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**ORIGINAL ARTICLE** ***Infertility***

# Outcome of *in-vitro* oocyte maturation in patients with PCOS: does phenotype have an impact?

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**STUDY QUESTION:** Does the phenotype of patients with polycystic ovary syndrome (PCOS) affect clinical outcomes of ART following *in-vitro* oocyte maturation?

**SUMMARY ANSWER:** Cumulative live birth rates (CLBRs) after IVM were significantly different between distinct PCOS phenotypes, with the highest CLBR observed in patients with phenotype A/HOP (= hyperandrogenism + ovulatory disorder + polycystic ovaries), while IVM in patients with phenotype C/HP (hyperandrogenism + polycystic ovaries) or D/OP (ovulatory disorder + polycystic ovaries) resulted in lower CLBRs (OR 0.26 (CI 0.06–1.05) and OR 0.47 (CI 0.25–0.88), respectively, $P = 0.03$).

**WHAT IS KNOWN ALREADY:** CLBRs in women with hyperandrogenic PCOS phenotypes (A/HOP and C/HP) have been reported to be lower after ovarian stimulation (OS) and ART when compared to CLBR in women with a normo-androgenic PCOS phenotype (D/OP) and non-PCOS patients with a PCO-like ovarian morphology (PCOM). Whether there is an influence of the different PCOS phenotypes on success rates of IVM has been unknown.

**STUDY DESIGN, SIZE, DURATION:** This was a single-centre, retrospective cohort study including 320 unique PCOS patients performing their first IVM cycle between April 2014 and January 2018 in a tertiary referral hospital.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Baseline patient characteristics and IVM treatment cycle data were collected. The clinical outcomes following the first IVM embryo transfer were retrieved, including the CLBR defined as the number of deliveries with at least one live birth resulting from one IVM cycle and all appended cycles in which fresh or frozen embryos were transferred until a live birth occurred or until all embryos were used. The latter was considered as the primary outcome. A multivariate regression model was developed to identify prognostic factors for CLBR and test the impact of the patient's PCOS phenotype.

**MAIN RESULTS AND THE ROLE OF CHANCE:** Half of the patients presented with a hyperandrogenic PCOS phenotype (n = 140 A/HOP and n = 20 C/HP vs. n = 160 D/OP). BMI was significantly different between phenotype groups (27.4 ± 5.4 kg/m$^2$ for A/HOP, 27.1 ± 5.4 kg/m$^2$ for C/HP and 23.3 ± 4.4 kg/m$^2$ for D/OP, $P < 0.001$). Metformin was used in 33.6% of patients with PCOS phenotype A/HOP, in 15.0% of C/HP patients and in 11.2% of D/OP patients ($P < 0.001$). Anti-müllerian hormone levels differed significantly between groups: 12.4 ± 8.3 µg/l in A/HOP, 7.7 ± 3.1 µg/l in C/HP and 10.4 ± 5.9 µg/l in D/OP patients ($P = 0.01$). The number of cumulus-oocyte complexes (COC) was significantly different between phenotype groups: 25.9 ± 19.1 COC in patients with phenotype A/HOP, 18.3 ± 9.0 COC in C/HP and 19.8 ± 13.5 COC in D/OP ($P = 0.004$). After IVM, patients with different phenotypes also had a significantly different number of mature oocytes (12.4 ± 9.3 for A/HOP vs. 6.5 ± 4.2 for C/HP vs. 9.1 ± 6.9 for D/OP, $P < 0.001$). The fertilisation rate, the number of usable embryos and the number of cycles with no embryo available for transfer were comparable between

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the three groups. Following the first embryo transfer, the positive hCG rate and LBR were comparable between the patient groups (44.7% (55/123) for A/HOP, 40.0% (6/15) for C/HP, 36.7% (47/128) for D/OP, $P = 0.56$ and 25.2% (31/123) for A/HOP, 6.2% (1/15) for C/HP, 26.6% (34/128) for D/OP, respectively, $P = 0.22$). However, the incidence of early pregnancy loss was significantly different across phenotype groups (19.5% (24/123) for A/HOP, 26.7% (4/15) for C/HP and 10.2% (13/128) for D/OP, $P = 0.04$). The CLBR was not significantly different following univariate analysis (40.0% (56/140) for A/HOP, 15% (3/20) for C/HP and 33.1% (53/160) for D/OP ($P = 0.07$)). When a multivariable logistic regression model was developed to account for confounding factors, the PCOS phenotype appeared to be significantly correlated with CLBR, with a more favourable CLBR in the A/HOP subgroup (OR 0.26 for phenotype C/HP (CI 0.06–1.05) and OR 0.47 for phenotype D/OP (CI 0.25–0.88), $P = 0.03$)).

