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ASSISTED REPRODUCTION TECHNOLOGIES

Do young women with unexplained infertility show manifestations of decreased ovarian reserve?

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Abstract

Purpose To investigate whether unexplained infertility at a young age demonstrates manifestations of decreased ovarian reserve.

Methods A total of 100 women were divided into two equally sized groups. The study group comprised women aged ≤ 37 years diagnosed with unexplained infertility, and the control group included age-matched women with either mechanical factor or severe male factor infertility.

Results Both groups were comparable in their basic characteristics. Overall, women with unexplained infertility presented with inferior ovarian reserve results set against women of the control group. The number of ≥ 14-mm follicles on the day of hCG administration was significantly lower in the study compared with the control group (7.0 ± 4.5 vs. 10.4 ± 4.1 follicles, respectively, $P < 0.001$). Likewise, basal serum FSH was higher in the study compared with the control group (8.4 ± 5.5 vs. 6.4 ± 1.7 IU/L, respectively, $P = 0.015$), while antral follicle count was lower (10.9 ± 6.6 vs. 16.2 ± 6.6 follicles, respectively, $P < 0.001$). Furthermore, women with unexplained infertility required a higher total dose of FSH for ovarian stimulation (2,923 ± 1,701 vs. 2,196 ± 941 IU/L, respectively, $P = 0.010$), but exhibited a lower number of retrieved oocytes (9.3 ± 6.3 vs. 15.6 ± 7.9 oocytes, respectively, $P < 0.001$), alongside a lower number of achieved embryos (5.3 ± 4.0 vs. 8.0 ± 4.7 embryos, respectively, $P = 0.002$). Interestingly, the cumulative clinical pregnancy rate was not significantly different between the two groups (44% vs. 58%, respectively, $P = 0.163$).

Conclusions Young women ≤ 37 years of age with unexplained infertility have clear manifestations of sub-optimal ovarian reserve set against controls. Our findings suggest that unexplained infertility at a young age may be a risk factor for developing poor ovarian response, specifically as a quantitative, rather than a qualitative, risk factor.

Keywords Unexplained infertility · Ovarian reserve · (Basal) Ovarian reserve tests · Ovarian hyperstimulation · Bologna criteria

Introduction

Up to 30% of couples who are unable to conceive are determined to have unexplained infertility [1]. It is generally established as a diagnosis of exclusion in women who have tried to conceive for 1 year without success, despite evidence of ovulation, tubal patency, and normal semen parameters [2]. Without treatment, couples with unexplained infertility experience both diminished and delayed fecundity with an average live birth of 1.8–3.8% per cycle [3]. Moreover, pregnancy rates in these couples are lower with increasing age of the female partner and duration of infertility [4].

Several steps of the fertility process are not thoroughly explored or cannot be accurately investigated when reaching the diagnosis of unexplained infertility. Consequently, this diagnosis may encompass a heterogeneous group of conditions [5]. These may include a potential malfunction of the cervical [6], endometrial [7], tubal [8], peritoneal [9], or male [10] concealed factors that could be involved, albeit normal basic evaluation.

Except for age, risk factors for POR in women undergoing controlled ovarian stimulation (COS) for assisted reproduction are still poorly defined [11]. The publication of the

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Bologna criteria in 2011 provided the minimal criteria required to define POR. At least two of the three following criteria must exist to establish POR: (1) Advanced maternal age (>40 years) or any other risk factor for POR. (2) A previous POR (≤3 oocytes with a conventional stimulation protocol). (3) An abnormal ovarian reserve test [i.e. antral follicle count (AFC) <5-7 follicles or Anti-Müllerian hormone (AMH) <0.5-1.1 ng/mL]. They did not, however, delineate the risk factors included in the first out of three advocated criteria [12]. Several suggestions were made for more specific risk factors; among these and the least studied was unexplained infertility in women under the age of 40 years [13]. To the best of our knowledge, since the publication of the Bologna criteria, no study has been performed to target the potential implication of unexplained infertility on the development of POR. Moreover, the literature lack targeted studies that explored the association between unexplained infertility and decreased ovarian reserve (DOR) in young infertile women.

