, the chemical agents, PTEN inhibitor and Akt stimulator, are avoided. Their potential adverse effect on the long-term health of patients and their offspring should be taken into consideration. Longitudinal follow-up and risk evaluation are further warranted.

Interestingly, the patients presenting follicle development had higher levels of baseline E$_2$ before biopsy/scratch compared to women without follicle development ($P < .01$). It was consistent with previous report that higher E$_2$ level could predict ovarian resumption.[6,19] In addition, higher baseline LH level was found in women without follicle development compared to those with follicle activation, although without statistical significance. As is known, elevated LH associated with premature luteinization of follicles, which might elicit the follicle atresia. In this study, only 3 oocytes were retrieved successfully, although 11 follicles developed dominantly. Therefore, it may be helpful to downregulate LH level using gonadotropin-releasing hormone agonist to improve the maturation of oocytes.[20] The regimes for triggering follicles development into matured and preovulatory stage still need to be optimized.

Our preliminary data with biopsy/scratch procedure in patients with POI for follicle activation seem promising, although the efficiency needs to be improved. Premature ovarian insufficiency is highly heterozygous in etiology and phenotype. Undeniable, activation by biopsy/scratch is not applicable for all patients. Patients who had residual primary follicles might benefit more from this approach, whereas those with genetic defect, such as abnormal chromosome or

Table 4. Reproductive Outcome of Women With POI With Follicle Development.

Patient No.	Age	Follicle-TVB Prebiopsy	Follicle-TVB Postbiopsy	Histologic Follicle	COH Protocol	Follicle Development (No.)	E$_2$ Level	Follicular Retrieval No.	Embryo Transfer No.	Reproductive Outcome
1	33	ROV	LOV	Y	HMG	LOV(1)	132.4	1	1	Single live birth
2	37	Bilateral	Bilateral	N	Natural	LOV(1)	220	0		
3	32	NO	Bilateral	Y	HMG	ROV(1)	206.8	0		
4	29	Bilateral	Bilateral	Y	HMG	ROV(2), LOV(1)	64.5	1	0	
5	29	ROV	ROV	N	HMG	ROV(2)	317.3	0 (premature ovulation)		
6	27	NO	LOV	N	HMG	LOV(1)	445.7	1	1	No pregnancy
7	26	NO	ROV	N	HMG	LOV(1)	238.6	0		
8	26	ROV	ROV	N	HMG	ROV(1)	34.7	NO (cancel)		
9	25	NO	NO	Y	HMG	LOV(1)	173.5	0		
10	26	Bilateral	Bilateral	Y	Natural	LOV(1)	123.8	0		
11	27	Bilateral	ROV	N	Natural	ROV(1)	309.9	0		

Abbreviations: E$_2$, estradiol; COH, controlled ovarian hyperstimulation; HMG, human menopausal gonadotropin; LOV, left ovary; POI, premature ovarian insufficiency; ROV, right ovary; TVB, transvaginal ultrasonography.

causative gene mutations, might be ineffective. Future exploration should optimize this approach within more suitable patients, such as a shorter amenorrhea duration (eg, >2 years), higher baseline E$_2$ level, or visible follicles by ultrasound. As for the ovarian biopsy, how to and how much tissues biopsied are still need to discuss for the practice criteria.

In conclusion, the traditional technique—ovarian biopsy/scratch with a new effect on follicle activation in vivo was proposed in women with POI. This approach seemed much simpler, safer, and could induce less damage. Future improvement is warranted to improve the activation efficiency and practice criteria.

Authors' Note

Xiruo Zhang and Ting Han are considered similar in author order.

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Declaration of Conflicting Interests

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