**LIMITATIONS, REASONS FOR CAUTION:** These data should be interpreted with caution as the retrospective nature of the study holds the possibility of unmeasured confounding factors and misassignment of the PCOS phenotype. Moreover, the sample size for phenotype C/HP was too small to draw conclusions for this subgroup of patients.

**WIDER IMPLICATIONS OF THE FINDINGS:** Caucasian infertile patients with a PCOS phenotype A/HOP who undergo IVM achieved a higher CLBR than their counterparts with C/HP and D/OP. This is in strong contrast with previously reported outcomes following OS where women with PCOS and hyperandrogenism (A/HOP and C/HP) performed significantly worse. For PCOS patients who require ART, the strategy of OS followed by an elective freeze-all strategy remains to be compared with IVM in a prospective fashion; however, the current data provide support for IVM as a valid treatment option, especially in the most severe PCOS phenotypes (A/HOP). Our data suggest that proper patient selection is of utmost importance in an IVM programme.

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**Key words:** PCOS / Phenotype / in-vitro maturation / cumulative live birth / hyperandrogenism

# Introduction

Oocyte IVM is a reproductive laboratory technique that has been used as an alternative to conventional ART in women with PCOS for more than 25 years (Trounson *et al.*, 1994). Since IVM obviates the need for ovarian stimulation (OS) with gonadotropins in order to obtain oocytes that can be fertilised *in vitro*, the treatment burden for the patient is limited. IVM was originally advocated as a strategy to avoid ovarian hyperstimulation syndrome (OHSS) in high responders; however, this indication has lost part of its appeal because of the emergence of gonadotropin-releasing hormone (GnRH) antagonist protocols and GnRH agonist ovulation triggering in patients with a high risk of OHSS (Engmann *et al.*, 2008; Devroey *et al.*, 2011; Humaidan, 2012). Nevertheless, IVM remains a valid alternative in experienced hands, with live birth rates (LBRs) approaching those of standard IVF/ICSI treatment, especially when embryos generated using IVM are not transferred when fresh, but are instead vitrified and transferred in a subsequent frozen/warmed cycle (Teede *et al.*, 2018; Vuong *et al.*, 2018). Because of the reduced developmental potential of oocytes that have matured in currently available IVM systems compared to their *in-vivo* matured counterparts, the availability of relatively high numbers of oocytes is required to compensate for the success rate gap compared to OS (Guzman *et al.*, 2013). In other words, women with a high antral follicle count, especially those with polycystic ovary syndrome (PCOS), achieve higher clinical success rates with IVM compared to those with a normal functional ovarian reserve (Guzman *et al.*, 2013). In a subset of infertile women with PCOS who require ART, OS can be cumbersome because of the narrow window of optimal ovarian response (Oudshoorn *et al.*, 2017). In view of this, IVM can be an alternative option for those women with PCOS in whom ovarian response to OS may be unpredictable or for women who prefer to avoid the side effects of OS.

Since the establishment of the diagnostic Rotterdam criteria of PCOS, with a more recent extension of these criteria to include a phenotypical classification of the pathology (2012), several studies have shown that women with a hyperandrogenic PCOS phenotype have a less favourable prognosis after OS compared to their normoandrogenic counterparts (Ramezanali *et al.*, 2016; De Vos *et al.*, 2018). Indeed, an unfavourable impact of biochemical hyperandrogenism has been suggested at several levels of the ART process, including adverse effects both on oocyte/embryo quality (Qiao and Feng, 2011) and endometrial receptivity (Schulte *et al.*, 2015). However, previously published outcomes of IVM in PCOS patients by our own (De Vos *et al.*, 2011; Ortega-Hrepich *et al.*, 2013) and other research groups (Walls *et al.*, 2015; Ho *et al.*, 2019) have not taken into account the different PCOS phenotypes. Hence, it has remained unclear whether cumulative live birth rates (CLBRs) after IVM differ among women with different PCOS phenotypes. This retrospective cohort study was conducted to address this question.

