**In vitro maturation is associated with increased early embryo arrest without impairing morphokinetic development of useable embryos progressing to blastocysts**

M.L. Walls\(^1,2,\*)\(^,\) J.P. Ryan\(^1,2\), J.A. Keelan\(^2\), and R. Hart\(^1,2\)

\(^1\)Fertility Specialists of Western Australia, Bethesda Hospital, 25 Queenslea Drive, Claremont, Perth, WA 6010, Australia \(^2\)School of Women’s and Infant’s Health, University of Western Australia, King Edward Memorial Hospital, 374 Bagot Road, Subiaco, Perth, WA 6008, Australia

\(^\*)\)Correspondence address. Tel: +61-4-18-92-13-80; E-mail: melanie@fertilitywa.com.au

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**STUDY QUESTIONS:** Does polycystic ovarian syndrome (PCOS) or in vitro maturation (IVM) treatment affect embryo development events and morphokinetic parameters after time-lapse incubation?

**SUMMARY ANSWER:** There was an increase in some abnormal phenotypic events in PCOS-IVM embryos as well as an increase in early arrest of PCOS-IVM and PCOS-ICSI embryos; however, IVM treatment or PCOS status did not alter morphokinetic development of embryos suitable for transfer of vitrification.

**WHAT IS KNOWN ALREADY:** IVM has been less successful than standard IVF in terms of clinical pregnancy, implantation and live birth rates. There is currently no information available about the development of IVM embryos according to time-lapse analysis.

**STUDY DESIGN, SIZE AND DURATION:** This article represents a prospective case–control study. The study involved 93 participants who underwent 93 treatment cycles. Cycles were completed between January 2013 and July 2014.

**PARTICIPANTS/MATERIALS, SETTING AND METHODS:** Participants were recruited for the study at Fertility Specialists of WA and Fertility Specialists South, Perth, Western Australia. Of the PCOS diagnosed patients, 32 underwent IVM treatment (PCOS-IVM) and 23 had standard ICSI treatment (PCOS-ICSI). There were 38 patients without PCOS who underwent standard ICSI treatment comprising the control group (control-ICSI).

**MAIN RESULTS AND THE ROLE OF CHANCE:** The PCOS-IVM group showed significantly more embryos with multinucleated two cells (\(P = 0.041\)), multinucleated four cells (\(P = 0.001\)) and uneven two cells (\(P = 0.033\)) compared with the control-ICSI group, but not the PCOS-ICSI group. There were no significant differences in the rates of any abnormal events between the PCOS-ICSI and control-ICSI groups. Embryo arrest between Days 2 and 3 was higher in the PCOS-IVM and PCOS-ICSI groups compared with the control-ICSI group (\(P < 0.001\) and \(P = 0.001\)). Embryo arrest from Days 3 to 4 was higher in the PCOS-IVM group compared with both the PCOS-ICSI and control-ICSI groups (\(P < 0.001\)). There were no differences in embryo arrest rates across all three groups at the compaction or blastulation stages. Cumulative rates of embryo arrest, from the time to second polar body extrusion (tPB2) to the time to formation of a blastocyst (tB), result in a decreased proportion of useable PCOS-IVM blastocysts compared with the other two treatment groups; however, of the embryos remaining, there was no significant difference in morphokinetic development between the three groups.

**LIMITATIONS AND REASONS FOR CAUTION:** This was a small study using time-lapse analysis of embryo development as the primary end-point. Larger, randomized, clinical trials are required to clarify the implications of time-lapse incubation of IVM embryos and the effects on implantation and ongoing pregnancy.

**WIDER IMPLICATIONS OF THE FINDINGS:** This is the first study to compare the time-lapse analysis of IVM with standard ICSI for patients with and without PCOS. This allows for a more detailed and specific timeline of events from embryos generated using this approach.
for patients diagnosed with PCOS and shows that embryos generated from IVM have an increased rate of early embryo arrest, however, morphokinetic development is not impaired in embryos that progress to the usable blastocyst stage.

**STUDY FUNDING/COMPETING INTEREST(S):** The study was supported by the Women’s and Infant’s Research Foundation of Western Australia. R.H. is the Medical Director of Fertility Specialists of Western Australia and a shareholder in Western IVF. He has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals. The other authors have no competing interests.

