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Retrospective analysis of reproductive outcomes in women with primary ovarian insufficiency showing intermittent follicular development

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Abstract The aim of this retrospective study was to explore the reproductive outcomes of IVF treatment in women with primary ovarian insufficiency (POI) showing intermittent follicular development. A total of 44 POI women with normal karyotype and absent autoimmunity, attending the centre for fertility treatment at Nanfang Hospital, Guangzhou from March 2009 to March 2011, were identified as suitable for inclusion in this study. Out of 44 women, 20 (20/44; 45.5%) had growing follicles and 13 underwent 27 oocyte retrievals. The empty follicle rate per oocyte retrieval was 70.4% (19/27); eight oocytes were recovered: one (12.5%) germinal vesicle (GV), two (25.0%) metaphase I (MI), one (12.5%) metaphase II (MII), and four (50.0%) atretic. One MI oocyte matured *in vitro* and two women had embryo transfer. Only the woman with the MI oocyte matured *in vitro* conceived, giving birth to a healthy baby at term. These results suggest that intermittent follicular development is common in women with POI but most of the developed follicles are empty or contain atretic oocytes. The pregnancy rate remains very low for IVF treatment.

KEYWORDS: Empty follicle, In vitro fertilization, Primary ovarian insufficiency

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Introduction

Primary ovarian insufficiency (POI) is diagnosed when women, before the age 40 years, have amenorrhoea for 4 months or more and two elevated serum FSH concentrations in the menopausal range at intervals of at least 1 month apart (Nelson, 2009). It affects about 1% of women under 40 years old and 0.1% of women under 30 years old (Laml et al., 1999). The causes include 45X, autoimmunity, chemotherapy and ovarian surgery but remain unknown in many patients.

POI is usually associated with infertility, but the phenomenon of intermittent follicular development is commonly seen in these women. Bidet et al. (2011) reported that the cumulative incidence of resumption of follicular growth was 22.5% in 12 months and 25% in 48 months in a cohort of 358 women with POI. About 3–10% of these women may conceive spontaneously after the diagnosis (Bidet et al., 2011). Oocyte donation is usually advised (Shah and Nagarajan, 2014) but is strictly regulated in China, leading to very limited availability. Moreover, the great majority of Chinese women want to have their own genetic children because a couple without their own child may end up in divorce. Therefore, many women with POI strongly request IVF treatment in order to get pregnant as soon as possible, before the depletion of the few follicles remaining in the ovaries.

In literature, only a few reports described IVF treatment in patients undergoing autotransplantation of frozen-thawed ovarian tissue to orthotopic or heterotopic sites for fertility preservation (Biasin et al., 2015; Burmeister et al., 2013; Dolmans et al., 2009; Donnez et al., 2011; Kim, 2012; Revel et al., 2011; Revelli et al., 2013; Schmidt et al., 2011). To date, there is no information regarding reproductive outcomes of IVF treatment in POI women with intermittent follicular development. The aim of this retrospective study was to report the reproductive outcomes of these women.

Materials and methods

This is a retrospective analysis of all POI women with normal karyotype and absent autoimmunity attending the Centre for Reproductive Medicine, Department of Gynecology and Obstetrics, Nanfang Hospital from March 2009 to March 2011. They were followed up until pregnancy and delivery or for 48 months if they did not conceive. Patients gave their informed consent for the treatment given. The Medical Ethics Committee of Nafang Hospital, Southern Medical University approved this study in December 2015, indicating that subject-informed consent was not required for this retrospective analysis of anonymized patient data.