# Materials and methods

## Ethical approval

The study was approved by the Ethics Committee of Universitair Ziekenhuis Brussel (B.U.N. 143201938570) and performed in accordance with the endorsed guidelines.

## Study design and participants

This is a single-centre, retrospective cohort study encompassing data from all consecutive PCOS patients performing a first IVM treatment in a tertiary university-affiliated centre between April 2014 and January 2018. Patients were excluded from the analysis when pre-implantation

genetic testing had been performed and/or when IVM was prescribed in the context of fertility preservation, oocyte donation, oocyte maturation defects or FSH resistance. PCOS was diagnosed according to the Rotterdam criteria, based on parameters, that were recorded at the patient's first visit to the fertility clinic, regarding specific cycle length, ovarian morphology, hormone profile and signs of hyperandrogenism. Patients were subdivided into the following phenotype categories: A (HOP)= hyperandrogenism + ovulatory dysfunction + polycystic ovaries, B (HO)= hyperandrogenism + ovulatory dysfunction, C (HP)= hyperandrogenism + polycystic ovaries and D (OP)= ovulatory dysfunction + polycystic ovaries (Lizneva *et al.*, 2016). None of the patients in this study had PCOS phenotype B (HO). Clinical hyperandrogenism was defined as the presence of hirsutism (Ferriman–Gallwey score > 8) and/or severe acne and alopecia; biochemical hyperandrogenism was defined as total serum testosterone >52 ng/dl and calculated free testosterone >0.64 ng/dl, based on the distribution (mean ± 2 SD) of these parameters in our population. Analysis of serum testosterone was performed using validated automated immunoassay methods (Elecsys electrochemiluminescence immunoassays on Cobas 6000, Roche Diagnostics). Serum AMH was analysed using the automated Elecsys® AMH assay, Roche Diagnostics. All PCOS patients in this study underwent ART either because they had previously failed to become pregnant after ovulation induction using clomiphene, letrozole or gonadotropins (including failure to ovulate with this hormone treatment) or because of male factor infertility. In case of the latter, IVM was performed either as a first-line ART treatment or after failed OS. Specific exclusion criteria were severe male factor with non-obstructive azoospermia or other known reasons for impaired implantation (i.e. endometriosis, hydrosalpinx, fibroid distorting the cavity, Asherman's syndrome, thrombophilia and endometrial tuberculosis).

## In-vitro maturation treatment

Non-triggered IVM was performed after a short 3-day course of highly purified human menopausal gonadotropin injections (Menopur, Ferring Pharmaceuticals, Aalst, Belgium) at a daily dose of 225, 225 and 150 IU HP-hMG, respectively, as previously described (Sanchez *et al.*, 2017). Cumulus-oocyte complexes (COC) were retrieved 42–44 h after the last HP-hMG injection. All oocyte retrieval procedures in this study were performed using a 17-gauge single lumen needle (Cook Medical, K-OPS-1230-VUB, Limerick, Ireland) and a suction pressure of −70 mmHg. Follicular aspirates were collected in Human Tubal Fluid (HTF) (IVF Basics® HTF HEPES, Gynotec B.V. Malden, the Netherlands) supplemented with heparin (5000 IU/ml, Heparin Leo, Leo Pharma, Belgium; final heparin concentration 20 IU/ml) and filtered through a cell strainer (Falcon®, 70 µm mesh size, BD Biosciences, CA, USA). After collection, COC were washed in LAG medium (IVM System, Medicult, Origio) and incubated in IVM medium (IVM System, Medicult, Origio) supplemented with 75 mIU/ml HP-hMG (Menopur, Ferring, Saint-Prex, Switzerland), 100 mIU/ml hCG (Pregnyl, Organon, MSD, Haarlem, The Netherlands) and 10 mg/ml HSA (Vitrolife) for 30 h in a four-well dish with oil overlay (Ovoil, Vitrolife, Göteborg, Sweden). COC were cultured in groups of 10 COCs per well in 500 µl IVM medium at 37°C under 6% $CO_2$ in air. After IVM culture, oocytes were mechanically and enzymatically denuded from their cumulus layers under a stereomicroscope and oocyte maturation was assessed under the inverted microscope. Matured oocytes were inseminated using ICSI with partner sperm. Fertilisation was assessed 16–18 h post-insemination by the presence of two pronuclei. Fertilised oocytes and embryos were cultured in individual droplets of 25 µl medium with oil overlay until Day 3 or until Day 5 (or Day 6) after ICSI, depending on the number of embryos available on Day 3; in cycles with at least four embryos on Day 3 that were classified as transferable/good-quality embryos according to the criteria described by Van Landuyt *et al.* (2013), embryos were cultured until Day 5 or 6. Blastocysts were categorised according to the Gardner and Schoolcraft (1999) classification system.