**Key words:** IVM / ICSI / PCOS / morphokinetics

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**Introduction**

Morphokinetics is the study of embryo cleavage kinetics according to the annotation of time-lapse sequences (Meseguer et al., 2011; Montag et al., 2011). The recent availability and uptake of time-lapse embryo microscopy/incubation systems have enabled the identification of specific morphokinetic time points and development events associated with embryo viability and arrest. Traditional morphological grading systems are unable to detect abnormal phenotypic events and lack the ability to precisely define specific events. Time-lapse incubation technology facilitates the identification of aspects of embryo development such as early cleavage or abnormal phenotypic events, which have either a negative or positive impact on embryo development. These can be used as an embryo selection or deselection tool after incorporation into prediction models that have been associated with an increase in successful reproductive outcomes (Rubio et al., 2014). Morphokinetic analysis is now also being used as an outcome variable to compare results from different patient groups (Wissing et al., 2014) and assess environmental contributors to infertility (Fréour et al., 2013). These methods could also be employed to compare the effects of different treatments across patient groups.

Polycystic ovarian syndrome (PCOS) is a significant cause of infertility for patients seeking assisted reproductive procedures and occurs in ~4–12% of the general population (Costello et al., 2012). A range of treatment options including administration of gonadotrophin-releasing hormone (GnRH) antagonists (Kolibianakis et al., 2006; Al-Inany Hesham et al., 2011), metformin (Costello et al., 2006) and dopamine agonists (Cabergoline) (Tang et al., 2012); ‘freeze all’ precautions are recommended for these patients to minimize the risk of ovarian hyperstimulation syndrome (OHSS) or to eliminate the risk completely by using in vitro maturation (IVM) (Lindenberg, 2013; Walls et al., 2014). A recent morphokinetic analysis comparing hyperandrogenic PCOS, normoandrogenic PCOS and non-PCOS patients (controls) found that the differences in embryo developmental timings were primarily associated with hyperandrogenemia (Wissing et al., 2014).

Embryo development kinetics may be influenced by a variety of culture conditions such as oxygen concentration (Kirkegaard et al., 2013); these may result in differences in embryo development characteristics between laboratories. There is currently no literature that reports embryo cleavage kinetics or the rates of abnormal phenotypic events following IVM treatment. We hypothesized that in embryos developing to the blastocyst stage, the morphokinetic time points and/or occurrence of abnormal early cleavage/phenotypic events of PCOS-IVM versus standard intra cytoplasmic sperm injection (PCOS-ICSI) embryos would be similar, whereas differences in morphokinetics between embryos from patients with or without PCOS would be more significant.

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**Materials and Methods**

**Patient diagnosis and recruitment**

Participants were recruited for the study at Fertility Specialists of WA and Fertility Specialists South, Perth, Western Australia between January 2013 and July 2014. All PCOS patients, defined according to the Rotterdam criteria (Rotterdam, 2004), who were referred by their clinician for IVM or standard IVF with ICSI treatment were assessed for inclusion into the study. Patients were deemed eligible for inclusion if the following criteria were met: patients about to embark on their first cycle of IVM or ICSI treatment who were under 37 years of age at the initiation of their treatment cycle and who had a body mass index (BMI) of <35 kg/m². Patients were then attributed to one of three groups: IVM (PCOS-IVM), ICSI patients diagnosed with PCOS (PCOS-ICSI) and age equivalent, non-PCOS-ICSI patients were recruited as the control group (control-ICSI).

**Stimulation, oocyte collection and endometrial preparation**

The protocol for PCOS-IVM or PCOS-ICSI stimulation, oocyte collection and endometrial preparation has been previously described (Walls et al., 2014). Briefly, the PCOS-IVM patients received 3–5 days of recombinant follicle-stimulating hormone (rFSH) priming without a human chorionic gonadotrophin (hCG) trigger, and immature oocytes were cultured for 24 h in G-2 Plus culture medium (Vitrolife, Sweden), supplemented with 10% maternal serum, 0.1 IU/mL rFSH (Puregon, Merck Sharp and Dohme, South Granville, NSW, Australia) and 0.5 IU/mL hCG (Pregnyl, Merck Sharp and Dohme, South Granville, NSW, Australia). In the PCOS-ICSI group, 21 patients underwent a GnRH antagonist treatment cycle and 2 underwent a GnRH agonist protocol. In the control-ICSI group, 35 patients underwent a GnRH antagonist treatment cycle and 3 underwent a GnRH agonist protocol. Mature oocytes were collected 36 h after ovulation trigger.