With the ongoing postponement of first birth in modern societies [14], it seems that the higher the woman's age, the harder it becomes to distinguish between unexplained infertility and age-related infertility [5]. Female's age is one factor that does contribute to the unexplained category. When a woman's age of more than 37 years is the only reason for infertility, test results are likely to be normal [15]. That is to suggest that only younger women ≤ 37 years of age may adequately be referred to as having "genuine" unexplained infertility.

The present study aims to evaluate whether young women aged ≤ 37 years, with an established diagnosis of unexplained infertility, demonstrate manifestations of DOR, as compared to adequate matched controls, in an assisted reproduction setting.

Materials and methods

Study population

The present study was designed as a retrospective, case-control study. It included young infertile women aged ≤ 37 years eligible for in vitro fertilization (IVF)-embryo transfer (ET) at our center chosen by a consecutive systematic review of patients' data on files. All included women had normal cycles with no symptoms of dysmenorrhea or dyspareunia. The study group comprised women meticulously diagnosed with unexplained infertility who had all of the following: (1) Verified ovulation, demonstrated by regular menstrual cycles and ovulatory mid-luteal progesterone level, (2) A normal uterine cavity and patent tubes, demonstrated by hysterosalpingography, and (3) A normal semen analysis according to the World Health Organization (WHO) reference values [35] The control group included women with either mechanical factor infertility or severe male factor infertility treated at our unit during the same time interval. Mechanical factor infertility was defined in women following bilateral salpingectomy or with bilateral tubal occlusion diagnosed by hysterosalpingography and/or laparoscopy. Severe male factor infertility was defined in couples with a pre-wash total motile sperm of $\leq 5 \times 10^6$ in accordance with Hamilton et al. [16] or previously failed fertilization in conventional in vitro fertilization treatment.

Women with hypogonadotropic hypogonadism, polycystic ovarian syndrome, or endometriosis or cases employing testicular sperm were excluded from the study. In addition, women who underwent ovarian surgery (cystectomy or oophorectomy), known FMR1 premutation, or following chemotherapy and/or radiotherapy were excluded. To minimize the effect of potential causative "hidden" conditions, women who had more than five unsuccessful trials of IVF-ET were also omitted from this study. The included women in both the study and control groups could participate in the study only once.

Data were collected in two separate stages in order to increase the reliability of this case-control study. The first stage aimed to look for infertile women who met the inclusion and exclusion criteria. All women who were reviewed during this stage were treated in our unit between April 2015 and November 2016. To ensure similar basal characteristics of both the study and control groups, specifically age and duration of infertility, management of the initial selection was blinded to clinical results. Following completion of the first stage, the second stage included the collection of all related clinical parameters relevant to ovarian reserve, in both groups.

Study conduct

This study was authorized beforehand by the Institutional Review and Ethical Committee of Poriya Medical Center. The first and last authors, NA and JSY, conducted the search, as well as inclusion and exclusion of women to the study or control groups. All women participating in the study underwent ovarian reserve evaluation in a natural cycle prior to treatment initiation by means of endocrine and ultrasonographic (US) tests. Endocrine tests included basal serum follicle-stimulating hormone (FSH) level, serum luteinizing hormone (LH) level, serum estradiol (E_2) level, and FSH/LH ratio. US tests included antral follicle count (AFC) and ovarian volume. The sonographic assessment was performed by two US technicians, both of whom are highly proficient with more than 25 years of experience in reproductive US

evaluation. Of note, both technicians were blinded to the strategy, as well as the target of the study, as it was retrospective in nature.

COS was individually prescribed on a case-to-case basis according to standard clinical practice. The employment of the long/short GnRH agonist or the GnRH antagonist protocols was based on clinical judgment. Recombinant human chorionic gonadotropin (hCG) dosage of 250 μg was administered when the transvaginal scan (TVS) showed at least two follicles with a diameter of ≥ 17 mm each. Oocyte pick-up (OPU) was planned 34–35 h following hCG administration. Conventional IVF and/or intra-cytoplasmic sperm injection (ICSI) were performed according to the cause of infertility. Detailed descriptions of sperm, oocyte, zygote, and embryo handling, as well as embryo transfer and luteal phase supplementation in our unit, have been described previously [17]. Top embryo quality was defined as embryos with 4 or 5 blastomeres on days 2 and 7 or more cells on day 3, with ≤ 20% of fragmentation and absence of multinucleated blastomeres during the whole observation period in accordance with Van Royen et al. [18].