## Embryo transfer

Embryos were transferred freshly or vitrified electively, as previously described (Mostinckx *et al.*, 2019). Specifically, embryos were cultured to the blastocyst stage, and fresh embryo transfer was performed on Day 5 if at least four cleavage-stage embryos of good morphological quality were observed on Day 3; if not, embryos were vitrified electively on Day 3 after ICSI. Day 6 blastocysts were not transferred freshly but only after vitrification/warming, to achieve a more appropriate embryo-endometrial synchronisation.

The protocol for endometrium preparation in IVM cycles with fresh embryo transfer involved two daily unit doses of Oestrogel® (Besins Healthcare; one unit dose of the Oestrogel® metered-dosing pump corresponds to 1.5 mg of gel and contains 0.75 mg oestradiol) administered three to six times daily until seven weeks' gestation, after which the dose was gradually reduced and discontinued 1 week later. Administration of Oestrogel® was started on the day before oocyte retrieval or on the day of oocyte retrieval. Luteal support with intravaginal micronised progesterone (P, 200 mg three times a day; Utrogestan®, Besins Healthcare) was started on the evening of the day of ICSI.

Vitrified-warmed embryo transfer (frozen embryo transfer, FET) after IVM was performed in an artificial endometrium priming cycle initiated when basal hormone levels were reached after the IVM cycle. Briefly, the endometrium was primed with transdermal Oestrogel® (2 units administered three times a day) or with oral oestradiol valerate (Progynova®, Bayer-Schering Pharma AG, Berlin, Germany) at a dose of 2 mg three times daily, based on the clinician's preference. When an endometrial thickness of more than 6 mm was reached, luteal support was started using intravaginal micronised progesterone tablets (P, 200 mg three times a day; Utrogestan®, Besins Healthcare), and the transfer of one or two embryos was scheduled between 5 and 7 days later, depending on the stage of the embryo. The transfer of Day 3 vitrified embryos was performed 1 day after warming, whereas vitrified blastocysts were transferred on the day of warming. Administration of oestrogens and P was continued until a pregnancy test was performed and was continued until 7 weeks of gestation if the pregnancy test was positive, after which the dose was gradually reduced and discontinued 1 week later.

## Outcome parameters

The primary outcome parameter was CLBR, defined as the number of deliveries with at least one live birth resulting from one aspirated IVM cycle, including all cycles in which fresh and/or frozen embryos were transferred, until a live birth occurred or until all embryos were used (Zegers-Hochschild *et al.*, 2017). Secondary outcome parameters were biochemical pregnancy rate, ongoing pregnancy rate, early

pregnancy loss (as defined by Kolte *et al.*, 2015), i.e. as an intrauterine pregnancy loss <10 weeks) and LBR, all following the first embryo transfer.

### Statistical analysis

Continuous data were presented as mean ± standard deviation and categorical data were described by number of cases and corresponding percentages. Categorical data and continuous data that did not show normal distribution were analysed by Pearson's $\chi^2$ test/Fisher's exact test or Kruskal–Wallis test as appropriate. Univariate regression analyses were performed to identify candidate factors that predicted CLBR. The candidate variables were age, BMI, number of previously failed OS attempts, use of metformin, number of mature oocytes, transfer policy and transfer stage. Variables showing a *P*-value <0.25 in the univariate analyses were included in the multivariate logistic regression model with CLBR per started cycle as the dependent variable and the PCOS phenotype as the main independent variable. All variables were simultaneously entered into the logistic regression model. The likelihood of CLBR is presented as an odds ratio (OR) with standard error (SE) and 95% confidence interval (CI). All statistical analyses were performed using Stata 13.0 (Stata Statistical Software: Release 13; StataCorp., College Station, TX, USA).