**Fertilization and embryo culture**

For patients in all treatment groups, insemination was performed using ICSI in G-IVF medium (Vitrolife, Sweden). After the ICSI procedure, all oocytes were placed individually in a culture slide (Unisense Fertilitech, Vitrolife, Sweden) containing pre-equilibrated G-1 media (Vitrolife, Sweden) covered with sterile mineral oil and cultured in the Embryoscope time-lapse incubation system (Unisense Fertilitech, Vitrolife, Sweden) in an atmosphere of 5.0% O₂, 6.0% CO₂ and 89% N₂. Media change was performed on the third day of culture, whereby embryos were moved into pre-equilibrated G2Plus Media (Vitrolife, Sweden), reinserted into the incubator and cultured continuously until transfer or vitrification.

**Time-lapse analysis**

For each embryo, images were taken in seven focal planes, every 10 min after insemination and insertion into the incubator. Only embryos displaying normal fertilization (presence of two pronuclei) were included in the study.
Embryo transfer or vitrification was performed at the blastocyst stage on Day 5 or 6 depending on embryo development, after which time the embryo was removed from the incubator. Embryoviewer software (Unisense Fertitech, Vitrolife, Sweden) was used to analyse all images. All annotations were performed by one individual (MW) to avoid user bias and were recorded in the first image frame at which they occurred. Annotations included the extrusion of the second polar body (tPB2), the appearance of two pronuclei (tPNa), the disappearance of the two pronuclei (tPNf), cell cleavage time points two cells (t2), three cells (t3), four cells (t4), five cells (t5), six cells (t6), seven cells (t7), eight cells (t8) and nine cells (t9+), time to formation of a morula (tM), start of blastulation (tSB) and the formation of a blastocyst (tB). Cell cycle durations were calculated for the second cell cycle, cc2 (t3–t2), third cell cycle, cc3 (t5–t3), ss2: synchrony of the second cell cycle (t4–t3) and ss3: synchrony of the third cell cycle (t8–t5). Additionally, the following abnormal cleavage and phenotypic events were recorded: irregular division, no division, direct cleavage from one to three cells, other direct cleavage and reverse cleavage.

Analysis of embryo arrest
Embryos were grouped into five stages of development according to the morphokinetic time point at which they were arrested (Fig. 1). These groups included the following morphokinetic events: first cytokinesis (Days 0–2) tPB2, tPNa, tPNf and t2; second cytokinesis (Days 2 and 3) t3 and t4; third cytokinesis (Days 3 and 4) t5, t6, t7 and t8; compaction (Days 4 and 5) t9+ and tM; and blastulation (Days 5 and 6) tSB and tB.

Statistics
All statistical analysis was performed using the statistical package SPSS version 20 (SPSS, Inc., Chicago, USA). Patient demographics and embryology outcomes were analysed using ANOVA procedures. Analysis of the rates of abnormal phenotypic and early embryo cleavage events as well as rates of embryo arrest were tested using generalized estimating equations (GEE) for binomial data to include an adjustment for repeated measures as each patient contributed multiple embryos. This was also true for analysis of the morphokinetic time points of useable embryos using generalized linear mixed modelling (GLMM) to account for the interaction effect of patient ID and treatment group. All tests were two tailed, and P-values <0.05 were considered statistically significant.

Ethics approval
This study received ethics approval from the University of Western Australia Human Research Ethics Committee (reference: RA/4/1/5629) and the Western Australia Reproductive Technology Council (RTC) committee in line with the guidelines established at Fertility Specialists of Western Australia (FSWA). All patients gave written, informed consent.