Briefly, clinical evaluations included age at POI diagnosis, clinical presentation, family history, history of autoimmunity, ovarian surgery, chemotherapy or pelvic radiotherapy. They were screened for thyroid antibody, antinuclear antibody, antiendometrium antibody, and antiovarian antibody. Laboratories in Nanfang hospital use chemiluminescence to measure the thyroid antibody (Roche Diagnostics [Shanghai] Ltd., China), immunofluorescence to measure the antinuclear antibody (Roche Diagnostics [Shanghai] Ltd.) and enzyme-linked immunosorbent assay (ELISA) to measure the antiendometrium antibody (Dade Behring Inc., USA) and the antiovarian antibody (Dade Behring Inc.). Serum FSH, LH, oestradiol and progesterone concentrations were checked using electrochemiluminescence immunoassay (Roche Diagnostics (Shanghai) Ltd.). The inter-assay coefficients of variation (CV) for FSH, LH, oestradiol and progesterone were 4.5, 2.2, 4.9 and 4.8%, respectively. The intra-assay CV for FSH, LH, oestradiol and progesterone were 2.8, 1.2, 3.3 and 2.9%, respectively. Anti-Müllerian hormone (AMH) concentrations were measured using ELISA with the Beckman Coulter assay. The inter-assay CV for AMH was less than 14.2% and the intra-assay CV for AMH was less than 12.3%. The sensitivity was 0.14 ng/ml.

POI was diagnosed when a woman who was less than 40 years old had amenorrhoea for 4 months or more, with two serum FSH concentrations (obtained at least 1 month apart) above 40 IU/l, a normal karyotype and absence of thyroid antibody, antinuclear antibody, antiendometrium antibody and antiovarian antibody. Women who had a history of autoimmunity or of chemotherapy or pelvic radiotherapy were excluded from the study group.

All women received 4 mg/day of oestradiol valerate (Tablet Progynova, Bayer Healthcare Co. Ltd., China) for 4 weeks. Transvaginal ultrasounds were performed once a week in order to check the presence of growing follicles. If no growing follicle was detected for 3 weeks, in the last week they were supplemented with oral dydrogesterone (Tablet Duphaston, Solvay Pharma China Ltd.) 10 mg daily for withdrawal bleeding, followed by another cycle of hormone replacement and ultrasound monitoring.

When the diameter of a follicle exceeded 10 mm, the women were scanned daily with the transvaginal ultrasound in the morning. Once the follicle exceeded 14 mm in diameter, serum FSH, LH, oestradiol and progesterone concentrations were measured. The scheduling strategy is summarized in **Table 1**. When serum hormone concentrations met one of the following three conditions: (i) progesterone > 1.0 ng/ml; (ii) progesterone < 1.0 ng/ml and LH > 30 IU/l; or (iii) progesterone < 1.0 ng/ml, LH was 25–30 IU/l and oestradiol > 250 pg/ml, 10,000 IU human chorionic gonadotrophin (HCG) (Livzon pharmaceutical group inc., Zhuhai, China) were given instantly and oocyte retrieval was scheduled the next morning between 15 and 24 h after the HCG injection. Others were given 10,000 IU HCG at night and oocyte retrieval was scheduled 36 h later. A 16-gauge double-lumen–needle (Cook Ireland Ltd., Limerick, Ireland) was used and 20 ml flush medium was re-injected for more than five times and re-aspirated for each punctured follicle. When the aspirated oocyte was mature, intracytoplasmic sperm injection (ICSI) was performed. Three days after ICSI, an early cleavage embryo was transferred into the uterine cavity. Luteal phase was supported by 6 mg of oestradiol valerate (Bayer Healthcare Co. Ltd., China) daily, 40 mg daily of intramuscular progesterone (Shanghai General Pharmaceutical Co. Ltd., Shanghai, China) and 2000 IU HCG (Livzon pharmaceutical group inc., Zhuhai, China) every 2 days from the day of oocyte retrieval to 12 days after embryo transfer, and continued in case of pregnancy diagnosed by serum β-HCG concentration >50 IU/l 12 days after embryo transfer.

Table 1 Oocyte retrieval scheduling according to serum hormone concentrations.

Hormone concentrations on HCG day	*HCG triggering*	*Time of oocyte retrieval (h after HCG)*	*Number of cycles*	*Oocyte retrieval rates*
Progesterone > 1.0 ng/ml	Between 10:00 AM and 5:00 PM	15–24	13	38.46% (5/13)
Progesterone < 1.0 ng/ml and LH > 30 IU/l	Between 10:00 AM and 5:00 PM	15–24	4	25% (1/4)
Progesterone < 1.0 ng/ml, LH 25–30 IU/l and oestradiol > 250 pg/ml	Between 10:00 AM and 5:00 PM	15–24	2	0 (0/2)
Others	At night	36	8	25% (2/8)

HCG = human chorionic gonadotrophin.