## Results

In total, 320 unique PCOS patients who had their first IVM treatment were included. Among them, 50% presented with a hyperandrogenic phenotype (n = 140 type A (HOP), n = 0 type B (HO), n = 20 type C (HP)) and 50% had a normo-androgenic PCOS phenotype (n = 160 type D (OP)).

Relevant patient parameters are presented in Table I. No differences were observed in terms of maternal age, nor in the number of failed OS cycles prior to the IVM cycle included in this study. BMI was significantly different between phenotype groups (27.4 ± 5.4 kg/m$^2$ for A/HOP, 27.1 ± 5.4 kg/m$^2$ for C/HP and 23.3 ± 4.4 kg/m$^2$ for D/OP, $P < 0.001$). Furthermore, significant differences were observed concerning the use of metformin. More specifically, 33.6% of patients with PCOS phenotype A/HOP used metformin versus 15.0% for C/HP and 11.2% for D/OP ($P < 0.001$). Antimüllerian hormone levels differed significantly between phenotype groups (12.4 ± 8.3 µg/l for A/HOP, 7.7 ± 3.1 µg/l for C/HP and 10.4 ± 5.9 µg/l for A/OP, $P = 0.01$).

The IVM cycle characteristics are shown in Table II. In accordance with the different AMH levels, significantly different numbers of COC were retrieved in the different groups: 25.9 ± 19.1 COC in patients with phenotype A/HOP versus 18.3 ± 9.0 in phenotype C/HP versus 19.8 ± 13.5 in phenotype D/OP patients ($P = 0.004$). Similarly, the number of mature oocytes following IVM differed significantly (12.4 ± 9.3 for A/HOP vs. 6.5 ± 4.2 for C/HP vs. 9.1 ± 6.9 for D/OP, $P < 0.001$), as well as the maturation rates. The fertilisation rates were comparable between groups, as was the total number of usable embryos, i.e. available for fresh transfer followed by vitrification of surplus embryos or vitrification only (in case of freeze all) (2.8 ± 2.5 for A/HOP, 1.8 ± 1.7 for C/HP, 2.5 ± 1.9 for D/OP, $P = 0.08$). In 17 patients with PCOS phenotype A/HOP (12.1%), in 5 patients with PCOS phenotype C/HP (25.0%) and in 32 patients with PCOS phenotype D/OP (20.0%), no embryo was available for transfer or vitrification ($P = 0.12$).

The cycle characteristics of the first IVM embryo transfer are presented in Table III. The embryo transfer stage (cleavage vs. blastocyst) and the transfer policy (fresh transfer vs. elective freeze-all strategy followed by FET) were equally distributed in the three groups. Single embryo transfer was performed in 106/123 (86.2%) of PCOS A/HOP patients, in 12/15 (80.0%) of PCOS C/HP patients and in 119/128

**Table I Baseline patient characteristics.**

| | PCOS A (HOP) n = 140 | PCOS C (HP) n = 20 | PCOS D (OP) n = 160 | *P*-value |
|---|---|---|---|---|
| Age (years), mean (SD) | 29.2 (3.8) | 28.8 (3.5) | 29.6 (3.5) | 0.44[a] |
| Previously failed OS cycles, (%) | | | | |
| 0 | 72.9 | 55.0 | 73.1 | |
| 1 | 9.3 | 10.0 | 6.2 | |
| ≥2 | 17.8 | 35.0 | 20.7 | 0.30[b] |
| BMI (kg/m$^2$), mean (SD) | **27.4 (5.4)** | **27.1 (5.4)** | **23.4 (4.4)** | **<0.001**[a] |
| AMH (µg/l), mean (SD) | **12.4 (8.3)** | **7.7 (3.1)** | **10.4 (5.9)** | **0.01**[a] |
| Total testosterone (µg/l), mean (SD) | **0.63 (0.23)** | **0.53 (0.18)** | **0.32 (0.13)** | **<0.001**[a] |
| SHBG (nmol/l), mean (SD) | **58.0 (42.4)** | **59.7 (40.3)** | **102.7 (57.0)** | **<0.001**[a] |
| Calculated free testosterone (ng/l), mean (SD) | **8.94 (4.05)** | **7.85 (4.03)** | **3.25 (1.58)** | **<0.001**[a] |
| Use of OCP*, n (%) | 119 (85.0) | 13 (65.0) | 137 (85.6) | 0.055[c] |
| Use of metformin, n (%) | **47 (33.6)** | **3 (15.0)** | 18 (**11.2**) | **<0.001**[c] |

[a]Kruskal–Wallis test.
[b]Fisher exact test.
[c]Pearson $\chi^2$ test.
*Short course (14-21 days) of OCP pretreatment for IVM cycle scheduling.
BMI, body mass index; AMH, anti-müllerian hormone; SHBG, sex hormone-binding globulin; OCP, oral contraceptive pill; PCOS, polycystic ovary syndrome.
Bold values are the outcomes with statistically significant differences.

