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# *In vitro* maturation as an alternative to standard *in vitro* fertilization for patients diagnosed with polycystic ovaries: a comparative analysis of fresh, frozen and cumulative cycle outcomes

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**STUDY QUESTION:** Is *in vitro* maturation (IVM) as successful as standard *in vitro* fertilization (IVF) for the treatment of patients with polycystic ovaries (PCO) in terms of fresh, frozen and cumulative pregnancy outcomes?

**SUMMARY ANSWER:** There was no difference in clinical pregnancy rates in fresh or frozen embryo transfer (FET) cycles between the two treatment groups however, the IVM group showed a lower clinical pregnancy rate cumulatively. There was significantly fewer live births resulting from IVM treatment for both fresh and cumulative cycle outcomes however, there was no difference in live birth rates resulting from FETs between IVM and IVF treatment.

**WHAT IS KNOWN ALREADY:** IVM is well recognized as the only treatment option to eliminate completely the incidence of ovarian hyperstimulation syndrome. However, historically IVM has been less successful than standard IVF in terms of clinical pregnancy, implantation and live birth rates.

**STUDY DESIGN, SIZE, AND DURATION:** This paper represents a retrospective case–control study. The study involved 121 participants who underwent 178 treatment cycles. Cycles were completed between March 2007 and December 2012. All fresh cycles and subsequent FET cycles were included in the analysis to calculate cumulative outcomes.

**PARTICIPANTS/MATERIALS, SETTING, AND METHODS:** All participants were prospectively diagnosed with PCO morphology or polycystic ovarian syndrome (PCOS) and underwent either IVM or standard IVF treatment. Their treatment outcomes were analysed with regard to embryological data, and the rate of biochemical pregnancy, clinical pregnancy and live birth, in addition maternal and neonatal outcomes were assessed. Fifty-six patients underwent 80 cycles of IVM treatment and 65 patients underwent 98 cycles of standard IVF treatment.

**MAIN RESULTS AND THE ROLE OF CHANCE:** For fresh cycles, the differences in the biochemical pregnancy, clinical pregnancy or miscarriage rates between the two treatment groups were not statistically significant. The IVM group showed significantly lower live birth rates in fresh cycles in comparison to standard IVF treatment (18.8 versus 31.0%, $P = 0.021$). For frozen embryo transfer (FET) cycles the differences in biochemical pregnancy, clinical pregnancy, live birth or miscarriage rates between the two treatments groups were not statistically significant. The cumulative biochemical pregnancy (67.5 versus 83.7%, $P = 0.018$), clinical pregnancy (51.3 versus 65.3%, $P = 0.021$) and live birth rates (41.3 versus 55.1%, $P = 0.005$) were significantly lower in the IVM group in comparison to the standard IVF treatment group. There was no overall difference in the cumulative miscarriage rates between the two treatment groups. There was no difference between treatment methods with regard to the neonatal outcomes, and the IVM group had a significantly lower rate of ovarian hyperstimulation syndrome (0 versus 7.1%, $P < 0.001$).

**LIMITATIONS, REASONS FOR CAUTION:** This was an observational study and further randomized clinical trials are required to clarify the difference in outcomes between standard IVF and IVM for patients with PCO/PCOS.

**WIDER IMPLICATIONS OF THE FINDINGS:** This is the first study to compare IVM with standard IVF in PCO/PCOS patients using blastocyst development and single embryo transfer. Furthermore, it is the first study to show the results of fresh, frozen and cumulative treatment cycle outcomes between the two groups. Our results show similar success rates to those reported from other groups, particularly in relation to the incidence of miscarriage in fresh IVM cycles and improved success from FET cycles. Maternal and neonatal outcomes are consistent with the limited literature available.

**STUDY FUNDING/COMPETING INTEREST(S):** The study was supported by the Women's and Infant's Research Foundation of Western Australia. Professor Hart is Medical Director of Fertility Specialists of Western Australia (FSWA) and a shareholder Western IVF. He has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals. T.H. is a consultant with FSWA and a shareholder in Western IVF. She has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals. The other authors have no competing interests.

**Key words:** IVM / IVF / PCOS / OHSS

# Introduction

*In vitro* maturation (IVM) as a clinical treatment option has delivered success in terms of reducing the risks and side effects involved with gonadotrophin stimulation for women embarking on standard *in vitro* fertilization (IVF) treatment, while offering an acceptable chance of conception (Lindenberg, 2013). IVM treatment has repeatedly shown to eliminate the risk of ovarian hyperstimulation syndrome (OHSS) (Fadini *et al.*, 2009; Gremeau *et al.*, 2012; Junk and Yeap, 2012). Patients with polycystic ovaries (PCO) or polycystic ovarian syndrome (PCOS) are at a much higher risk of developing OHSS than those without the conditions (MacDougall *et al.*, 1993). These are patients who respond well to IVM treatment due to their increased antral follicle count (Child *et al.*, 2001).

Owing to the lack or minimal use of stimulation, IVM may also significantly reduce the costs associated with fertility treatment (Hovatta and Cooke, 2006). These reduced costs and side effects usually come at the expense of clinical pregnancy success rates when compared with standard IVF techniques. To date, there have only been two studies which directly compared the outcomes of standard IVF with IVM treatment in PCO/PCOS patients, both of which reported significantly lower clinical implantation in the IVM group (Child, 2002; Gremeau *et al.*, 2012). Neither of these papers reported on blastocyst development comparisons between the two treatment groups. Furthermore, the studies did not separately assess fresh and frozen treatment cycles or use a single embryo transfer strategy to reduce the risks associated with multiple pregnancies.