Results
Patient demographics and embryology outcomes are displayed in Table I. There were no significant differences in the mean age or BMI between patients in different treatment groups. The PCOS-IVM group had significantly higher mean serum anti-Mullerian hormone (AMH) levels (P = 0.15, P < 0.001), fewer days of stimulation required (P < 0.001, P = 0.037), lower mean consumption of gonadotrophins (P < 0.001) and lower peak serum estradiol concentrations (P < 0.001) than either the PCOS-ICSI or control-ICSI group, respectively. Additionally, the PCOS-ICSI group had a significantly higher mean serum AMH level (P < 0.001) and fewer days of stimulation required (P = 0.037) than the control-ICSI group. The PCOS-ICSI group had a significantly higher incidence of moderate OHSS (P < 0.001) compared with the PCOS-IVM and control-ICSI groups (Table I). The PCOS-ICSI group also had four cases of mild OHSS, and the control-ICSI group had one case of mild OHSS that required a precautionary freeze all embryo protocol. The PCOS-IVM group had no cases of mild OHSS, and there were no cases of severe OHSS in any of the treatment groups.

The PCOS-IVM group had significantly greater mean oocytes collected per patient than the control-ICSI group (P = 0.031), but not the PCOS-ICSI group (Table I). There were no differences in the mean number of mature or normally fertilized oocytes per patient between any of the treatment groups (Table I). However, the PCOS-IVM group had significantly fewer useable (P = 0.045) and total blastocysts (P = 0.029) per patient compared with the PCOS-ICSI group. Overall, the PCOS-IVM group had significantly fewer oocytes mature (P < 0.001), fertilize normally (P = 0.024), useable blastocysts (P < 0.001) and total blastocysts (P < 0.001) compared with both the PCOS-ICSI and control-ICSI groups.

There were no differences between groups in the rates of direct cleavage of one to three, or three to five cells or reverse cleavage (Table II). However, the PCOS-IVM group showed significantly more cases of multinucleated two cells (P = 0.041), multinucleated four cells (P = 0.001) and uneven two cells (P = 0.033) compared with the control-ICSI group, but not the PCOS-ICSI group. There were no significant differences in the rates of any abnormal events between the PCOS-ICSI and control-ICSI groups.
The data in Table III show that there were no significant differences in the incidence of embryo arrest rates across the three groups from fertilization to Day 2 during the first cytokinesis. The PCOS-IVM and PCOS-ICSI groups showed significantly higher rates of embryo arrest between Days 2 and 3, during the second cytokinesis, compared with the control-ICSI group \((P < 0.001)\) and \(P = 0.001\). The PCOS-IVM group also showed significantly higher rates of embryo arrest at the third cytokinesis stage from Days 3 to 4, compared with both other treatment groups \((P < 0.001)\). There was no difference in the number of embryos arresting at the third cytokinesis stage between the PCOS-ICSI and the control-ICSI groups, and there was no difference in embryo arrest rates across all three groups at the compaction or blastulation stages. Figure 2 shows the cumulative rate of embryo arrest, from time \(tPB2\) to time \(tB\), demonstrating an overall reduction in the proportion.

