BMJ Open Vitamin D supplementation prior to in vitro fertilisation in women with polycystic ovary syndrome: a protocol of a multicentre randomised, double-blind, placebo-controlled clinical trial

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ABSTRACT

Introduction Polycystic ovary syndrome (PCOS) is one of the leading causes of female infertility, affecting around 5% of women of childbearing age in China. Vitamin D insufficiency is common in women with PCOS and is associated with lower live birth rates. However, evidence regarding the effectiveness of vitamin D supplementation in women with PCOS is inconclusive. This multicentre randomised, double-blinded, placebo-controlled trial aims to evaluate the effectiveness of vitamin D supplementation prior to in vitro fertilisation (IVF) on the live birth rate in women with PCOS.

Methods and analysis We plan to enrol women with PCOS scheduled for IVF. After informed consent, eligible participants will be randomised in a 1:1 ratio to receive oral capsules of 4000 IU vitamin D per day or placebo for around 12 weeks until the day of triggering. All IVF procedures will be carried out routinely in each centre. The primary outcome is live birth after the first embryo transfer. The primary analysis will be by intention-to-treat analysis. To demonstrate or refute that treatment with vitamin D results in a 10% higher live birth rate than treatment with placebo, we need to recruit 860 women (48% vs 38% difference, anticipating 10% loss to follow-up and non-compliance, significance level 0.05 and power 80%).

Ethics and dissemination This study has been approved by the Ethics Committee in Women's Hospital of Zhejiang University on 2 March 2020 (reference number: IRB-20200035-R). All participants will provide written informed consent before randomisation. The results of the study will be submitted to scientific conferences and a peer-reviewed journal.

Trial registration number NCT04082650.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous clinical condition characterised by androgen excess, oligo-ovulation

Strengths and limitations of this study

- This is a multicentre randomised, double-blinded, placebo-controlled trial.
- The intended sample size is large with 860 participants.
- Despite the large sample size, our study is not designed to detect a small difference.

or anovulation, and polycystic ovary morphology.¹ PCOS is one of the common causes of infertility and it affects around 5.6% of women with childbearing age in China.² In women with PCOS, the proportion of severe vitamin D deficiency (less than 10 ng/mL) was significantly higher than that in women without PCOS (44% vs 11%).³

The most reliable determinant of serum vitamin D is the serum concentration of 25-hydroxyvitamin D (25-OHD), with a half-life of 2–3 weeks.^{4 5} Serum vitamin D levels show seasonal variations, with the highest levels in autumn and the lowest levels in spring.⁶ The optimal concentration of serum 25-OHD is 30 ng/mL or above, whereas concentrations below 20 ng/mL (50 nmol/L) and 30 ng/mL (75 nmol/L) are considered deficiency and insufficiency, respectively. In China, around 88% of individuals aged over 15 years have vitamin D deficiency or insufficiency.^{7 8} PCOS women with vitamin D deficiency are less likely to ovulate and have a lower live birth rate, whereas in women with unexplained infertility, vitamin D deficiency

Figure 1 Flowchart of recruitment. IVF, in vitro fertilisation; PCOS, polycystic ovary syndrome.

does not significantly reduce the live birth rate.⁹

Recent studies have shown that vitamin D supplementation improves insulin resistance and lipid metabolism, reduces testosterone levels, inflammatory indicators and serum anti-müllerian hormone (AMH) levels, and regulates the menstrual cycle in women with PCOS.^{10–21} In contrast, these effects are not significant in non-PCOS women with vitamin D insufficiency.^{10–21} A recent study indicates that serum vitamin D levels are associated positively with fertilisation rate but not clinical pregnancy and live birth rates following in vitro fertilisation (IVF).²² These findings indicate that vitamin D treatment functions mainly in women with PCOS.