A modified IVM protocol recently demonstrated clinical pregnancy success rates as high as 44.7% for patients with PCOS (Junk and Yeap, 2012). Similar success rates were achieved when using this protocol to compare IVF and intra cytoplasmic sperm injection (ICSI) fertilization techniques in IVM (Walls *et al.*, 2012). This paper reports on the use of this modified protocol for patients undergoing IVM treatment in comparison to standard IVF treatment. To compare the embryological characteristics, pregnancy and live birth rates, as well as the incidence of OHSS, after IVM and standard IVF treatment in women with PCO/PCOS, we analysed the results of 6 years of fertility treatment data from a single clinic from women with prospectively recorded PCO ovaries or with PCOS who underwent either IVM or standard IVF treatment.

# Materials and Methods

## Patient cohort and data collection

All patients who underwent either standard IVF or IVM treatment in the years 2007–2012 at Fertility Specialists of Western Australia (FSWA) had been prospectively recorded. Subfertile patients were eligible for inclusion in the study if they had been prospectively diagnosed with either PCO or PCOS. The Rotterdam criteria were used to define PCOS and PCO morphology (Rotterdam, 2004) with the diagnosis being confirmed by examination of the patient's clinical record. There were 18 patients who received both treatment methods over this time period and were therefore excluded from the analysis to ensure independent treatment groups were analysed.

## IVM treatment regime

*Hormonal priming*

All IVM patients had a blood test on Day 2 of their cycle for circulating hormone levels and were considered ready to commence treatment once the following were achieved; estrogen ($\leq$250 pmol/l), progesterone ($\leq$3.5 nmol/l), FSH ($\leq$10 IU/l), luteinizing hormone (LH) ($\leq$10 IU/l) and prolactin ($\leq$500 μU/l). A transvaginal ultrasound scan was performed to determine the number of antral follicles on each ovary. Gonadotrophins were administered for 3–6 days, subcutaneously (FSH priming). The normal dosage being 100–150 IU of recombinant FSH (rFSH) using either Gonal-F (Merck-Serono, Frenchs Forest, NSW, Australia) or Puregon (Merck Sharp and Dohme, South Granville, NSW, Australia). The patient underwent an additional transvaginal ultrasound scan on Day 6 of their cycle, all follicles >4 mm were recorded. Once a follicle ~10 mm in diameter was observed, the patient was considered ready for oocyte collection within the following 72 h.

*Oocyte collection*

All patients were administered a general anaesthesia. A 16 gauge double lumen needle (Cook Medical, Brisbane, QLD, Australia) was then inserted through the vaginal wall into the ovary using transvaginal ultrasound guidance. A Cook vacuum pump (Cook Medical) was used with pressure maintained at 175 mmHg. Each immature follicle was drained and flushed up to three times using compound sodium lactate (Hartmann's) solution (Baxter Healthcare, Toongabbie, NSW, Australia) supplemented with heparin (Pfizer, West Ryde, NSW, Australia). Cumulus–oocyte complexes (COCs) were identified by sight, removed from the collection fluid using a sterile glass pipette and washed in G-2Plus media (Vitrolife, Sweden) supplemented with 10% heat inactivated maternal serum, in a small petri dish. The COCs were then transferred to the laboratory.

*Oocyte maturation culture and preparation for insemination*

Once in the laboratory, the oocytes were individually distributed for maturation culture into 20 μl droplets of G-2Plus culture medium, supplemented with 10% v/v maternal serum, 0.1 IU/ml rFSH (Puregon, Merck Sharp and Dohme) and 0.5 IU/ml hCG (Pregnyl, Merck Sharp and Dohme) under sterile mineral oil. The immature oocytes were cultured for 24 h at 37°C in an atmosphere of 6% $CO_2$, 5% $O_2$ and 89% $N_2$. After this time, the oocytes were denuded to assess maturation status if undergoing ICSI insemination, and were deemed mature by the presence of the first polar body. If the oocytes were to be inseminated by IVF, they were transferred with

cumulus cells intact, to a new dish containing G-IVF (Vitrolife, Sweden) and covered in sterile mineral oil.

*Endometrial preparation*

Hormone replacement therapy (HRT) was initiated 2 days prior to egg collection, whereby patients were administered 3 mg of estradiol valerate, Progynova (Merck Sharp and Dohme) orally, three times per day. On the day of egg collection the dose was decreased to 2 mg estradiol valerate orally, three times per day. Twenty-four hours post-egg collection the patient commenced 400 mg progesterone pessaries, three times per day or Crinone (Merck-Serono) 90 mg twice a day. The progesterone regime continued until the pregnancy test 15 days post-embryo transfer. If the test was positive, the regime continued for 12 weeks of pregnancy, if negative the regime was ceased.

## Standard IVF treatment regime

*Hormonal stimulation*

For standard IVF patients, 67 received a gonadotrophin releasing hormone (GnRH) antagonist treatment cycle and 60 received a GnRH agonist protocol, 47 of which were performed prior to 2010 with publications demonstrating the benefit of antagonist cycles leading to a reduction in the incidence of OHSS, our protocol for PCO/PCOS patients changed to predominantly antagonist only cycles.