### Table I  Patient demographics and embryology outcomes.

<table>
<thead>
<tr>
<th></th>
<th>PCOS-IVM, (n = 32)</th>
<th>PCOS-ICSI, (n = 23)</th>
<th>Control-ICSI, (n = 38)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) ((95% \text{ CI}))</td>
<td>31.9 ((30.9—32.9))</td>
<td>32.0 ((30.4—33.7))</td>
<td>32.9 ((31.2—33.8))</td>
<td>NS</td>
</tr>
<tr>
<td>BMI ((95% \text{ CI}))</td>
<td>27.2 ((20.8—33.6))</td>
<td>22.9 ((21.6—24.3))</td>
<td>23.6 ((22.5—24.8))</td>
<td>NS</td>
</tr>
<tr>
<td>AMH ((\text{pmol/L})) ((95% \text{ CI}))</td>
<td>75.5 ((61—90.1))</td>
<td>50.1 ((39.4—60.8))</td>
<td>18.9 ((15.6—22.1))</td>
<td>(0.015^a)</td>
</tr>
<tr>
<td>Duration of stimulation ((\text{days})) ((95% \text{ CI}))</td>
<td>5.3 ((4.5—6))</td>
<td>11 ((9.9—12.1))</td>
<td>9.9 ((9.3—10.4))</td>
<td>(&lt;0.001^b)</td>
</tr>
<tr>
<td>Mean gonadotrophin intake ((\text{IU})) ((95% \text{ CI}))</td>
<td>697 ((605—789))</td>
<td>1535 ((1250—1820))</td>
<td>1892 ((1600—2184))</td>
<td>(&lt;0.001^b)</td>
</tr>
<tr>
<td>Mean peak estradiol ((\text{E2})) ((\text{pmol/L})) ((95% \text{ CI}))</td>
<td>1144 ((857—1432))</td>
<td>6528 ((4841—8215))</td>
<td>5860 ((5027—6694))</td>
<td>(&lt;0.001^b)</td>
</tr>
<tr>
<td>Moderate OHSS, (n (%))</td>
<td>0 ((0))</td>
<td>4 ((17.4))</td>
<td>0 ((0))</td>
<td>(&lt;0.001^b)</td>
</tr>
<tr>
<td>Number of oocytes collected</td>
<td>498</td>
<td>338</td>
<td>438</td>
<td></td>
</tr>
<tr>
<td>Mean (p/p)</td>
<td>15.6 ((13—18))</td>
<td>14.7 ((11—18))</td>
<td>11.5 ((10—13))</td>
<td>(0.03^a)</td>
</tr>
<tr>
<td>Number of oocytes matured/inseminated</td>
<td>308</td>
<td>265</td>
<td>351</td>
<td></td>
</tr>
<tr>
<td>Mean (p/p)</td>
<td>9.6 ((8—11))</td>
<td>11.5 ((9—14))</td>
<td>9.2 ((8—10))</td>
<td>NS</td>
</tr>
<tr>
<td>Number of oocytes normally fertilized</td>
<td>195 ((63.3))</td>
<td>195 ((73.6))</td>
<td>246 ((70.1))</td>
<td>(0.02^b)</td>
</tr>
<tr>
<td>Mean (p/p)</td>
<td>6.1 ((5—8))</td>
<td>8.5 ((6—11))</td>
<td>6.5 ((6—7))</td>
<td>NS</td>
</tr>
<tr>
<td>Number of useable blastocysts developed</td>
<td>76 ((39))</td>
<td>110 ((56.4))</td>
<td>137 ((55.7))</td>
<td>(&lt;0.001^b)</td>
</tr>
<tr>
<td>Mean (p/p)</td>
<td>2.4 ((1—3))</td>
<td>4.8 ((3—7))</td>
<td>3.6 ((3—4))</td>
<td>(0.045^a)</td>
</tr>
<tr>
<td>Total number of blastocysts developed</td>
<td>94 ((48.2))</td>
<td>132 ((67.7))</td>
<td>167 ((67.9))</td>
<td>(&lt;0.001^b)</td>
</tr>
<tr>
<td>Mean (p/p)</td>
<td>2.9 ((2—4))</td>
<td>5.7 ((4—8))</td>
<td>4.4 ((4—5))</td>
<td>(0.029^a)</td>
</tr>
</tbody>
</table>

Values represent the mean per patient \((p/p)\) \((95\% \text{ confidence interval})\) and total \(\%\) \((across all patients)\). Differences were calculated using ANOVA. Superscript letters represent significant differences \((correlating to \(P\)-value)\). NS represents \(P > 0.05\). AMH, anti-mullerian hormone; BMI, body mass index; OHSS, ovarian hyperstimulation syndrome.