The role of vitamin D supplementation in IVF is inconclusive. Vitamin D deficiency does not significantly impair pregnancy rates among infertile women undergoing frozen-thawed embryo transfer cycles.^{23–24} A trial showed that a single dose of 300 000 IU vitamin D supplementation 2 months prior to ovarian stimulation significantly increases the endometrium thickness in PCOS women who undergo intrauterine insemination but does not significantly improve ongoing pregnancy rate (38% vs 33%).²⁵ However, another trial showed that concurrent use of vitamins D and E for 2 months prior to embryo transfer significantly increased the clinical pregnancy rate from 23% to 62% and live birth rate from 16% to 43% in women with PCOS undergoing IVF.²⁶ Due to the inconsistent evidence, no consensus has been reached regarding whether vitamin D should be routinely used in women with PCOS before IVF. In China, the current recommended upper limit for vitamin D supplements in

reproductive women is 2000 IU/day.²⁷ However, this dose recommendation is conservative as 60% of Chinese adults with vitamin D deficiency taking 2000 IU/day for 3 months fail to reach the optimal 25-OHD levels (≥ 75 nmol/L).^{27–28} Therefore, we plan a multicentre randomised, double-blind, placebo-controlled trial to evaluate whether the treatment of vitamin D at a dose of 4000 IU for around 12 weeks prior to IVF improves live birth rates in women with PCOS.

MATERIALS AND METHODS

Design, setting and participants

We plan a multicentre randomised, double-blind, placebo-controlled trial. The participating centres include Women's Hospital of Zhejiang University (Hangzhou), Peking University Third Hospital (Beijing), Jiangxi Maternal and Child Health Hospital (Nanchang), Fujian Maternity and Child Health Hospital (Fuzhou), Jinhua People's Hospital (Jinhua) and Huzhou Maternity and Child Health Care Hospital (Huzhou).

The detailed inclusion and exclusion criteria for participants are presented below.

Inclusion criteria:

1. Women aged 20–42 years.
2. Diagnosed with PCOS (Rotterdam criteria).¹
3. Scheduled for IVF.
4. Written informed consent.

Exclusion criteria:

1. Women who had three or more failed IVF cycles.
2. Women scheduled for preimplantation genetic testing.
3. Known vitamin D allergy.
4. Women with a history of chronic absorption syndrome or bile dysplasia, or parathyroid dysfunction, or kidney stones, or blood calcium ion concentration greater than 2.6 mmol/L (normal value: 2.25–2.75 mmol/L (9–11 mg/dL), or hyperphosphataemia (1.61 mmol/L), metabolic-related bone disease, or chronic diseases that may cause bone abnormalities (liver and kidney insufficiency).
5. Women receiving treatments for tuberculosis, convulsions and epilepsy because medications treating these diseases may affect the metabolism of vitamin D.
6. Women undergoing an IVF treatment with donor oocytes.

Recruitment and randomisation

PCOS women scheduled for IVF will be invited to participate in this study if they fulfil the eligibility criteria. Researchers in each centre will explain the study protocol to potential participants. After signing informed consent, participants will be enrolled and randomised to the vitamin D group or the placebo group (figure 1). We will use a computer-generated randomisation list in a 1:1 ratio, with a variable block size of four or six stratified by study centre. The randomisation list will be prepared by an independent statistician who will not participate in the recruitment and the list will not be available to

investigators in any centre. The randomisation list will be implemented with the use of sequentially numbered identical study-drug containers. The containers will be labelled centrally by two researchers who will not be involved in the participants' recruitment.

Blinding

Only the two researchers who labeled the containers will be aware of the allocation. Participants and other researchers will be unaware of the treatment allocation. Vitamin D capsules are produced by a licensed pharmaceutical company (Sinopharm Xingsha Pharmaceuticals (Xiamen) Co). Placebo capsules, with identical package, size, colour and appearance to Vitamin D capsules, are produced by the same manufacturer. In general, there should be no need to unblind the allocation. If urgent unblinding to participants is necessary, the allocation will be disclosed to the treating physician.

Intervention and comparator

The vitamin D group

Participants in the intervention group will be treated with vitamin D 4000 IU/day (800 IU per capsule, take five capsules once each day) for around 12 weeks (begins from 6-10 weeks prior to the ovarian stimulation till the triggering day).

The placebo group

Participants in the placebo group will be treated with five placebo capsules per day for the same duration.

Outcomes

Primary outcome

The primary outcome is live birth after the first embryo transfer. Live birth is defined as the delivery of at least one baby after 24 weeks of gestation that exhibits any sign of life. Pregnancies that occurs before the first embryo transfer but after randomisation will be included.