For antagonist treatment, patients commenced gonadotrophin injections on Day 3 of their menstrual cycle at the prescribed dose according to the treating clinician's choice. Serum estradiol, progesterone and LH measurements were reviewed on the sixth day of stimulation and gonadotrophin dose adjusted if required. Hormonal profile and ovarian ultrasound examination monitored follicular development with all follicles >9.0 mm recorded and 250 μg of Cetrotide (Merck-Serono) or Orgalutran (Merck Sharp and Dohme) 0.25 mg was administered daily when a leading follicle of 14 mm or serum estradiol level of 1000 pmol/l was achieved. Ovulation was triggered by the use of a recombinant hCG trigger, Ovidrel 250 μg (Merck-Serono) or Pregnyl 5000 IU (Merck Sharp and Dohme) when at least one leading follicle was above 17 mm with two to three other follicles >14 mm.

*Oocyte collection and preparation for insemination*

Transvaginal oocyte aspiration (TVOA) was performed 34–36 h after the trigger injection. All patients were administered a general anaesthesia. A 16 gauge double lumen needle (Cook Medical) was then inserted through the vaginal wall into the ovary using transvaginal ultrasound guidance. Using a Cook vacuum pump (Cook Medical), with pressure maintained at 125 mmHg, each mature follicle was drained and flushed up to three times using compound sodium lactate (Hartmann's) solution (Baxter Healthcare) supplemented with Heparin (Pfizer). COCs were identified by sight, removed from the collection fluid using a sterile glass pipette and washed in G-IVFPlus (Vitrolife, Sweden) in a small petri dish. The COCs were then transferred to G-IVFPlus media (Vitrolife, Sweden) and transported to the laboratory. Oocytes undergoing IVF insemination were moved into a dish containing G-IVF (Vitrolife) and covered in sterile mineral oil. Oocytes undergoing ICSI insemination underwent the same procedure of denuding as IVM oocytes with the presence of the first polar body indicating maturity.

*Endometrial preparation*

Luteal support was provided using either progesterone pessaries or Crinone (Merck-Serono), commenced 3 days post-trigger with an option of additional 1500 IU recombinant hCG, Pregnyl (Merck Sharp and Dohme) prescribed in the luteal phase 6 days post-trigger.

## Fertilization and embryo culture

For both standard IVF and IVM treatments, insemination was performed using either IVF or ICSI (with the IVM group predominantly using ICSI). Approximately 16–18 h post-insemination/injection all oocytes from both treatment groups were checked for signs of fertilization. Fertilization was defined by the presence of two pronuclei and two polar bodies. The embryos were transferred to G-1Plus medium (Vitrolife, Sweden) for a further 48 h of culture. The embryos were then transferred to 20 μl droplets of G2Plus medium (Vitrolife, Sweden) and cultured for an additional 48 h. After this time, embryo development was assessed and blastocyst stage embryos graded (Dokras *et al.*, 1993). Grade one blastocysts have good cellular development of both the inner cell mass (ICM) and trophectoderm (TE), grade two blastocysts have average development of the ICM and TE, while grade three embryos have poor cellular development. For embryo transfer or vitrification, only grade one or grade two embryos were selected.

In the standard IVF group there was one patient who underwent a double embryo transfer in a fresh cycle. For the remaining standard IVF patients and all IVM patients, a single blastocyst was transferred. The embryo with the best morphological grade was selected for transfer. Serum β-hCG was measured 10 days post-embryo transfer and the results recorded. A biochemical pregnancy was recorded as a serum hCG level ≥30 IU and a clinical pregnancy was classified by the presence of a fetal sac on ultrasound scan at 6–7 weeks gestation. Supernumerary grade one and grade two blastocysts were cryopreserved by vitrification (Cook Medical) for use in future treatment cycles.

## FET treatment regime

The protocols used for FET cycles utilized either a low-dose rFSH stimulation or HRT. With low-dose rFSH stimulation, patients commenced stimulation on Day 3 of their menstrual cycle. The usual starting dose of recombinant rFSH was 50 IU using either Gonal-F (Merck-Serono) or Puregon (Merck Sharp and Dohme) and this was titrated according to serum estradiol. Response to stimulation was assessed with serum monitoring of estradiol and ultrasound assessment of follicular development and endometrial thickness, commencing on Days 7–10 of the menstrual cycle (depending upon normal cycle length) with adjustment of the dose of recombinant rFSH if required. When there was evidence of a LH surge, or when good follicular and endometrial development was recorded, 250 μg of recombinant hCG, Ovidrel, (Merck-Serono) or Pregnyl 5000 IU (Merck Sharp and Dohme) was administered subcutaneously to trigger ovulation.

For a HRT cycle, a patient commenced estradiol orally from the second day of the menstrual cycle at a dose of 2 mg, three times per day. Monitoring of endometrial thickness was performed with transvaginal ultrasound monitoring and a serum measurement of progesterone was performed when ultrasound assessment demonstrated a minimal endometrial thickness of 7 mm. If the endometrial thickness was <8 mm, estradiol was continued for a further week and endometrial thickness re-evaluated. When the endometrial thickness exceeded 8 mm, 400 mg progesterone pessaries or Crinone (Merck-Serono) were commenced three times per day and embryo transfer was scheduled.