### Table II  The frequency of abnormal early cleavage events (all embryos).

<table>
<thead>
<tr>
<th>Abnormal event</th>
<th>PCOS-IVM = 194</th>
<th>PCOS-ICSI = 183</th>
<th>Control-ICSI = 243</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct cleavage 1–3 cells (%)</td>
<td>57 ((29))</td>
<td>35 ((19))</td>
<td>55 ((23))</td>
<td>NS</td>
</tr>
<tr>
<td>Direct cleavage 3–5 cells (%)</td>
<td>30 ((15))</td>
<td>31 ((17))</td>
<td>32 ((13))</td>
<td>NS</td>
</tr>
<tr>
<td>Multinucleated two cells (%)</td>
<td>62 ((32)^a)</td>
<td>35 ((19))</td>
<td>48 ((20)^a)</td>
<td>(0.041^a)</td>
</tr>
<tr>
<td>Multinucleated four cells (%)</td>
<td>49 ((26)^a)</td>
<td>28 ((15))</td>
<td>28 ((12)^a)</td>
<td>(0.001^a)</td>
</tr>
<tr>
<td>Uneven two cells (%)</td>
<td>36 ((19)^a)</td>
<td>24 ((13))</td>
<td>24 ((10)^a)</td>
<td>(0.033^a)</td>
</tr>
<tr>
<td>Reverse cleavage (%)</td>
<td>4 ((2))</td>
<td>3 ((2))</td>
<td>11 ((5))</td>
<td>NS</td>
</tr>
<tr>
<td>No division (%)</td>
<td>20 ((10))</td>
<td>26 ((14))</td>
<td>32 ((13))</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data represent the number of events and the incidence as \(\%\) \((total embryos)\). Differences in rates were calculated using GEE for binomial data with an adjustment for multiple comparisons. Superscript letters represent significant differences \((correlating to \(P\)-value)\). NS represents \(P > 0.05\).
of useable PCOS-IVM blastocysts compared with both other treatment
groups.

After analysing the data from only those top/good quality blastocysts, which were deemed suitable for transfer or vitrification, we found that there were no significant difference in embryo development between any of the groups according to the morphokinetic time points at which specific events occurred (Supplementary Table S1). Poor-quality blastocysts, which were unsuitable for transfer or vitrification, were not included in this analysis. Additionally, there were no significant differences between any of the groups in the timing of the second or third cell cycles, synchrony of the second or third cell cycles, or the time for completion of blastulation. Combined fresh and frozen embryo transfers resulted in 15 clinical pregnancies from 43 embryo transfers in the PCOS-IVM group (35%), 16 clinical pregnancies from 38 embryo transfers in the PCOS-ICSI group (42%) and 28 clinical pregnancies from 53 embryo transfers in the control-ICSI group (53%).

Discussion

The emergence of time-lapse systems for embryo culture has revolutionized the ART laboratory as embryos can now be monitored at all times throughout their development. The main focus for this technology has been to develop embryo selection models (Wong et al., 2010; Meseguer et al., 2011; Campbell et al., 2013). However, time-lapse incubation can provide other benefits to IVF culture and research to potentially increase success. Morphokinetic analysis has been used to assess patient groups of normal weight and obese infertile patients (Bellver et al., 2013) and determine the impacts of PCOS on morphokinetic development (Wissing et al., 2014). The technology has also been used to assess stimulation protocols (Gryshchenko et al., 2014) as well as culture conditions such as oxygen concentration (Kirkegaard et al., 2013) and culture media (Desai et al., 2014). In this study, we have incorporated the use of morphokinetics as an outcome variable to compare both patient groups and treatment types.

The PCOS-IVM treatment group showed a significantly shorter duration of stimulation, less total gonadotrophin consumption and lower mean peak serum estradiol levels, compared with both other treatment groups. Additionally, there were no cases of moderate OHSS in the PCOS-IVM and control-ICSI groups, which was significantly lower than the PCOS-ICSI group (P < 0.001). These results are the same as previously reported in a retrospective analysis by our group (Walls et al., 2014) and prospectively confirms IVM treatment as a more patient-friendly treatment, in terms of stimulation. OHSS remains a significant adverse outcome of standard IVF treatment, which can result in a range of adverse outcomes from mild abdominal discomfort to significant morbidity and even mortality in rare and extreme cases (Delvigne and Rozenberg, 2002). IVM remains the only treatment method to completely eliminate this risk (Lindenberg, 2013). While we did not have a case of OHSS in this study, some embryological outcomes in the PCOS-IVM group were less successful than the PCOS-ICSI and control-ICSI groups, and by utilizing time-lapse incubation, we were able to monitor individual embryo development more closely.