Secondary outcomes

Pregnancies after the first embryo transfer will not be considered in the following pregnancy-related secondary outcomes except for cumulative live birth.

- ▶ Cumulative live birth: defined as live birth resulting from pregnancies that occur within 6 months after randomisation.
- ▶ Clinical pregnancy: defined as at least one gestational sac on ultrasound at around 7 weeks gestation with the detection of heart-beat activity.
- ▶ Ongoing pregnancy: defined as pregnancy with detectable heart rate at 12 weeks gestation or beyond.
- ▶ Biochemical pregnancy: defined as a positive pregnancy test.
- ▶ Ectopic pregnancy: defined as embryo implanted at any site other than the endometrial lining of the uterus cavity.
- ▶ Miscarriage: defined as a positive pregnancy test but no detectable heart rate before 24 weeks gestation.
- ▶ Number of available embryos for transfer;

- ▶ Moderate or severe ovarian hyperstimulation syndrome (OHSS): defined according to the Golan's criteria.²⁹ Moderate OHSS is diagnosed by the presence of ascites on ultrasonography in addition to abdominal distension and discomfort with or without nausea, vomiting and/or diarrhoea. Severe OHSS is diagnosed when there is clinical evidence of ascites and/or hydrothorax or breathing difficulties with or without haemoconcentration, coagulation abnormalities and diminished renal function.

In case of pregnancy, we will report the following endpoints:

- ▶ Pre-eclampsia: defined as the development of gestational hypertension with proteinuria (≥ 300 mg/24-hour urine collection or 30 mg/dL in single urine sample) after 20 weeks of gestation.
- ▶ Gestational hypertension: defined as the development of blood pressure greater than 140/90 mm Hg after pregnancy without proteinuria or other signs of pre-eclampsia.
- ▶ Gestational diabetes mellitus: defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy as determined from the diagnosis in the obstetrical medical record.
- ▶ Premature rupture of membrane (PROM) rate: defined as rupture of the amniotic membranes before the onset of labour, including PROM at term and preterm PROM.
- ▶ Preterm delivery: defined as delivery of a fetus at less than 37 and more than 24 weeks' gestational age (classified as spontaneous or iatrogenic);
- ▶ Placenta previa: defined as a placenta that is implanted over or very close to the internal cervical orifice.
- ▶ Postpartum haemorrhage: defined as the loss of 500 mL of blood or more after completion of the third stage of labour.
- ▶ Birth weight.
- ▶ 1-minute and 5-minute Apgar score.
- ▶ 5-minute Apgar score < 7 .
- ▶ Stillbirth: defined as the absence of signs of life at or after birth.
- ▶ Serum 25-OHD levels at the triggering day and the pregnancy test day.
- ▶ Vitamin D binding protein, insulin and calcium ion concentration at the triggering day.

Standard IVF procedure

The oral contraceptive capsule will be used for participants with an irregular menstrual cycle (longer than 35 days or shorter than 21 days) at least one cycle prior to ovarian stimulation. The long or short Gonadotrophin-Releasing Hormone (GnRH) agonist or GnRH antagonist protocol will be used. In the long GnRH agonist protocol, GnRH agonist will be administered in the mid-luteal phase of the cycle preceding ovarian stimulation. In the short GnRH agonist protocol, GnRH agonist will be administered on day 2 of the ovarian stimulation cycle. In the GnRH antagonist protocol, GnRH antagonist will

Evaluation	Enrollment	Allocation	Online supervision	Oocyte collecting day	Embryo transfer		Pregnancy test day	35 days after ET	12 weeks gestation	24 weeks gestation	37 weeks gestation	Delivery day	Six weeks after delivery
					Fresh ET	FET							
Informed consent		▲											
Medical/treatment history	▲												
Physical exam	▲			▲									
Ultrasonography	▲			▲				▲	▲				
Steroid hormone		▲		▲									
Liver/renal function, metabolic test		▲											
SEQ within 3 months				▲									
FFQ within 3 months				▲									
Allocation		▲											
IVF-ET protocol					▲	▲							
Pregnancy test							▲						
Record of pregnancy/obstetric complications								▲	▲	▲	▲		
Record of maternal and infant health after delivery												▲	▲
Serum sample of female	▲			▲			▲						
Drug recycling and reporting				▲									
Report form of OHSS					▲	▲	▲						
Report form of concomitant drug use		▲	▲	▲	▲	▲	▲	▲					
Report form of adverse event			▲	▲	▲	▲	▲	▲					
Phone call follow up									▲	▲	▲	▲	▲
Medical record review												▲	