Outcome measures and sample size

The primary outcome measure was a priori set as the number of ≥ 14-mm follicles on the day of hCG administration. This measure was selected, in accordance with the available literature, as an indicator for ovarian response to conventional COS [36–43] to perform a power analysis. A preliminary literature search was conducted to look for other measures of ovarian reserve, such as AFC and Anti-Müllerian hormone (AMH). We also inquired parameters of ovarian response to COS, including serum E_2 levels and the number of retrieved oocytes. Yet such reports were not found in studies addressing the topic of unexplained infertility.

The secondary outcome measures incorporated parameters of ovarian reserve, including AFC and ovarian volume [12],-basal FSH level [33, 44], as well as FSH/LH ratio [19, 45–50]. Also pertained as secondary outcome measures are parameters of ovarian response to COS, including the total dosage of FSH required during COS, serum E_2 level on the day of hCG administration, the total number of oocytes, MII oocytes, the total number of embryos, number of top-rated embryos, number of frozen embryos and cumulative clinical pregnancy rate (per the entire cycle). The number of cycles in which all embryos were frozen due to excessive ovarian response, to prevent ovarian hyperstimulation syndrome (OHSS), was also chosen as a secondary outcome measure. Clinical pregnancy was defined as an intrauterine gestational sac with a fetal echo and pulse.

Power analysis was conducted to determine statistical power, based on a previously published study analyzing the ovarian response to COS in women with unexplained infertility [20]. To demonstrate a statistical power of at least 80%, a total of 78 women, i.e., 39 in each group, is required to detect a difference in means of 1.3 follicles ≥ 14 mm in diameter on the day of hCG administration, with a standard deviation of 2 follicles using a two-sample *t* test with a 0.05 two-sided significance level. A requirement target of 50 women per group was set to exemplify a higher statistical power.

Hormonal assays and ultrasound evaluation

Sera obtained for basal FSH and LH measurements were analyzed by microparticle enzyme immunoassay (AxSYM®, Abbott, Abbott Park, IL, USA). The intra-assay and inter-assay coefficients of variation were < 5% and < 11%, respectively, for FSH and < 7% and < 8%, respectively, for LH. Serum E_2 and P levels were assayed by solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000, DPC, Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation were < 10% and < 16%, respectively, for E_2 and < 18% and < 22%, respectively, for P. The same endocrine assays were employed throughout the duration of the research and were regularly calibrated to minimize variation of the findings associated with time and reagent batch renewal.

Ovarian ultrasonography was performed employing a trans-vaginal probe (5–9 MHz) (Voluson E-8; General Electric Medical System, Milwaukee, WI). AFC, comprising the total number of follicles measured 2–10 mm in both ovaries, was evaluated as recommended [21]. Ovarian volume was computed as the volume of an ellipsoid (length × width × depth × π/6). The total and mean ovarian volumes were subsequently calculated in each woman.

Statistical analysis

Data were analyzed using the Software Package for Social Sciences (SPSS) for Windows version 20.0 (SPSS Inc. 2013, Chicago, IL, USA). Patients' basic characteristics were evaluated using the independent samples Student *t* test, and each variable is presented as mean ± SD. To analyze ovarian reserve test (ORT) results and ovarian response to stimulation and laboratory IVF results, the independent samples Student *t* test was used as well. Analyses of chronic smoking rate, stimulation protocol, and the clinical pregnancy rate were performed by the Chi-square test. A *P* value of < 0.05 was considered as statistically significant.