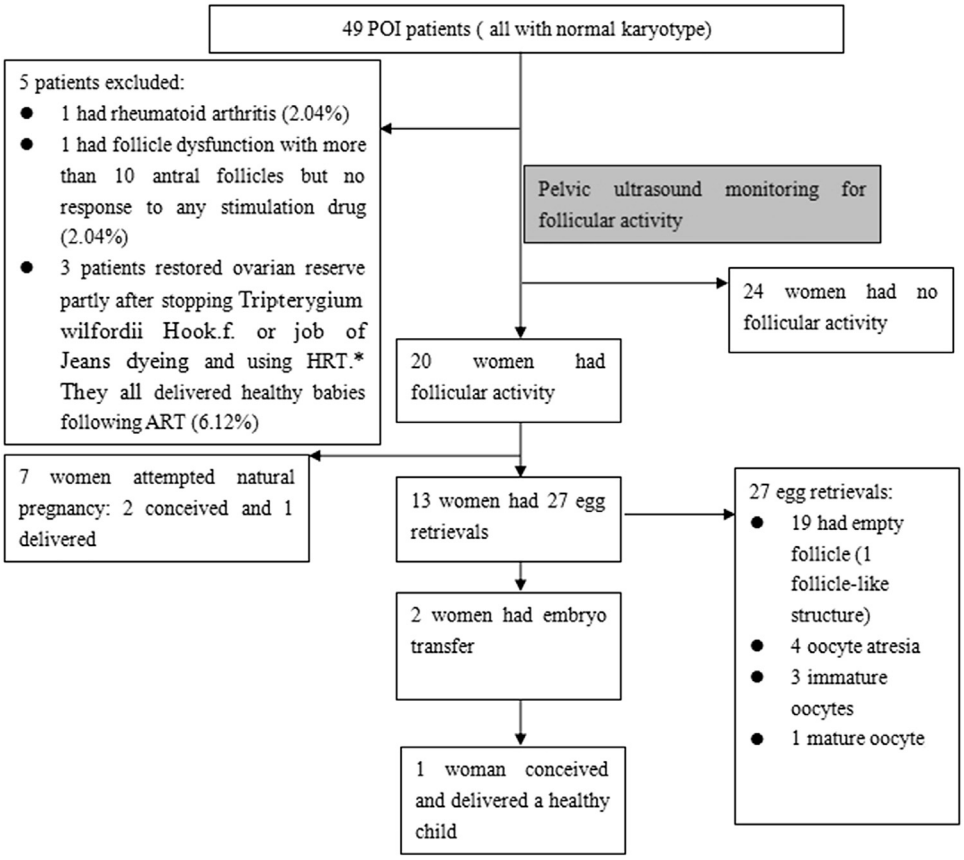

Figure 1 Flow chart for the study. * One patient took Tripterygium wilfordii Hook F for 3 months because of urticaria, which appeared suddenly after having seafood, whealing from head to foot, flaky skin, and ridges or bumps on the skin with local acute itchiness. The patient showed hypomenorrhoea, which escalated into amenorrhoea. She stopped medication, but menstruation was still not restored after about 3 months. The other two patients had a job dyeing jeans for more than one year, then showed amenorrhoea. They all restored ovarian reserve partly after stopping Tripterygium wilfordii Hook F or their job (dyeing jeans) and using hormonal replacement treatment. They delivered healthy babies following assisted reproduction treatment.

Results

During the study period, 49 women with POI were identified and five were excluded due to rheumatoid arthritis (n = 1, 2.04%), follicle dysfunction (n = 1, 2.04%), and chemical factors (n = 3, 6.12%) (Figure 1). All of them had no POI family history. There was no patient included in this study experiencing spontaneous pregnancies before the diagnosis of POI. In addition, there was no patient included in this study experiencing normalization of their FSH concentrations and/or resumption of their spontaneous menstrual cycles after the diagnosis of POI, except three patients excluded due to chemical factors (Figure 1).