Figure 2 Study visits. ET, embryo transfer; FFQ, food frequency questionnaire; IVF, in vitro fertilisation; OHSS, ovarian hyperstimulation syndrome; SEQ, sunshine exposure questionnaire.

be administered according to the follicle development and serum estradiol levels (around 5 days or 6 days after ovarian stimulation). The starting dose of gonadotrophins will be 75–225 IU. The dose of gonadotrophins will be adjusted according to the ovarian response evaluated by follicle development and serum estradiol levels. HCG 4000–8000 IU will be used to trigger ovulation when at least 3 follicles with a diameter of at least 18 mm are present, and oocyte retrieval will be carried out 36–37 hours after triggering.

Fertilisation, embryo transfer and luteal support

IVF will be performed 4–6 hours after oocyte retrieval by using either conventional IVF or intracytoplasmic sperm injection. Embryo transfer strategies will be used according to local protocol. Up to two embryos will be transferred at the cleavage or blastocyst stage. Endometrium thickness will be measured by transvaginal ultrasound 1 day prior to luteal support. Luteal support begins from the beginning of ovulation day. Embryo transfer will be performed on day 3, day 5 or day 6 after ovulation.

Research visits

Detailed study visits are presented in [figure 2](#). After informed consent, baseline data, including 25-OHD levels, will be collected. We will monitor and promote compliance by encouraging participants to record the daily intake of the dosage of study medication during the trial through an online tool. Meanwhile, trial coordinators will contact participants regularly to monitor compliance.

Vitamin D measurement

Serum vitamin D levels will be measured before the intervention, on the triggering day and at the pregnancy test day for all participants. We also plan to measure vitamin D levels in each trimester for participants who will get delivery in two centres, Peking University Third Hospital and Women's Hospital of Zhejiang University. These data will be used to generate secondary hypotheses.

Sample size calculation

The current live birth rate of women with PCOS after the first embryo transfer per started cycle is 38% in Women's Hospital of Zhejiang University. To demonstrate an increase of 10% in the live birth rate in the vitamin D treatment group (48%), a total of 768 participants are required (power 80% and α error: 5%). Taking into account possible protocol violation and loss of follow-up of around 10%, we plan to enrol 860 participants (430 per arm).

Statistical methods

Statistical analysis will be conducted according to the intention-to-treat principle. We will present descriptive statistics for baseline variables. For continuous characteristics, distribution between the two groups will be presented with means and SDs or medians and IQRs according to the normality of data. For categorical characteristics, we will present the proportions of the two arms. The incidence of binary outcomes will be estimated for each treatment group and the differences between groups will be presented using χ^2 test and risk ratios and

their 95% CIs. For continuous outcomes, means and SDs or medians and IQRs will be presented for each treatment group and compared using the Student's t-test or the Mann-Whitney test. Finally, a per-protocol analysis will be conducted but these results would be considered exploratory only. Details of the analysis will be described in a separate statistical analysis plan which will be signed off prior to data-lock.

Study duration

The trial is expected to start in July 2020 with a duration of 18 months for recruitment. It is expected to recruit the last participant in December 2021.

Patient and public involvement

Patients and the public were not involved in the development of the research question or study design. They will not be involved in the recruitment, conduct, or report of the study. The study results will be disseminated to study participants by social media.

Data monitoring committee and interim analysis

An independent Data Safety Monitoring Committee (DSMC) formed by at least two clinicians and one statistician who are independent of the sponsor without competing interests will monitor the conduct of the trial. An interim analysis will be performed 6 months after the recruitment of the first 300 participants. The DSMC will be asked to assess ongoing pregnancy, as the primary endpoint live birth would not be achieved in most participants at the interim analysis. The study could be stopped prematurely based on the advice of the DSMC.