Results

Between April 2015 and November 2016, the records of 285 consecutive infertile women attending the ART unit at the Poriya Medical Center for IVF-ET treatment were surveyed

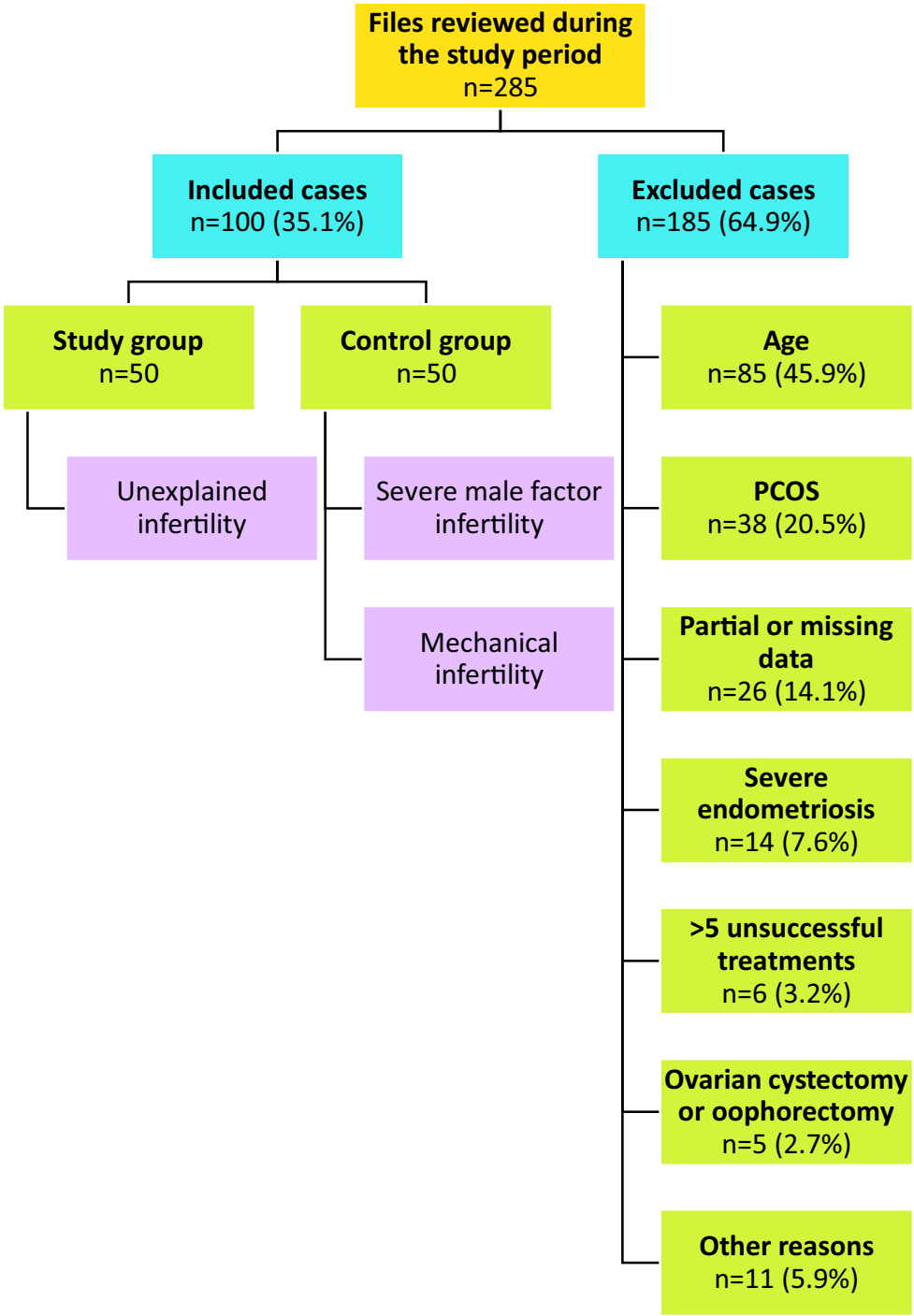

Fig. 1 Selection of Study Population

to match the objectives of the research. Among these cases, 185 were excluded as shown in Fig. 1, on the basis of the exclusion criteria.

Among 285 cases systematically reviewed, 100 were eligible to participate in the research. A study group of 50 infertile women with unexplained infertility was compared with the control group of 50 women with either male factor or mechanical factor infertility. The participants' basic characteristics are summarized in Table 1. As shown, the study and control groups were similar in their initial fundamental features and were comparable.