## Ethics approval

Ethics approval for IVM treatment was granted by the Curtin University Human Research Ethics Committee (HREC) in line with the guidelines established at FSWA. All patients in both treatments groups consented to the use of non-identifiable data resulting from their treatment for possible future research as part of their routine cycle information and consenting procedures discussed with their treating clinician.

## Data analysis

Descriptive statistics for continuous data were based on means and standard deviations or medians, interquartile ranges and ranges, according to data normality. Frequency distributions were used to summarize categorical data. Univariate comparisons of demographic characteristics between treatment

groups were made using Mann–Whitney tests for continuous outcomes and $\chi^2$ tests for categorical outcomes. Embryology and pregnancy outcomes were analysed using multivariate mixed models with the generalized estimating equation approach to account for correlation between repeated measures. All treatment estimates were adjusted for differences in baseline characteristics and known influential factors, such as age at start of cycle, cycle number and pregnancy in the previous cycle. Treatment effects were presented as incidence rate ratios or odds ratios together with 95% confidence intervals (CIs). Stata statistical software: Release 12 (StataCorp 2011, College Station, TX, USA) was used for data analysis. All tests were two-tailed and *P*-values $<0.05$ were considered statistically significant.

# Results

Patient characteristics are displayed in Table I. The differences were not statistically significant in their age, body mass index (BMI) or the duration of infertility in months. The total number of fresh cycles ranged from one to four for the IVM group and one to five for the standard IVF group; there was no difference between treatment groups in the median number of fresh cycles. The IVM group had significantly higher rates of a PCOS diagnosis ($P = 0.047$) and the standard IVF group had higher rates of male factor infertility ($P = 0.028$). There were significantly more follicles identified per patient at the final ultrasound scan in the IVM group ($P < 0.001$), although this may be because small (<9 mm) follicles were often not recorded during standard IVF. The duration of stimulation, mean consumption of gonadotrophins and peak estrogen level were all significantly higher in the standard IVF group ($P < 0.001$).

Table II represents the embryology outcomes for the two treatment groups. The IVM group showed a significantly lower proportion of mature oocytes ($P < 0.001$), but the difference in the mean number of mature oocytes retrieved per patient was not statistically significant ($P = 0.055$). In the IVM group there were significantly fewer normally fertilized oocytes overall ($P < 0.001$) and mean per patient ($P = 0.015$). For those oocytes fertilized by ICSI, there was no statistically significant difference in degeneration rates between the two groups (7.2 versus 8.2%; $P = 0.472$). Per patient, the mean number of useable and total blastocysts was significantly lower in the IVM group ($P < 0.001$). However, overall useable and total blastocyst development rates were similar ($P = 0.505$, $P = 0.717$). There was also no difference between treatments in the rate of failed blastocyst development that resulted in no embryo being available for transfer.

Table III represents the pregnancy, miscarriage and live birth outcomes from fresh, frozen and cumulative cycle data. Of the 80 fresh cycles of IVM undertaken, 64 fresh single embryo transfers were performed; of the 98 standard Fresh IVF treatment cycles performed, 58 were fresh single embryo transfers. In the IVM group 14 cycles resulted in failed blastocyst development with no suitable embryos for either fresh or frozen transfer and two freeze-all cycles were performed, one due to a thin endometrium and one at the patient's request for social reasons. In the IVF group 13 cycles resulted in failed blastocyst development with no suitable embryos for either fresh or frozen transfer and 27 cycles resulted in a freeze-all, seven of which were due to moderate to severe OHSS and the remaining as a precaution to avoid OHSS. Of the 80 Fresh IVM cycles, 48 had more than one embryo suitable for transfer and of the 98 standard IVF cycles 68 had more than one embryo suitable for transfer. Of the 64 fresh IVM cycles resulting in an embryo transfer, 21 had one additional FET and 16 resulted in more than one FET from embryos generated in that cycle. Of the two freeze-all cycles in the IVM group, one patient had only one embryo frozen and has yet to return for an FET. The other patient fell pregnant from her first FET and has yet to return for a subsequent FET. Of the 58 standard IVF cycles resulting in an fresh embryo transfer, 18 had one additional FET and 16 had two or more subsequent FET's resulting from embryos generated in that cycle. Of the 27 freeze-all cycles in the standard IVF group, eight went on to have one FET and 16 had two or more subsequent FET's from embryos generated in that cycle.

After adjustment for age at treatment commencement, primary infertility, male factor infertility, PCO or PCOS status, cycle number and clinical pregnancy in the previous cycle, the differences were not statistically significant in the biochemical pregnancy, clinical pregnancy or miscarriage rates between the two treatment groups. There were,

**Table I Patient characteristics.**

| | IVM ($n = 56$) | IVF ($n = 65$) | *P*-value |
|---|---|---|---|
| Number of fresh cycles (Total) | 80 | 98 | |
| Age (Median, IQR) | 31.9 (28.0–34.3) | 32.6 (29.6–36.0) | 0.063 |
| BMI (Median, IQR) | 22.5 (20.0–26.3) | 23.0 (21.0–28.0) | 0.416 |
| Duration of infertility in months (Median, IQR) | 12.0 (7.3–36.0) | 21.0 (8.0–48.3) | 0.218 |
| Number of Fresh Cycles (Range) | 1 (1–4) | 1 (1–5) | 0.777 |
| Polycystic ovary syndrome[a] (%) | 71 | 54 | 0.047 |
| Male factor infertility (%) | 21 | 40 | 0.028 |
| Number of follicles at final ultrasound scan[b] | 38.8 ± 17.5 | 19.9 ± 9.2 | <0.001 |
| Duration of stimulation in days[b] | 4.4 ± 1.6 | 10.0 ± 2.1 | <0.001 |
| Mean consumption of gonadotrophins[b] | 594 ± 244.4 | 1505 ± 688.7 | <0.001 |
| Peak estrogen (E2) level[b] | 1329 ± 1436.1 | 8036 ± 5196.8 | <0.001 |

*P*-values were obtained from generalized linear regression analysis which accounted for the correlation between cycles on each woman.
[a]By Rotterdam criteria, all subjects had PCO morphology.
[b]Data represent mean ± standard deviation per cycle.