**Table II IVM cycle characteristics.**

| | PCOS A (HOP) n = 140 | PCOS C (HP) n = 20 | PCOS D (OP) n = 160 | *P*-value |
|---|---|---|---|---|
| Cumulus-oocyte complexes, mean (SD) | **25.9 (19.1)** | **18.3 (9.0)** | **19.8 (13.5)** | **0.004** |
| Matured oocytes, mean (SD) | **12.4 (9.3)** | **6.5 (4.2)** | **9.1 (6.9)** | **<0.001** |
| Maturation rate, % (SD) | **49.8 (17.0)** | **34.8 (16.2)** | **48.1 (21.8)** | **0.006** |
| Fertilisation rate, % (SD) | 61.0 (23.4) | 53.6 (31.8) | 63.6 (27.2) | 0.26 |
| Total number of usable embryos, mean (SD) | 2.8 (2.5) | 1.8 (1.7) | 2.5 (1.9) | 0.08 |
| Number of cycles with no embryo available, n (%) | 17 (12.1) | 5 (25.0) | 32 (20.0) | 0.12 |

Kruskal–Wallis test. Bold values are the outcomes with statistically significant differences.

**Table III Outcome after the 1st IVM embryo transfer.**

| | PCOS A (HOP) n = 123 | PCOS C (HP) n = 15 | PCOS D (OP) n = 128 | *P*-value |
|---|---|---|---|---|
| Embryos transferred, n (%) | | | | |
| SET | 106 (86.2) | 12 (80.0) | 119 (93.0) | 0.08[a] |
| DET | 17 (13.8) | 3 (20.0) | 9 (7.0) | |
| Embryo stage, n (%) | | | | |
| Cleavage | 59 (48.0) | 10 (66.7) | 69 (53.9) | 0.32[b] |
| Blastocyst | 64 (52.0) | 5 (33.3) | 59 (46.1) | |
| Transfer policy, n (%) | | | | |
| Fresh ET | 59 (48.0) | 5 (33.3) | 50 (39.1) | 0.27[b] |
| Elective FET | 64 (52.0) | 10 (66.7) | 78 (60.9) | |
| hCG + rate, n (%) | 55 (44.7) | 6 (40.0) | 47 (36.7) | 0.43[b] |
| Early pregnancy loss, n (%)= (biochemical losses + clinical miscarriages) per embryo transfer | **24 (19.5)** | **4 (26.7)** | **13 (10.2)** | **0.04**[a] |
| Ongoing pregnancy rate, n (%) | 31 (25.2) | 2 (12.5) | 34 (26.6) | 0.56[a] |
| Live birth rate, n (%) | 31 (25.2) | 1 (6.2) | 34 (26.6) | 0.22[a] |

[a]Fisher exact test.
[b]Pearson $\chi^2$ test.
SET, single embryo transfer; DET, double embryo transfer; FET, frozen embryo transfer; ET, embryo transfer.
Bold values are the outcomes with statistically significant differences.

(93.0%) of PCOS D/OP patients ($P = 0.08$). When evaluating unadjusted clinical outcomes following the first IVM embryo transfer, biochemical pregnancy rate, ongoing pregnancy rate and LBR were comparable between the three PCOS phenotype groups (Table III), although the incidence of early pregnancy loss was significantly different (19.5% (24/123) for A/HOP, 26.7% (4/15) for C/HP and 10.2% (13/128) for D/OP, $P = 0.04$). Unadjusted CLBRs calculated per started IVM cycle were 40.0% (56/140) for A/HOP, 15.0% (3/20) for C/HP and 33.1% (53/160) for D/OP (*P*-value = 0.07; Table IV). Crude data separated for fresh versus elective FET can be retrieved in Supplementary Table SI.