Primary outcome measure

The number of follicles $\geq$ 14 mm on the day of hCG administration was defined as the primary outcome measure. The

Table 1 Women's basic characteristics

	Study group ($n = 50$)	Control group ($n = 50$)	P	95% CI
Age	32.1 ± 2.8	31.3 ± 2.9	0.16*	− 0.3 to 2.0
BMI (kg/m^2)	25.1 ± 5.5	24.3 ± 3.9	0.45*	− 1.3 to 2.8
Chronic smoking (rate)	5 (10%)	6 (13%)	0.67**	–
Infertility duration (months)	52.1 ± 33.7	54.3 ± 37.6	0.76*	− 16.7 to 12.2
Gravidity	1.2 ± 1.1	1.2 ± 1.8	0.98*	− 0.6 to 0.6
Parity	0.6 ± 0.7	0.5 ± 0.8	0.45*	− 0.2 to 0.4
No. of living children	0.6 ± 0.7	0.5 ± 0.8	0.44*	− 0.2 to 0.4

Values are presented as mean ± SD

CI confidence interval, *BMI* body mass index

*Independent two-samples *T* test

**Chi-Square test

obtained result was significantly lower in the study group as compared with the control group, corresponding to 7.0 ± 4.5 vs. 10.4 ± 4.1 follicles, respectively ($P < 0.001$, 95% CI 1.7–5.1).

Secondary outcome measures

Basal ORTs

Overall, women with unexplained infertility demonstrated poorer ORTs than the controls, as shown in Table 2. Basal FSH level was significantly higher in the study group compared with the control group, corresponding to 8.4 ± 5.5 vs. 6.4 ± 1.7 IU/L, respectively ($P = 0.015$, 95% CI 0.4–3.6). On a similar note, FSH/LH ratio was significantly higher in the study group compared with the control group, corresponding to 1.8 ± 0.9 vs. 1.3 ± 0.6, respectively ($P = 0.001$, 95% CI 0.2–0.8). In the same manner, ultrasonographic parameters of ovarian reserve, AFC and ovarian volume, were significantly lower in the study group as compared with the control group, with AFC corresponding to 10.9 ± 6.6 vs. 16.2 ± 6.6 follicles, respectively ($P < 0.0001$, 95% CI 2.6–7.9).

Ovarian response to COS

The results obtained for ovarian response to treatment are presented in Table 3. As opposed to controls, women with unexplained infertility necessitated significantly higher total dose of FSH, corresponding to 2923 ± 1701 vs. 2196 ± 941 IU, respectively ($P = 0.010$, 95% CI 179–1274), yet demonstrated a lower ovarian response. With regard to the aforementioned result, it should be noted that the total number of follicles on the day of hCG administration was significantly lower in the study group as compared with the control group, corresponding to 11.6 ± 6.5 vs. 17.6 ± 7.5 follicles, respectively ($P < 0.001$, 95% CI 3.2–8.7). Likewise, serum E_2 level on the day of hCG administration was significantly lower in the study group compared with the control groups, corresponding

Table 2 Ovarian reserve tests

	Study group ($n = 50$)	Control group ($n = 50$)	P	95% CI
FSH (IU/L)	8.4 ± 5.5	6.4 ± 1.7	0.015*	0.4–3.6
LH (IU/L)	5.6 ± 4.3	5.7 ± 2.9	0.969*	− 1.5 to 1.4
FSH/LH ratio	1.8 ± 0.9	1.3 ± 0.6	0.001*	0.2–0.8
E_2 (pg/mL)	78 ± 76	66 ± 55	0.382*	− 14.8 to 38.4
AFC	10.9 ± 6.6	16.2 ± 6.6	< 0.001*	2.6–7.9
Mean ovarian volume (cm^3)	8.8 ± 4.0	11.4 ± 6.1	0.018*	0.5–4.7
Total ovarian volume (cm^3)	17.6 ± 7.9	22.8 ± 12.2	0.018*	0.9–9.3

Values are presented as mean ± SD

CI confidence interval, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, E_2 estradiol, AFC antral follicle count