**Table II Embryology outcomes of IVM versus IVF treatment.**

| | IVM | IVF | IRR (95% CI)[a] | *P*-value |
|---|---|---|---|---|
| Number of oocytes | | | | |
| Total | 1058 | 1528 | | |
| Mean per patient ± SD | 13.2 ± 6.07 | 15.6 ± 7.81 | 0.87 (0.75–1.01) | 0.073 |
| Number of mature oocytes available | 767 | 1215 | | |
| Total (%) | 73 | 80 | | <0.001 |
| Mean per patient ± SD | 9.6 ± 4.87 | 12.5 ± 8.04 | 0.81 (0.66–1.00) | 0.055 |
| Number of mature oocytes fertilized | 525 | 937 | | |
| Total (%) | 68% | 77% | | <0.001 |
| Mean per patient ± SD | 6.6 ± 3.59 | 9.7 ± 5.89 | 0.67 (0.55–0.81) | 0.015 |
| Number of useable blastocysts formed | 198 | 370 | | |
| Total (%) | 38 | 40 | | 0.505 |
| Mean per patient ± SD | 2.5 ± 2.1 | 3.9 ± 3.40 | 0.51 (0.39–0.67) | <0.001 |
| Total number of blastocysts formed | 238 | 434 | | |
| Total (%) | 45 | 46 | | 0.717 |
| Mean per patient ± SD | 3.0 ± 2.41 | 4.6 ± 3.63 | 0.52 (0.41–0.67) | <0.001 |
| Number of failed blastocyst development | 14/80 | 13/98 | | |
| Total (%) | 16 | 14 | 2.40 (0.90–6.41)[b] | 0.323 |

[a]IRR represents the incidence rate ratio of the outcome in the IVM group when compared with the IVF group (reference). All incidence rates were adjusted for age at start of cycle, primary infertility, male factor, PCOS status, cycle number and clinical pregnancy in the previous cycle.
[b]Estimates represent odds ratios and 95% CIs.

**Table III Pregnancy, miscarriage and live birth outcomes from fresh, frozen and cumulative cycle data.**

| | IVM | | IVF | | Odds ratio (95% CI) | *P*-value |
|---|---|---|---|---|---|---|
| Fresh transfers (Per ET) | *n* = 64 | | *n* = 58 | | | |
| Biochemical pregnancy | 28/64 | 43.8% | 23/58 | 39.7% | 0.71 (0.29–1.71) | 0.446 |
| Clinical pregnancy | 19/64 | 29.7% | 21/58 | 36.2% | 0.52 (0.21–1.28) | 0.158 |
| Live birth | 12/64 | 18.8% | 19/58[a] | 31.0% | 0.59 (0.38–0.92)[b] | 0.021 |
| Miscarriage | 7/19 | 36.8% | 4/21 | 19.0% | 1.50 (0.27–8.21) | 0.642 |
| Frozen transfers (Per ET) | *n* = 62 | | *n* = 117 | | | |
| Biochemical pregnancy | 26/62 | 41.9% | 59/117 | 50.4% | 0.61 (0.28–1.30) | 0.199 |
| Clinical pregnancy | 22/62 | 35.5% | 43/117 | 36.8% | 0.77 (0.37–1.60) | 0.484 |
| Live birth | 21/62 | 33.9% | 35/117 | 29.9% | 1.01 (0.49–2.09) | 0.986 |
| Miscarriage | 1/22 | 4.5% | 8/43 | 18.6% | 0.36 (0.06–2.32) | 0.285 |
| Cumulative (Per egg collection) | *n* = 80 | | *n* = 98 | | | |
| Biochemical pregnancy | 54/80 | 67.5% | 82/98 | 83.7% | 0.64 (0.45–0.93) | 0.018 |
| Clinical pregnancy | 41/80 | 51.3% | 64/98 | 65.3% | 0.64 (0.44–0.94) | 0.021 |
| Live birth | 33/80 | 41.3% | 54/98[a] | 55.1% | 0.57 (0.39–0.84) | 0.005 |
| Miscarriage | 8/41 | 19.5% | 12/64 | 18.8% | 1.01 (0.45–2.23) | 0.987 |

ET, embryo transfer.
Odds ratio compares the IVM group to the IVF group (reference). All estimates were adjusted for age at start of cycle, primary infertility, male factor, PCOS status, cycle number and clinical pregnancy in the previous cycle.
[a]Includes two sets of twins.
[b]Represents incidence rate ratio (some transfers produced >1 live birth).

however, significantly fewer live births in the IVM treatment group ($P = 0.021$). From these fresh cycles a further 62 FET cycles were performed in the IVM group and 117 in the standard IVF group, resulting in no statistically significant differences in the biochemical pregnancy, clinical pregnancy, live birth or miscarriage rates between the two treatments groups. Cumulatively, there were 126 embryos transferred in both

fresh and frozen cycles resulting from the 80 cycles initiated in the IVM group and 175 embryos transferred from the 98 cycles initiated in the standard IVF group, resulting in significantly lower biochemical pregnancy ($P = 0.018$), clinical pregnancy ($P = 0.021$) and live birth rates ($P = 0.005$) in the IVM treatment group, respectively (Table III). However, there was no overall difference in the miscarriage rate between the two treatment groups.