### Univariate and multivariate regression analysis for CLBR

Univariate logistic regression analysis showed a significant association between CLBR and BMI, number of previously failed OS cycles, use of metformin, number of matured oocytes, transfer policy and embryo stage (Supplementary Table SII). The multivariate logistic regression model, taking into consideration the above-mentioned confounders, demonstrated that the PCOS phenotype was an independent predictive factor of CLBR in patients treated with IVM (with A/HOP considered as the reference group (OR = 1), phenotype C/HP had an OR of 0.26 (CI 0.06–1.05) and phenotype D/OP had an OR of 0.47 (CI 0.25–0.88), $P = 0.03$). Alongside the PCOS phenotype, BMI was identified as a negative predictor of CLBR (OR 0.88, CI 0.83 – 0.94, $P \leq 0.001$) (Table V).

## Discussion

To our best knowledge, this is the first study investigating whether success rates of IVM may differ among categories of patients with different PCOS phenotypes. According to our results, PCOS phenotype

**Table IV Cumulative IVM treatment outcome.**

| | PCOS A (HOP) n = 140 | PCOS C (HP) n = 20 | PCOS D (OP) n = 160 | *P*-value |
|---|---|---|---|---|
| Cumulative live birth rate per started cycle, n (%) | 56 (40.0) | 3 (15.0) | 53 (33.1) | 0.07 |

Pearson $\chi^2$ test.

**Table V Multivariate logistic regression analysis for the cumulative live birth rate per started cycle.**

| | OR (SE) | 95% CI | *P*-value |
|---|---|---|---|
| PCOS phenotype | | | |
| A (HOP) (ref) | **1** | – | |
| C (HP) | **0.26 (0.18)** | **0.06 – 1.05** | |
| D (OP) | **0.47 (0.15)** | **0.25 – 0.88** | **0.03** |
| Previously failed OS cycles | | | |
| 0 (ref) | 1 | – | |
| 1 | 0.30 (0.18) | 0.09 – 0.97 | |
| ≥2 | 0.66 (0.24) | 0.33 – 1.35 | 0.09 |
| BMI | **0.88 (0.03)** | **0.82 – 0.93** | **0.001** |
| Use of metformin | | | |
| No (ref) | 1 | – | |
| Yes | 0.64 (0.24) | 0.31 – 1.32 | 0.23 |
| Number of matured oocytes | 1.01 (0.02) | 0.98 – 1.05 | 0.44 |
| Transfer policy | | | |
| Fresh ET (ref) | 1 | – | |
| Elective FET | 0.51 (0.24) | 0.21 – 1.27 | 0.15 |
| Transfer stage | | | |
| Cleavage (ref) | 1 | – | |
| Blastocyst | 0.87 (0.21) | 0.54 – 1.39 | 0.56 |

Bold values are the outcomes with statistically significant differences.

A/HOP seems to confer the highest CLBR within the IVM programme. This observation is in strong contrast with what has been described in previous studies of PCOS patients who underwent OS, where hyperandrogenic PCOS phenotypes (A/HOP and C/HP) were associated with worse CLBR (Ramezanali *et al.*, 2016; De Vos *et al.*, 2018).

Since the establishment of the diagnostic Rotterdam criteria for PCOS in 2003, most studies investigating outcomes of reproductive treatment have considered patients with PCOS as one entire group, although there is growing evidence suggesting that PCOS is a heterogenous condition (Doherty *et al.*, 2015). Therefore, in order to be able to assess the efficiency of reproductive treatment approaches in PCOS patients, it is of paramount importance to precisely evaluate the specific PCOS phenotypes. Nevertheless, studies that have reported pregnancy rates after standard IVM (Walls *et al.*, 2015) and novel biphasic IVM systems (Vuong *et al.*, 2020) have not described the phenotypic status of patients in sufficient detail.