*Independent two-samples *T* test

**Chi-square test

Table 3 Ovarian response to controlled ovarian stimulation

		Study group ($n = 50$)	Control group ($n = 50$)	P	95% CI
Stimulation protocol	Short agonist	15 (30%)	9 (18%)	0.277**	–
	Long agonist	29 (58%)	31 (62%)		
	Antagonist	6 (12%)	10 (20%)		
Follicular phase duration (days)		11.4 ± 3.0	11.8 ± 2.8	0.508*	− 1.5 to 0.8
FSH total dosage (IU)		2923 ± 1701	2196 ± 941	0.010*	178.8–1274.0
LH total dosage (IU)		740 ± 1940	502 ± 1009	0.445*	− 378.6 to 853.7
No. of follicles ≥ 14 mm		7.0 ± 4.5	10.4 ± 4.1	< 0.001*	1.7–5.1
Total no. of follicles		11.6 ± 6.5	17.6 ± 7.5	< 0.001*	3.2–8.7
Serum E_2 (pg/mL)		1419 ± 1056	2172 ± 1143	0.001*	316.6–1190.1
Serum P (ng/mL)		0.8 ± 0.4	1.0 ± 0.7	0.111*	− 0.4 to 0.04
Endometrial thickness		10.7 ± 2.4	11.3 ± 2.2	0.208*	− 1.5 to 0.3

Values are presented as mean ± SD

CI confidence interval, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, E_2 estradiol, *P* progesterone

*Independent two-samples *T* test

**Chi-square test

to 1419 ± 1056 vs. 2172 ± 1143 pg/mL, respectively ($P = 0.001$, 95% CI 317–1190).

IVF-ET results

The outcome of IVF-ET treatments is presented in Table 4, where the unexplained infertility group exhibits inferior results as contrasted with controls. Significantly lower results were demonstrated in the number of retrieved oocytes, corresponding to 9.3 ± 6.3 vs. 15.6 ± 7.9 oocytes, respectively ($P < 0.0001$, 95% CI 3.5–9.2); the total number of embryos achieved, corresponding to 5.3 ± 4.0 vs. 8.0 ± 4.7 embryos, respectively ($P = 0.002$, 95% CI 1.0–4.5); the number of frozen embryos, corresponding to 2.5 ± 2.9 vs. 5.1 ± 4.4 embryos, respectively ($P = 0.0001$, 95% CI 1.1–4.1); as well as the number of top-quality embryos, corresponding to 3.6 ± 3.0 vs. 5.4 ± 3.4, respectively ($P = 0.005$, 95% CI 0.6–3.1). Another noteworthy finding refers to treatment cycles in which all embryos were frozen to prevent OHSS. The rate of such cycles was significantly lower in the study group in comparison to the control group, corresponding to 8% and 26%, respectively ($P = 0.017$, OR = 4.041, 95% CI 1.2–13.4).

Interestingly, in spite of the aforementioned, cumulative clinical pregnancy rate was not statistically different between the study and control groups, albeit the similar number of embryos transferred in both groups,

Table 4 IVF treatment results

	Study group ($n = 50$)	Control group ($n = 50$)	P	95% CI
Total no. of retrieved oocytes	9.3 ± 6.3	15.6 ± 7.9	< 0.001*	3.5–9.2
MII	4.7 ± 2.8	11.3 ± 6.5	< 0.001*	4.5–8.6
2PN	4.9 ± 3.5	7.3 ± 4.6	0.004*	0.8–4.1
Total no. of achieved embryos	5.3 ± 4.0	8.0 ± 4.7	0.002*	1.0–4.5
No. of transferred embryos	1.6 ± 0.6	1.5 ± 0.6	0.301*	− 0.1 to 0.4
No. of frozen embryos	2.5 ± 2.9	5.1 ± 4.4	0.001*	1.1–4.1
No. of top embryos	3.6 ± 3.0	5.4 ± 3.4	0.005*	0.6–3.1
Freeze all to prevent OHSS	4 (8%)	13 (26%)	0.023**	1.2–13.4
Cumulative clinical pregnancy rate	22 (44%)	29 (58%)	0.163**	0.3–1.3

Values are presented as mean ± SD

CI confidence interval, *OHSS* ovarian hyperstimulation syndrome

*Independent two-samples *T* test

**Chi-square test

corresponding to 44% vs. 58%, respectively ($P = 0.163$, OR = 0.596, 95% CI 0.3–1.3).