Twenty (45.5%, 20/44) women presented intermittent follicular development. Seven women attempted natural pregnancy and two patients conceived. One delivered a healthy child and another had a missed miscarriage diagnosed at 8 weeks of gestation.

Table 2 Characteristics of women with follicular activity at the first presentation.

Characteristics	*Study group (n = 20)*
Age at first presentation (years)	31.30 (27–38)
BMI (kg/m^2)	20.4 ± 1.4
Secondary amenorrhea	20
FSH (IU/l)	93.69 (51.87–205)
LH (IU/l)	52.86 (21.53–103.55)
Oestradiol (pg/ml)	36.78 (5.00–45.58)
AMH (ng/ml) ($n = 5$)[a]	0.48 (0.14–0.88)
Interval between two consecutive follicular activities (months) ($n = 12$)	3.5 (2–8)

AMH = anti-Müllerian hormone; BMI = body mass index.
[a]Data for five women with detectable AMH. Data given as median (range) or mean ± standard deviation.

At the first presentation, the mean age of 20 women with intermittent follicular development was 31.3 years (**Table 2**) and their mean body mass index was 20.4 kg/m^2. All of them presented with secondary amenorrhoea. As anticipated, mean FSH and LH concentrations were high (93.69 and 52.86 IU/l). Serum AMH was available for all patients, and five women had detectable concentrations. The presence of follicles with a diameter of at least 2 mm was observed in 50.0% (10/20) of women at the first presentation. The median interval between two consecutive follicular developments was 3.5 months (range: 2–8 months).

In total, 57 cycles of follicular development were found: follicles < 12 mm stopped growing in 21 cycles (36.8%), natural pregnancy attempted in nine cycles (15.8%) in seven women according to their requests and oocyte retrieval in 27 cycles (47.4%) of 13 women.

The reproductive outcomes in women who underwent oocyte retrieval are summarized in **Table 3**. The hormone concentrations on the day of HCG injection in these patients are shown in supplementary **Tables 1–3** according to whether they underwent one, two or more than two oocyte retrievals. The results of follicular puncture were 20 empty follicles, four atretic oocytes, three immature oocytes (one GV and two MI) and one mature oocyte. Two women (number 1 and number 11) had one 8-cell embryo transferred and one woman conceived.

Woman number 1 was 27 years old. She had amenorrhoea for 5 years and primary infertility for 4 years. Her serum FSH concentration ranged from 40 to 80 IU/l in the last 3 years. She had received gonadotrophin stimulation previously but no follicle was growing. Her left ovary was 20 × 9 mm and the right ovary was 16 × 16 mm. There were two follicles in both ovaries. She was given oestrogen replacement therapy as stated earlier. In the conception cycle, her day 2 serum FSH concentration was 79.89 IU/l, LH concentration was 22.74 IU/l and the serum oestradiol concentration was 42.94 pg/ml. Seven days later (day 9) she had a follicle of 10 mm in diameter. Two days later (day 11) the follicle grew to 14.5 mm in diameter and serum oestradiol, LH and progesterone concentrations on that day were 575.1 pg/ml, 33.66 IU/l and 0.423 ng/ml, respectively. 10,000 IU of HCG were given instantly and oocyte retrieval was performed the next morning. One MI oocyte was collected and matured *in vitro* to a MII oocyte. One 8-cell embryo was transferred 3 days after ICSI. The luteal phase support was continued up to 12 weeks of gestation. A healthy boy with birth weight of 3150 gm was delivered by Caesarean section at term.

Discussion

This retrospective analysis showed that nearly half of women with POI showed intermittent follicular development but about one-third of follicles stopped growing beyond 12 mm. In those proceeding to retrieval, no oocytes were obtained in 70% of cycles and a mature oocyte was found in one out of 27 retrievals. There seemed no difference in the success between natural pregnancy and IVF.