The IVM treatment group had a significantly lower rate of OHSS than the standard IVF group ($P < 0.001$; Table IV), with no cases of OHSS recorded in the IVM group, with two patients having all of their embryos frozen, one for social reasons and one due to poor endometrial development. In the standard IVF treatment group there were seven cases of moderate to severe OHSS (7.1%), two of which arose from GnRH agonist cycles and five of which resulted from GnRH antagonist cycles. There were no multiple pregnancies or births reported in the IVM group with two sets of twins resulting in the standard IVF group, one resulting from the only double embryo transfer performed across the two treatment groups and the other resulting in a monozygotic twin pregnancy.

There was no difference in preterm birth rates ($P = 0.070$), with 6.0% of infants in the IVM group delivered before 37 weeks gestation and 22.0% of standard IVF infants delivered before 37 weeks (Table III). Owing to the low sample size, an analysis of preterm birth rates between fresh and frozen transfers was not performed; however, there were six preterm babies (including two sets of twins) from fresh transfers in the standard IVF group and seven preterm babies resulting from FETs. There was one preterm baby delivered resulting from a fresh embryo transfer and one resulting from a FET in the IVM group. IVM infants had a mean birthweight of 3.364 kg and standard IVF infants had a mean birthweight of 3.199 kg ($P = 0.262$; Table IV). There was one case of a horseshoe kidney in the IVM group and no congenital birth defects were reported in the standard IVF group (Table IV). Statistical analysis was not performed due to the small sample size.

# Discussion

Our results show that overall in the IVM treatment group compared with the standard IVF group, a smaller proportion achieved maturity and fewer of these were normally fertilized; however, the difference in the mean number of oocytes collected or matured per patient was not statistically significant. The primary objective for an IVM treatment cycle is for oocytes to successfully complete meiosis during maturation culture and gain both nuclear and cytoplasmic competence to enable fertilization, embryo development and ultimately a healthy live birth. Our results demonstrated that less mature oocytes were obtained using our IVM stimulation protocol than from standard IVF cycles at the time of oocyte retrieval, which is consistent with results from previous studies (Child, 2002; Gremeau *et al.*, 2012). While these mature oocytes have successfully undergone nuclear maturation, as assessed by the presence of the first polar body extrusion, cytoplasmic maturation may not yet be complete and this may be one of the factors contributing to their significantly lower fertilization rate compared with those from the standard IVF group.

Insemination in the IVM group was predominantly performed using ICSI, as it was originally thought to be necessary due to the hardening of the zona pellucida during maturation culture; however, a study with sibling oocytes showed rates of fertilization, embryo development and clinical pregnancy did not differ between those inseminated using ICSI and those inseminated with traditional IVF (Walls *et al.*, 2012). Even though in the current study, the number of normally fertilized oocytes in the IVM group was lower than that of the standard IVF group, the 68.4% fertilization rate observed is considered to be well within an acceptable range for clinical treatment. A number of modifications to IVM protocols to overcome poor maturation and fertilization issues have been suggested after successful animal trials. These include the addition to culture media of factors such as epidermal growth factor (EGF)-like peptides and cyclic adenosine monophosphate (cAMP) modulators (Richani *et al.*, 2014) as well as oocyte secreted factors (Gilchrist *et al.*, 2008; Mester *et al.*, 2014). If such additives increased maturation and fertilization rates of human IVM oocytes to levels comparable to standard IVF, there would be the potential to further increase the yield of useable blastocysts for embryo transfer.

This is the first comparative study between IVM and standard IVF to publish data on blastocyst development and suggests that the rates of development do not differ between the two groups. The use of blastocyst culture and transfer is now widely accepted as a means to optimize clinical pregnancy rates per embryo transferred, while reducing the need for multiple embryos to be transferred in order to successfully achieve a pregnancy (Gardner *et al.*, 1998; Blake *et al.*, 2007). The benefits of blastocyst culture appear to be equally applicable for IVM treatment with a publication from our group establishing this protocol of blastocyst culture and single embryo transfer to achieve high implantation rates with an excellent singleton live birth rate (Junk and Yeap, 2012). Following on from their research, the current study compared blastocyst development of IVM oocytes with standard IVF oocytes also from women with PCO morphology or with features of PCOS. The potential for asynchrony between nuclear and cytoplasmic maturation in IVM treatment was thought to negatively impact embryonic development (Trounson *et al.*, 1998). Our results have shown that while the mean number of blastocysts per patient was lower in IVM than standard IVF, this is a result of overall fewer oocytes collected, matured and fertilized in the IVM group.

**Table IV Maternal and neonatal outcomes.**

| | IVM ($n = 33$) | IVF ($n = 54$) | *P*-value |
|---|---|---|---|
| Ovarian hyperstimulation | 0 (0%) | 7 (7.1%) | <0.001 |
| Preterm birth (<37 weeks) | 2 (6%) | 12 (22%) | 0.070 |
| Birthweight (g) | 3364 ± 590 | 3199 ± 694 | 0.262 |
| Congenital birth defects | 1[a] | 0 | N/A |

[a]Represents a horseshoe kidney.