Discussion

Our study indicates that young women ≤ 37 years of age, diagnosed with unexplained infertility in an ART setting, exhibit sub-optimal ovarian reserve set against controls, as evidenced by the inferiority of their basal ORTs, ovarian response to COS, and IVF results. Along with a significantly higher FSH dosage required by the women in the study group, our findings imply that women with unexplained infertility may develop POR in the future. However, this should be targeted by a longitudinal study.

Another possible explanation to our observation of sub-optimal ovarian response to COS is FSH receptor polymorphism [51]. It seems that the diversity in receptor genotype is associated with different basal FSH levels [52, 53], and can affect the dosage required for ovarian stimulation [54, 55], as well as pregnancy rate [54, 56]. Although ORTs results of the study group show their ovarian reserve is lower in comparison to the control group, it should be emphasized that the absolute values of the unexplained infertility group are within normal limits. The AFC of young women with unexplained infertility in the study was 10.9 ± 6.6 follicles, superior to that defined by the Bologna criteria. It may be argued that to define a young woman with unexplained infertility as a potential POR in accordance with Bologna criteria, a second criterion should be fulfilled, i.e., a previous POR (≤ 3 oocytes with a conventional stimulation protocol), or an abnormal ovarian reserve test (AFC < 5–7 follicles) [12]. Nevertheless, the applicability of unexplained infertility as a risk factor for an expected POR, in accordance with the Bologna criteria, should be validated by well-designed studies.

Regardless, women with unexplained infertility exhibit cumulative clinical pregnancy rate similar to that of women in the control group. This finding implies that, unlike chronological age, unexplained infertility may be considered as a quantitative, rather than a qualitative, risk factor for POR in accordance with the Bologna criteria [13].

POR to conventional COS protocols does not portray a homogenous population of women. Instead, it incorporates several subpopulations, each with its distinct features. A need for the stratification of possible risk factors was suggested by our group to refine the definition of POR [11]. The ESHRE Working Group on Poor Ovarian Response Definition considered our proposal as the most complete for potential risk factors of low ovarian reserve in young women [12]. In a subsequent comprehensive critical appraisal of the Bologna criteria, it was realized that unexplained infertility was the least investigated among the proposed risk factors [13]. To the best of our knowledge, our study is the first to tackle the topic of unexplained infertility as a risk factor for POR since the release of the Bologna criteria.

The results of our present study are supported by previous studies published prior to the release of the Bologna criteria for POR. Randolph *et al.* conducted a prospective study in a small group ($n = 9$) of women, aged 25–40 and diagnosed with unexplained infertility, following an injection of gonadotropin-releasing hormone (GnRH) bolus. Their basal early follicular serum FSH was found to be significantly higher compared to a matched control, suggesting a DOR and negating an abnormality in pulsatile GnRH secretion or an abnormal pituitary sensitivity to GnRH [22].

Kok *et al.* examined the age of natural menopause as a proxy for accelerated ovarian aging in a cohort of 2,392 women, aged 50–63 years. The data was taken from an existing regional population-based program of breast cancer screening that explored the age of natural menopause. Several measures related to subfertility history were examined, all of which demonstrated an unequivocal association with early menopause. These findings support the notion that fertility problems are frequently followed by early menopause [23].

Goverde *et al.* performed a dual-design study in women between 21 and 41 years of age; one prospective ($n = 85$) and the other a retrospective study ($n = 1{,}155$) in an ART setting. The investigators found a reduced number of ≥ 14-mm follicles on the day of hCG administration in cohorts of women with unexplained and mild male factor subfertility but not in controls with mechanical factor infertility [20].