Total maturation, fertilization and blastocyst development were significantly lower in the PCOS-IVM group compared with the other two treatment groups. Blastocyst development rates after time-lapse incubation vary considerably in the literature, and it has suggested that time-lapse systems, such as the embryoscope, may be contributing to improved development (Meseguer et al., 2012; Rubio et al., 2014). A large, multicentre, randomized control trial recently reported 20.9% optimal and 52.3% total blastocyst formation (Rubio et al., 2014), and

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**Table III Percentage incidence of embryo arrest according to embryo development events.**

<table>
<thead>
<tr>
<th>Cell stage</th>
<th>PCOS-IVM (N)</th>
<th>PCOS-ICSI (N)</th>
<th>Control-ICSI (N)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First cytokinesis (Days 0–2) (%)</td>
<td>8/195 (4.1)</td>
<td>7/195 (3.6)</td>
<td>3/246 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Second cytokinesis (Days 2 and 3) (%)</td>
<td>11/187 (5.9)a</td>
<td>8/188 (4.3)b</td>
<td>0/243 (0)ab</td>
<td>&lt;0.001a, 0.001b</td>
</tr>
<tr>
<td>Third cytokinesis (Days 3 and 4) (%)</td>
<td>48/176 (27.3)a,b</td>
<td>16/180 (8.9)a</td>
<td>21/243 (8.6)b</td>
<td>&lt;0.001a, 0.001b</td>
</tr>
<tr>
<td>Compaction (Days 4 and 5) (%)</td>
<td>15/128 (11.7)</td>
<td>9/164 (5.5)</td>
<td>20/222 (9.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Blastulation (Days 5 and 6) (%)</td>
<td>19/113 (16.8)</td>
<td>23/155 (14.8)</td>
<td>34/202 (16.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data represent the number of events and the incidence as % of total embryos. P-values were obtained from GEE for binomial data with an adjustment for multiple comparisons. Superscript letters represent significant differences (correlating to P-value). NS represents P > 0.05.
Wissing et al. (2014) reported total blastocyst development at 34–35% for their PCOS patients and 43% for their controls. In this study, useable and total blastocyst development in PCOS-IVM embryos is comparatively high (39 and 48.2%), while still being significantly lower than the PCOS-ICSI (56.4 and 67.7%) and control-ICSI (55.7 and 67.9%) groups. Therefore, while blastocyst development rates were acceptable in the PCOS-IVM group, this treatment group was less successful in terms of embryological outcomes. The cause of this observation is unclear but may be related to the inadequate cytoplasmic maturation of a proportion of the PCOS-IVM oocytes. Alternatively, it may be attributed to deficiencies in the treatment regime or may be associated with inherent poorer quality of oocytes that otherwise would not have been collected as part of a standard IVF/ICSI treatment regime. Furthermore, our data demonstrate significantly higher levels of AMH and BMI in the PCOS-IVM group in comparison with the PCOS-ICSI group, suggesting the possibility that a more pronounced PCOS condition may be affecting embryonic outcomes rather than the IVM procedure itself.

We also found no difference in the rates of any abnormal events between the PCOS-ICSI and the control-ICSI groups. Wissing and colleagues did not include an analysis of any abnormal events, and so as far as we are aware it is the first time this has been reported for this patient group and also for PCOS-IVM. The literature is still limited on the diagnostic potential of these events, as they are not readily monitored using traditional incubation systems, and most are missed altogether when applying standard morphological assessment. A recent characterization of atypical embryo phenotypes showed that some events occur in up to 30% of embryos (Athayde Wirka et al., 2014). In our study, we found that PCOS-IVM embryos showed significantly higher rates of uneven two cells as well as multinucleation at the two and four cell stages, compared with the control group. Some studies not utilizing time-lapse incubation have suggested that multinucleation may contribute to embryo arrest (Van Royen et al., 2003; Ambroggio et al., 2011). This evidence may indicate that higher rates of these events in PCOS-IVM embryos are contributing to an increase in embryo arrest. Further research into the exact implications and diagnostic significance of these events is necessary to provide additional clarity.