Adverse events monitoring

Adverse events will be closely monitored during this study. If a participant shows suspected drug-related severe discomfort symptoms, abnormal signs, abnormal liver and kidney function or abnormal blood routine results, then she will be instructed to discontinue the trial medication. Researchers will continue to observe the symptoms and signs until they disappear or the test results are normal. A previous randomised controlled trial (RCT) showed that adults taking vitamin D 4000 IU/day for 3 months with serum vitamin D concentrations reaching 81.3–115.3 nmol/L did not present safety concerns.³⁰ Common adverse events related to vitamin D supplements are mainly bone and joint pain, swelling, itching of the skin, vomiting, constipation or diarrhoea, nausea, etc. Rare adverse events include allergic reactions. A severe adverse event is defined as an event that occurs during the study and meets one of the following criteria: death, life threatening, causing severe or permanent disability, congenital malformation or birth defect, and prolonged hospitalisation. No severe adverse event (SAE) in relation to vitamin D has been reported in the literature.

Ethics and dissemination

This study has been approved by the Ethics Committee in Women's Hospital of Zhejiang University on 2 March 2020

(reference number: IRB-20200035-R). Protocol modifications after the recruitment of the first participant will not be allowed unless it is necessary and additional ethical approval will be required. All participants must provide written informed consent before participating in the trial. Data handling will be done anonymously, with only the participant code available in the central database. All personal information on potential and enrolled participants out of the scope of this trial will not be collected, shared or maintained to protect confidentiality before, during and after the trial. The results of the study will be submitted to scientific conferences in reproductive medicine and a peer-reviewed journal.