Whereas overall rates of useable and total blastocyst development, as a percentage of normally fertilized oocytes, were not different between the two treatment groups. Additionally, the rate of failed blastocyst development, resulting in no embryo available for transfer, was not different between the two treatment groups. Therefore, in this study IVM did not specifically affect embryo development to the blastocyst stage. Culture to the blastocyst stage is a successful embryo selection tool that allows the use of single embryo transfers to yield high rates of implantation and ongoing clinical pregnancies and our data show that it can be combined with IVM.

In both fresh and frozen cycles, there was no difference in clinical pregnancy rates between the IVM and standard IVF treatment groups. Furthermore, for embryos transferred in frozen treatment cycles, there was no difference in the live birth rate. However, for fresh embryo transfers, there was a significantly lower live birth rate per embryo transferred than in the standard IVF treatment group. The scientific literature reports implantation rates for IVM to range between 0% (Mikkelsen and Lindenberg, 2001) and 14.8% (Chian *et al.*, 2000) for cycles with no hormonal priming with gonadotrophins and between 9.1% (Lin *et al.*, 2003) and 21.6% (Mikkelsen and Lindenberg, 2001) for cycles with rFSH priming. When retrospectively compared with standard IVF in two case–control studies of women with PCO, IVM implantation and live birth rates were significantly lower than those achieved with standard IVF (Child, 2002; Gremeau *et al.* 2012).

The same modified protocol of hormonal priming for larger follicle growth with a specified endometrial preparation, which has been shown to produce implantation rates of 43% (Walls *et al.*, 2012) and 44% (Junk and Yeap, 2012), was used to treat IVM patients in this retrospective study. Although we did not see implantation and live birth rates as high as these previous studies, we believe this to be a result of the differing inclusion and exclusion criteria, particularly involving our inclusion of patients who had multiple cycles of IVM and the exclusion of those who had previously received standard IVF treatment, to ensure independence of treatment groups when performing data analysis. Regardless of this decrease in efficacy in fresh embryo transfer cycles, in frozen cycles, we have shown comparable results for the IVM treated patients to those using standard IVF. IVM combined with blastocyst culture and single embryo transfer can therefore, be used as a first line clinical treatment without concerns for reduced pregnancy success.

Cumulative rates of biochemical pregnancy, clinical pregnancy and live birth were significantly lower in the IVM group compared with the standard IVF group. There are a number of factors, which may contribute to this difference. The primary reason for the decrease in success appears to be related to the smaller number of usable embryos generated in the IVM group. This is likely to be due to the additive effect of lower numbers of oocytes collected, matured and fertilized normally compared with the standard IVF group. This in turn resulted in a greater total number of frozen embryos for standard IVF patients, which contributed to an overall higher cumulative pregnancy rate. Another contributing factor for the reduced cumulative live birth rate could be the significantly higher number of patients with PCOS as opposed to just having a polycystic ovarian morphology in the IVM group. The literature shows that cumulative live birth rates are significantly higher in women with PCO, but not in women with PCOS, when compared with those without PCO/PCOS following standard IVF treatment (Li *et al.*, 2013). We can therefore conclude that with further research leading to improvements in the total oocyte yield and successful fertilization that cumulative success rates could be comparable between the two treatment groups.

The use of predominantly single embryo transfer led to no cases of multiple births in the IVM group and only two cases of twins in the standard IVF group, one monozygotic and one dizygotic, resulting from the only double embryo transfer performed across both treatment groups. Our clinic favours a single blastocyst transfer approach, which has been shown to maintain high implantation and ongoing pregnancy rates, while minimizing the risk of multiple pregnancy (Gardner *et al.*, 2004). In our study, only single embryo transfers were performed in the IVM group leading to an implantation rate equal to that of the clinical pregnancy rate. A study by Child and colleagues in 2002 demonstrated that IVM treatment has the potential to produce clinical pregnancy and live birth rates not significantly different to those in standard IVF (Child, 2002). However, this was a result of a higher mean number of embryos transferred (3.2 versus 2.7), leading to a decrease in implantation rates per embryo and an increase in multiple live births. Rates of multiple live births in the IVM treatment group were reported to be as high as 41.2%. Following on from their research, Gremeau and colleagues also transferred significantly higher mean number of embryos in the IVM group (1.9 versus 1.7) however; they still reported a significantly lower implantation, clinical pregnancy and live birth rate. In contrast, the present study shows no statistically significant difference in implantation rates in both fresh and frozen cycles between IVM and standard IVF treatment, while maintaining a zero per cent rate of multiple pregnancies for IVM.

As was used in the present study, a protocol of rFSH priming with no ovulation trigger and the collection of oocytes from slightly larger follicles, has been suggested as the main reason for optimising success rates in IVM (Junk and Yeap, 2012). The use of a more complex blastocyst culture medium, as used in the present study, as the basis of the maturation media could also have an influence on successful embryo development and implantation potential (Kim *et al.*, 2011). Such a medium may provide a better culture environment for both the oocyte and the associated cumulus cells than a medium designed for oocytes or early embryos alone. Another explanation for the overall increase in cumulative implantation rates in the IVM group may be due to the high rate of implantation and live births resulting from FETs.