The inability to conceive remains a major concern for women after diagnosis of POI. Many of them are strongly requesting IVF treatment, thinking that it will improve their chance of success. This explains why 13 women with intermittent follicular development opted for IVF treatment. However, there is a lack of relevant information in this aspect and accurate information is important for appropriate counselling.

This study revealed that intermittent follicular development in POI women was fairly common (45.5%). The cumulative incidence of POI women with resumption of ovarian function in the study is also higher than that of general POI women, which is 25% (Bidet et al., 2011). A higher rate of follicular development in this study may be related to a longer duration of observation (48 months in this study compared with 1–2 years of observation in the study of Bidet et al., 2011), continuous oestrogen-replacement treatment (Tartagni et al., 2007) and undergoing weekly transvaginal scanning during the whole study period.

The interval between two consecutive follicular developments ranged from 2 to 8 months in this study. The time is not predictable. AMH may have an impact on ovarian activity as the three patients who recovered ovarian function had AMH concentrations of more than 0.45 ng/ml. Although AMH may have an implication on the follicular pool, Bidet et al. (2011) found AMH concentrations were not predictive of the resumption of ovarian activity in their study. Spontaneous ongoing pregnancies have been reported in women with undetectable serum AMH concentrations (Fraisse et al., 2008).

In the present study, eight oocytes were recovered; one (12.5%) GV, two (25.0%) MI, one (12.5%) MII, and four (50.0%) atretic. The incidence of empty follicle syndrome in the current study (70%) is much higher than the range of 0.6% to 7% reported in the general IVF population (Kummer et al., 2013; Stevenson and Lashen, 2008). In the current study the percentage of GV and degenerated oocytes was 19%, which is slightly higher than the 11–17% reported for the general IVF population (Papaleo et al., 2009).

Dolmans et al. (2009) reported the largest series investigating the quality of oocytes and embryos of IVF treatment after transplantation of frozen-thawed ovarian tissue in patients who requested fertility preservation. They

Table 3 Reproductive outcomes in women with egg retrievals.

Patient no.	*Age (years)*	*Follicular puncture cycles*	*The outcome of follicular puncture (the number of oocytes, the type of cells)*	*Embryo transferred and embryo quality*	*Pregnancy*
1	27	1	1, MI and GC	Yes, an 8-cell embryo with 5% fragmentation	Yes
2	30	3	0, GC three times	No	N/A
3	35	1	0, GC	No	N/A
4	28	2	0, 2 follicle-like structures	No	N/A
			0, GC	No	N/A
5	38	1	1, GV and GC	No	N/A
6	31	2	1, atretic oocyte and GC	No	N/A
			1, atretic oocyte and GC	No	N/A
7	30	2	0, GC twice	No	N/A
8	29	5	0, GC four times	No	N/A
			1, atretic oocyte and GC	No	N/A
9	27	2	0, GC twice	No	N/A
10	33	2	0, GC twice	No	N/A
11	27	1	1, MII and GC	Yes, an 8-cell embryo with 5% fragmentation	No
12	33	1	0, GC	No	N/A
13	31	4	0, GC twice	No	N/A
			1, MI and GC	No	N/A
			1, atretic oocyte and GC	No	N/A

GC = granulosa cell; GV = germinal vesicle; MI = metaphase I; MII = metaphase II.

performed 21 oocyte retrievals in 15 cases and found an empty follicle rate per retrieval of 29% (6/21). Sixteen oocytes were recovered, of which 10 (63%) were in MII and six (38%) were abnormal or immature. Three MII oocytes failed to fertilize, two showed abnormal fertilization and five normal MII oocytes successfully fertilized with subsequent normal embryo development, resulting in an embryo transfer rate of 24% per retrieval. No pregnancy occurred. Results from this study of empty follicle syndrome and abnormal oocytes are in line with those reported in patients undergoing IVF treatment after transplantation of frozen-thawed ovarian tissue.