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REFERENCES

- 1 Group REA-SPCW. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25.
- 2 Li R, Zhang Q, Yang D, *et al.* Prevalence of polycystic ovary syndrome in women in China: a large community-based study. *Hum Reprod* 2013;28:2562–9.
- 3 Li HWR, Brereton RE, Anderson RA, *et al.* Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. *Metabolism* 2011;60:1475–81.
- 4 Pearce SHS, Cheetham TD. Diagnosis and management of vitamin D deficiency. *BMJ* 2010;340:b5664.
- 5 Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al.* Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:1911–30.
- 6 Niculescu DA, Capatina CAM, Dusceac R, *et al.* Seasonal variation of serum vitamin D levels in Romania. *Arch Osteoporos* 2017;12:113.
- 7 Li M, Lv F, Zhang Z, *et al.* Establishment of a normal reference value of parathyroid hormone in a large healthy Chinese population and evaluation of its relation to bone turnover and bone mineral density. *Osteoporos Int* 2016;27:1907–16.
- 8 Lu H-K, Zhang Z, Ke Y-H, HK L, YH K, *et al.* High prevalence of vitamin D insufficiency in China: relationship with the levels of parathyroid hormone and markers of bone turnover. *PLoS One* 2012;7:e47264.
- 9 Butts SF, Seifer DB, Koelper N, *et al.* Vitamin D deficiency is associated with poor ovarian stimulation outcome in PCOS but not unexplained infertility. *J Clin Endocrinol Metab* 2019;104:369–78.
- 10 Thys-Jacobs S, Donovan D, Papadopoulos A, *et al.* Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. *Steroids* 1999;64:430–5.
- 11 Dastorani M, Aghadavod E, Mirhosseini N, *et al.* The effects of vitamin D supplementation on metabolic profiles and gene expression of insulin and lipid metabolism in infertile polycystic ovary syndrome candidates for in vitro fertilization. *Reproductive Biology and Endocrinology* 2018;16:94.
- 12 Ostadmohammadi V, Jamilian M, Bahmani F, *et al.* Vitamin D and probiotic co-supplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome. *J Ovarian Res* 2019;12:5.
- 13 Seyyed Abootorabi M, Ayremlou P, Behrooz-Lak T, *et al.* The effect of vitamin D supplementation on insulin resistance, visceral fat and adiponectin in vitamin D deficient women with polycystic ovary syndrome: a randomized placebo-controlled trial. *Gynecol Endocrinol* 2018;34:489–94.
- 14 Nasri K, Akrami S, Rahimi M, *et al.* The effects of vitamin D and evening primrose oil co-supplementation on lipid profiles and biomarkers of oxidative stress in vitamin D-deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Endocr Res* 2018;43:1–10.
- 15 Pal L, Berry A, Coraluzzi L, *et al.* Therapeutic implications of vitamin D and calcium in overweight women with polycystic ovary syndrome. *Gynecol Endocrinol* 2012;28:965–8.
- 16 Asemi Z, Foroozanfar F, Hashemi T, *et al.* Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. *Clin Nutr* 2015;34:586–92.
- 17 Jamilian M, Foroozanfar F, Rahmani E, *et al.* Effect of two different doses of vitamin D supplementation on metabolic profiles of insulin-resistant patients with polycystic ovary syndrome. *Nutrients* 2017;9. doi:10.3390/nu9121280. [Epub ahead of print: 24 11 2017].
- 18 Foroozanfar F, Talebi M, Samimi M, *et al.* Effect of two different doses of vitamin D supplementation on metabolic profiles of insulin-resistant patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Horm Metab Res* 2017;49:612–7.
- 19 Maktabi M, Chamani M, Asemi Z. The effects of vitamin D supplementation on metabolic status of patients with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Horm Metab Res* 2017;49:493–8.
- 20 Karadağ C, Yoldemir T, Yavuz DG. Effects of vitamin D supplementation on insulin sensitivity and androgen levels in vitamin-D-deficient polycystic ovary syndrome patients. *J Obstet Gynaecol Res* 2018;44:270–7.
- 21 Javed Z, Papageorgiou M, Deshmukh H, *et al.* A randomized, controlled trial of vitamin D supplementation on cardiovascular risk factors, hormones, and liver markers in women with polycystic ovary syndrome. *Nutrients* 2019;11. doi:10.3390/nu11010188. [Epub ahead of print: 17 Jan 2019].
- 22 Liu X, Zhang W, Xu Y, *et al.* Effect of vitamin D status on normal fertilization rate following in vitro fertilization. *Reprod Biol Endocrinol* 2019;17:59.
- 23 van de Vijver A, Drakopoulos P, Van Landuyt L, *et al.* Vitamin D deficiency and pregnancy rates following frozen-thawed embryo transfer: a prospective cohort study. *Hum Reprod* 2016;31:1749–54.
- 24 Aflatoonian A, Arabjahvani F, Eftekhari M, *et al.* Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: a randomized clinical trial. *Iran J Reprod Med* 2014;12:595–600.
- 25 Asadi M, Matin N, Frootan M, *et al.* Vitamin D improves endometrial thickness in PCOS women who need intrauterine insemination: a randomized double-blind placebo-controlled trial. *Arch Gynecol Obstet* 2014;289:865–70.
- 26 Fatemi F, Mohammadzadeh A, Sadeghi MR, *et al.* Role of vitamin E and D₃ supplementation in Intra-Cytoplasmic sperm Injection outcomes of women with polycystic ovarian syndrome: a double blinded randomized placebo-controlled trial. *Clin Nutr ESPEN* 2017;18:23–30.
- 27 Yao P, Lu L, Hu Y, *et al.* A dose-response study of vitamin D₃ supplementation in healthy Chinese: a 5-arm randomized, placebo-controlled trial. *Eur J Nutr* 2016;55:383–92.
- 28 Yao P, Sun L, Lu L, *et al.* Effects of Genetic and Nongenetic Factors on Total and Bioavailable 25(OH)D Responses to Vitamin D Supplementation. *J Clin Endocrinol Metab* 2017;102:100–10.
- 29 Herman A, Ron-El R, Golan A, *et al.* Overstimulated cycles under low-dose gonadotropins in patients with polycystic ovary syndrome: characterization and management. *Hum Reprod* 1993;8:30–4.
- 30 Burt LA, Billington EO, Rose MS, *et al.* Effect of high-dose vitamin D supplementation on volumetric bone density and bone strength: a randomized clinical trial. *JAMA* 2019;322:736–45.