That being said, the studies mentioned above [20, 22, 23] did not target unexplained infertility in young women aged ≤ 37 years, nor were they designed to explore a possible association between unexplained infertility and low ovarian reserve. Additionally, in the study conducted in an ART setting [20], other measures of ovarian reserve such as ORTs, gonadotropin requirement, E_2 level on the day of hCG administration, IVF-ET results, and clinical pregnancy rate were not studied. In contrast, our study was specifically designed to examine the population of women ≤ 37 years of age diagnosed with unexplained infertility following a meticulous investigation as to the cause of infertility. Moreover, in our study, we obtained a vast spectrum of parameters, including ORTs, ovarian response to COS, IVF-ET treatment results, and achievement of clinical pregnancy. A power analysis was performed to determine the necessary sample size for the study to be statistically significant, based on the available literature.

Of note, former studies have suggested POR or a low number of retrieved oocytes as predisposing factors to premature ovarian failure or early menopause [24–28]; two of them were longitudinal studies [24, 27]. Since our study revealed manifestations of DOR in younger women diagnosed with unexplained infertility in an ART setting, it can be intriguing to explore whether unexplained infertility per se may predispose to natural menopause at an earlier age.

In addition, two recently published papers present noteworthy conclusions, showing that currently employed basal ORTs, AFC, and AMH do not discriminate women with infertility from healthy community-based women of similar demographic characteristics [29, 30], in particular Greenwood *et al.* who reported this conclusion based on a study which included women rigorously diagnosed with unexplained infertility [30]. The lead assumption in both papers stated that oocyte quantity predicts reproductive trajectory. Hence, both AMH levels and AFC were expected to be lower in infertile patients compared with reproductively healthy controls. ORTs are utilized to provide a relatively good measure of the quantitative aspect of ovarian reserve but have little contribution to the qualitative nature of the ovarian pool [13, 31, 32]. Fertility, pregnancy achievement, and maintenance are immensely complex issues that encompass various influential factors other than oocyte quantity or quality. Moreover, basal ORTs are only one proxy of ovarian reserve. Other significant parameters for ovarian reserve appraisal include ovarian response to COS, IVF-ET outcomes, and age at menopause. Therewith, it is known that infertility, POR to COS, and IVF-ET results are all implicated and associated with early age at menopause [33, 34].

Notwithstanding the obvious advantages of this work, some limitations must be addressed as well. Firstly, this study is retrospective in design. Since our goal was to uphold unexplained infertility as a risk factor for POR, we evaluated ovarian reserve parameters of young women rigorously diagnosed with unexplained infertility and compared them with controls. In that context, AMH measurements might have made a substantial contribution to support our results. However, AMH testing is not routinely performed in Israel. Though we looked for AMH test results during our data collection process, only a few patients performed such a test, not nearly enough to be statistically significant. Secondly, the present study was powered to look for the number of $\geq$ 14-mm follicles on the day of hCG administration as the primary outcome measure. Indeed, this was found to be significantly lower in the unexplained infertility group as compared with controls, thus supporting the primary hypothesis of our study. Nevertheless, since our study collectively included 100 women, further studies of higher order are needed to substantiate our results, mainly the secondary outcome measures. Consequently, since cumulative pregnancy achievement is related to the quality of ovarian reserve, larger prospective studies are needed to explore this finding. Our study may be employed as the basis for power analysis for such research.

Conclusions

Ultimately, young women $\leq$ 37 years of age, diagnosed with unexplained infertility, have clear manifestations of suboptimal ovarian reserve compared with controls, as evidenced by their inferior basal ORTs, ovarian response to COS, and IVF results. Our findings insinuate that unexplained infertility at a young age might be a risk factor for future POR, conforming with the Bologna criteria. Particularly, unexplained infertility appears to be a quantitative, rather than a qualitative, risk factor for POR. Having that said, further research is necessary to corroborate this finding and should be targeted in well-designed studies. Our study may have broader implications. Ideally, women with unexplained infertility should undergo ovarian reserve evaluation as part of their preparatory array of tests, which may as well tailor treatment plans. For that matter, identification of unexplained infertility in young women might guide them in their future family planning, and enable health care providers to start treatment at an appropriate time.

Compliance with ethical standards

Conflict of interests The authors declare they have no conflict of interest.

Ethical approval Approval and waiver of written informed consent to retrieve and analyze the data was obtained from the Institutional Review and Ethical Committee of Poriya Medical Center. No additional interventions were performed to the routine clinical and laboratory standards in our unit.

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