A possible cause of empty follicles in these patients may be dysfunctional folliculogenesis (Beck-Fruchter et al., 2012), which is due to increased apoptosis and follicular atresia, defective granulose cell function, faulty oocyte development and maturation. Therefore, the oocyte may be present originally and later experience atresia and apoptosis. Patient number 4 from this study was a good example of this condition. Two "follicle-like" structures were assessed immediately upon recovery for the presence of an oocyte (shown in **Figure 2**). However, zona pellucida was clearly visualized and no oocytes could be identified within these structures. There was also no evidence of a degenerated oocyte. Eberspaecher et al. (2001) concluded that the zona proteins are expressed and assembled exclusively by the oocyte and not by the granulosa cells in humans. They also suggested that the involvement of granulosa cells in zona pellucida formation observed in the previous studies may be due to the disruption of zona pellucida resulting from improper fixation of tissues. If this is true, the presence of the thin zona in the retrieved follicle-like structures in patient number 4 may be a proof of dysfunctional folliculogenesis, in the sense that the oocyte was originally present and then became atretic and apoptotic after the formation of a zona pellucida.

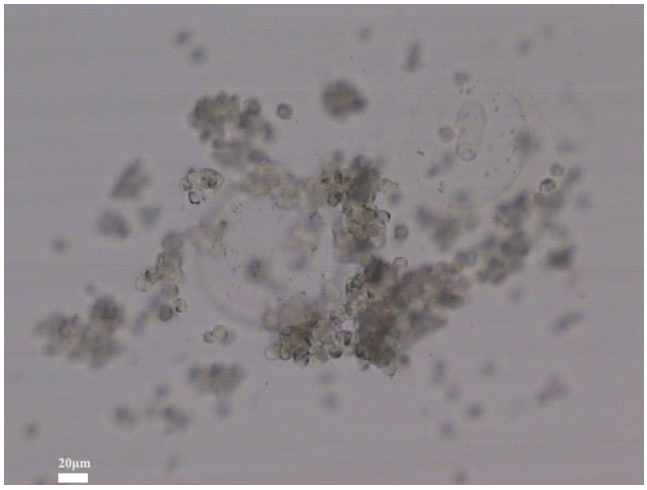

Figure 2 Photomicrographs of follicle-like structures observed at the time of oocyte retrieval of Patient number 4. Structures were devoid of oocytes but thin zona pellucida were clearly visable.

The rationale for the ovarian preparation strategy using Progynova (oestradiol valerate) ± Duphaston (dydrogesterone) in this study is based on the point that chronically high gonadotrophin concentrations in patients with POI down-regulate FSH receptors of granulosa cells, so that remnant

follicles become refractory to exogenous stimulation (Tartagni et al., 2007). The administration of exogenous oestradiol suppresses serum FSH concentrations and allows the restoration of FSH receptors in the remaining follicles. This strategy has been used previously by the present authors to good effect (Chen and Chen, 2011). Progynova, 17β-estradiol, a human sex hormone and steroid, is used because this oestradiol is safe for women wanting to be pregnant and has been widely used in the field of reproductive medicine. In addition, Progynova ± Duphaston is also a protocol for endometrial preparation in frozen embryo transfer (FET) cycles. Moreover, oestradiol is essential for the development and maintenance of female reproductive tissues, but it also has important effects in many other tissues including bone, especially for this unique subset of infertile patients, POI patients.

Ben-Nagi and Panay (2014) concluded that there was no conclusive evidence for which treatment is optimal for women with POI who wish to conceive using their own gametes. Spontaneous pregnancies have been reported to be 5–10% in POI women. This study reports the largest series investigating the quality of oocytes and embryos of IVF treatment in women with POI. Although many POI women in our practice strongly request IVF treatment, only one live birth has been achieved out of 27 retrievals. Women with POI should be discouraged from IVF treatment because of the low success rate, which may be similar to spontaneous pregnancy.

In conclusion, although intermittent follicular development is common in women with POI, most of the developed follicles are empty or found with atretic oocytes, which may be due to dysfunctional folliculogenesis or the altered follicular cell metabolism/environment. The pregnancy rate remains very low in these women following IVF treatment and may not be different from that of spontaneous pregnancies. Natural conception is as effective as IVF as long as there are no other mechanical or male factor problems. These findings have significant implications in patient counselling and management.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rbmo.2015.12.011.

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