Our finding may seem to contradict those of previous studies that suggested that hyperandrogenism may hinder oocyte/embryo quality (Lebbe and Woodruff, 2013) and/or endometrial receptivity (Gonzalez *et al.*, 2012; Rosas *et al.*, 2016). As a potential explanation for our findings, we hypothesise that IVM of oocytes in a patient with the most severe PCOS phenotype (A/HOP) may bypass the negative impact from OS on parameters that have been associated with success after OS, such as ovarian response, oocyte quality and endometrial receptivity. Alternatively, we hypothesise that the rather unexpected higher CLBR in PCOS patients with phenotype A/HOP following IVM might be linked to the significantly different number of COC retrieved ($P = 0.004$) which is reflected in a different number of matured oocytes ($P < 0.001$), although this was not confirmed by the multivariate logistic regression model (Table V; OR for number of mature oocytes 1.01, CI 0.98–1.05, $P = 0.44$). The currently available standard IVM systems require sufficient numbers of COC in order to compensate for the lower maturation rate and lower developmental potential of oocytes as compared to conventional OS and ART (Guzman *et al.*, 2013). Although the number of available embryos was not significantly different, we observed a trend towards higher number of available embryos in the phenotype A/HOP subgroup (Table II; $P = 0.08$), which may be clinically relevant. Although our data deserve further scrutiny in a future clinical trial with a prospective design, the observation that the PCOS phenotype A/HOP confers the highest CLBR after IVM compared to phenotypes C/HP and D/OP, despite this group has a higher BMI (Table I) negatively impacting the outcome (Table V), leads us to suggest that standard IVM may be a more suitable approach in patients with the most severe form of PCOS who require ART. While standard IVM has generally been reported to convey inferior reproductive outcomes in comparison to OS (Walls *et al.*, 2015; Ho *et al.*, 2019), a comparison with our own historical research data in patients with PCOS phenotype A/HOP seems to suggest that IVM may be considered as a first-line treatment for this specific patient population. Indeed, the CLBR of 40% per started IVM cycle in the study presented here is higher compared the CLBR per started OS cycle (25.8% for phenotype A/HOP (De Vos *et al.*, 2018)). However, it is possible that OS protocols in patients reported in our previous OS study (De Vos *et al.*, 2018) were suboptimal. Indeed, ovarian response in the hyperandrogenic subgroups in that study was rather modest, which seems to suggest that a higher BMI was not compensated by a higher daily dose of gonadotrophins. This can be explained by the fact that the OS cycles analysed in De Vos *et al.* (2018) had been performed before the more widespread adoption of the elective freeze-all policy in predicted high responders in order to avoid OHSS.

While this study has a large total sample size and included unique PCOS patients in their first IVM cycle, there are several important limitations to consider. First of all, our data may be subject to possible unmeasured confounding factors because of the retrospective nature of the study. Also, assignment of the PCOS phenotypes was not

consistently performed in the out-patient clinic. For patients whose PCOS phenotype was not evaluated at intake, we had to scrutinise the medical records in order to retrospectively assign the PCOS phenotype, which may hold risk for misassignment. Furthermore, given the relatively low prevalence of the milder hyperandrogenic phenotype C/HP compared to the 'full' phenotype A/HOP in our population and in PCOS populations studied by others in our geographical region (Fraissinet *et al.*, 2017), phenotype C/HP remained underrepresented in our study (20/320 patients), and the net difference in outcome for this group requires further investigation. Finally, the heterogeneity in embryo transfer protocols in terms of fresh and frozen, as well as cleavage and blastocyst embryo transfer renders the current data difficult to interpret in an overall manner. An essential endeavour to provide a more robust answer on which ART treatment holds the best chances for success in specific PCOS phenotype patient groups should be a prospective comparison of IVM using the currently most effective IVM culture system (Sanchez *et al.*, 2019) versus OS, followed by elective vitrification (Teede *et al.*, 2018) of good-quality blastocysts (Walls *et al.*, 2015) in a randomised-controlled trial design. The answers provided by such a trial would be of tremendous value to the current daily practice of ART in PCOS patients.

In conclusion, our current findings further stress the importance of keeping track of the phenotypic features when selecting a suitable ART treatment in PCOS patients and confirm that proper patient selection is the cornerstone of successful IVM treatment. PCOS phenotype A/HOP patients requiring ART treatment can be reassured that, although they may have a lower CLBR following OS, they may perform better compared to the other phenotypes with current IVM programmes.

## Supplementary data

Supplementary data are available at *Human Reproduction* online.

## Authors' roles

S.M. and M.D.V. were responsible for the concept and study design. S.P. and L.M. performed the data collection. P.D. and S.S.R. performed the statistical analyses. S.M., S.P. and M.D.V. drafted the manuscript. All authors contributed to the interpretation, discussion and editing of the manuscript. All authors approved the last version.

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## Conflict of interest

None of the authors have a conflict of interest to declare pertaining to this study.

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