Embryo arrest occurs at an increased rate in the PCOS-IVM and the PCOS-ICSI groups compared with the control-ICSI group during the second cytokinesis or the Day 2–3 stage. Additionally, PCOS-IVM embryos have a much higher rate of embryo arrest during the third cytokinesis compared with both other treatment groups, indicating that the PCOS-IVM procedure is impacting on development at the Day 3–4 stage. The early stages of embryo development are driven by the maternal genome, and embryonic genome activation (EGA) is essential for mammalian embryo development (Latham, 1999). A proposed model for human embryo development based on time-lapse observations suggested that degradation of maternal RNAs begins after fertilization, with transition to EGA beginning towards the end of the second cytokinesis and dominating during the third (Wong et al., 2010). Therefore, in this context, PCOS embryos may have a reduced capacity to complete earlier stages of development compared with non-PCOS embryos, and IVM treatment is impacting on embryonic development during the stages of transition and completion of EGA.

For those embryos that did progress past the third cytokinesis stage, there was no difference in the rates of embryo arrest during the compaction and blastulation stages. A number of improvements to IVM culture in animal models have been suggested to overcome the process of spontaneous maturation that affects ongoing development. These include the addition of recombinant oocyte-secreted factors (Gilchrist et al., 2008; Mester et al., 2014), epidermal growth factor (EGF)-like peptides and cyclic adenosine monophosphate (cAMP) modulators (Richani et al., 2014) to the culture media. Additionally, it has been suggested that adjusting the oxygen concentration during culture may have an effect on embryo development. (Banwell et al., 2007; Salavati et al., 2012). Further research into the improvement in early development of PCOS-IVM embryos may help to improve overall blastocyst and subsequent implantation rates.

Wissing and colleagues found that embryos from normoandrogenic PCOS patients took longer to reach the four cell stage than control patients, and embryos from hyperandrogenic PCOS patients took significantly longer to achieve a number of events compared with the normoandrogenic PCOS patients (Wissing et al., 2014). However, in their study, all fertilized embryos were included in their analysis, even though only 43% of them made it to the blastocyst stage. Additionally, embryo transfer was performed on Day 2, effectively removing the best morphological grade embryo from the cohort of embryos for analysis. We only included embryos that made it to the top/good quality blastocyst stage that were suitable for transfer or vitrification as poor-quality blastocysts and non-blastulating embryos take significantly longer time to reach almost all stages of development (Desai et al., 2014), and including these embryos in the analysis will affect morphokinetic outcomes. We acknowledge that there is the potential limitation in our results due to our patient cohort not being assessed according to their androgenic status, and this may require future investigation. However, in removing arrested embryos from the analysed cohort, we believe that we have shown a more accurate account of embryo morphokinetic development, effectively demonstrating the similarities between patient and treatment groups.

For those blastocysts that were deemed suitable for transfer or vitrification, the results of this study show that there is no difference in morphokinetic development to the blastocyst stage between the three groups. There is increasing evidence of improved success rates after IVF by incorporating embryo selection models, developed from time-lapse analysis, to choose a particular embryo for transfer (Meseguer et al., 2012; Conaghan et al., 2013; Rubio et al., 2014). It has been suggested to combine traditional morphological scoring systems with these newly developed time-lapse algorithms to maximize the potential of selecting the best embryo for transfer (Conaghan et al., 2013; Thornhill, 2014). So far, clinical outcomes in this study are similar to those previously reported by our group (Walls et al., 2014). However, data analysis on these results was not performed as this study was not powered to include pregnancy and implantation success as an outcome. The primary end-point was useable blastocyst development as blastocyst culture is widely accepted to improve implantation and ongoing pregnancy (Gardner et al., 1998; Blake et al., 2007). Therefore, IVM-derived embryos developing to the useable blastocyst stage should have the same implantation potential according to their morphokinetic development as embryos from standard ICSI, regardless of PCOS status. This study has shown that IVM can be safely and easily incorporated into a laboratory time-lapse incubation protocol, in an attempt to maximize successful outcomes while minimizing risks to the patients.
Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org.

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Authors’ roles
All of the authors have contributed to the manuscript in the following manner: (i) substantial contributions to conception and design, analysis and interpretation of data, (ii) drafting the article and revising it critically for important intellectual content and (iii) final approval of the version submitted for review by the Journal of Human Reproduction.

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Conflict of interest
R.H. is the Medical Director of Fertility Specialists of Western Australia and a shareholder in Western IVF. He has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals.

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