Significantly improved clinical pregnancy and implantation rates have previously been reported in IVM treatment using vitrified-warmed embryos replaced in a frozen embryo cycle (De Vos *et al.*, 2011). Additionally, a recent meta-analysis of fresh versus frozen embryo pregnancy rates in standard IVF treatment concluded that the rate of ongoing pregnancies from frozen embryos was significantly higher compared with fresh (Roque *et al.*, 2013). These findings may also account for the lack of miscarriages in the IVM group after successful frozen embryo pregnancies compared with a 36.8% miscarriage rate after fresh embryo transfers. This difference leads us to believe that while endometrial preparation has improved with this modified protocol, it is possibly still not as effective as it would be in a frozen embryo treatment cycle. Therefore, more research is needed to improve the uterine environment in IVM cycles following fresh embryo transfers to further increase the success rates of this IVM protocol.

With respect to birth outcomes, the difference was not statistically significant in the incidence of preterm birth or the mean birthweights of infants between the two treatment groups. Preterm birth is a significant contributor to neonatal morbidity and mortality, and the rates of preterm birth have increased in recent years (McDonald *et al.*, 2009).

One factor that may be implicated in this increasing incidence of prematurity is the increasing number of children born as a result of standard IVF procedures (Slattery and Morrison, 2002). Rates of preterm birth have been shown to be significantly higher, with significantly lower mean birthweights recorded, for infants born resulting from standard IVF treatment compared with than those spontaneously conceived (McDonald *et al.*, 2009; Henningsen *et al.*, 2011). This increased risk of preterm birth is even more prevalent in babies born to mothers diagnosed with PCOS (Boomsma *et al.*, 2006).

The incidence of preterm birth in our IVM group, although not significantly lower, was only 6% compared with 22% in the standard IVF group. While this may be due to the low sample size, it is an encouraging result and is consistent with the low rate of preterm birth reported in IVM infants in the literature (Söderström-Anttila *et al.*, 2006). This could potentially support the theory of a hyper-estrogenic environment in a fresh embryo transfer cycle contributing to lower recorded mean birthweights in standard IVF conceived infants, as cryopreserved embryos are typically transferred into a natural or minimally stimulated endometrium (Belva *et al.*, 2008). This is supported by the finding of higher mean birthweight of infants resulting from frozen embryo treatment cycles compared the birthweights of infants conceived in a fresh standard IVF cycles (Henningsen *et al.*, 2011). Therefore, while the mean birthweights of infants were similar between the two treatment groups, embryos transferred in a fresh IVM cycle are replaced in a hormonal environment similar to those of frozen embryos as demonstrated by the significantly higher mean peak estrogen in the standard IVF group compared with the IVM group. IVM, therefore, does not appear to pose any increase in the risk of adverse neonatal outcomes such as a lower mean birthweight or a higher prevalence of preterm birth, which are often reported following standard IVF assisted conception.

In terms of evaluating maternal risks resulting from IVM treatment, there were no cases of ovarian hyperstimulation, which was significantly lower than observed for the standard IVF treatment group. OHSS remains one of the most serious consequences of rFSH stimulation in standard IVF cycles. Severe OHSS resulting in additional hospital admission, not only results in further physical and mental distress to the patient, but also increases their costs associated with treatment and increases the financial burden on the public healthcare system. Even with improved monitoring, using antagonist protocols (Kolibianakis *et al.*, 2006; Al-Inany *et al.*, 2007), metformin (Costello *et al.*, 2006), dopamine agonists (Cabergoline) (Tang *et al.*, 2012) and 'freeze-all' precautions, the risk of OHSS cannot be completely eliminated in standard IVF cycles. This risk is the most significant for patients otherwise considered ideal candidates for IVM treatment.

Study results have consistently shown no recorded cases of OHSS following IVM (Child, 2002; Fadini *et al.*, 2009; Gremeau *et al.*, 2012; Junk and Yeap, 2012) with one of the main findings of a recent IVM review article stating that the risk of OHSS is no longer an issue for IVM treatment (Lindenberg, 2013). The decrease in OHSS rates in the IVM group is a direct result of this minimal-approach treatment by lowering the hormonal burden on the patient. This is evidenced by the significantly lower consumption of gonadotrophins, duration of stimulation and peak estrogen levels seen in this study. The question is then 'at what level of IVM success does the decrease in OHSS risk outweigh perceived higher success rates with standard IVF'? Our results are consistent with the literature in reporting zero cases of OHSS for IVM treatment while still achieving success rates similar to standard IVF treatment. Therefore, we conclude that IVM is a more patient friendly treatment. It can eliminate the risk of OHSS and should be highly recommended as a treatment option particularly for women with PCO who are at an increased risk of the significant morbidity resulting from OHSS after standard IVF treatment.

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## Authors' roles

M.L.W., T.H., J.A.K., E.N., J.P.R. and R.J.H. contributed to the manuscript in the following manner:

(i) Contributions to conception and design, analysis and interpretation of data.
(ii) Drafting the article and revising it critically for important intellectual content.
(iii) Final approval of the version submitted for review by *The Journal of Human Reproduction*.

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## Conflict of interest

T.H. is a consultant with FSWA and a shareholder Western IVF. She has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals. R.J.H. is Medical Director of FSWA and a shareholder of Western IVF. He has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals. The other authors have no conflict of interest to declare.

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