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Full length article

The effectiveness of Paraffin oil and Mineral oil for day-5 embryo culture in couples undergoing in vitro fertilisation

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ABSTRACT

Objective: This study evaluated the effectiveness of Paraffin oil versus Mineral oil for day-5 embryo culture in couples undergoing assisted reproductive technology (ART).
Methods: We performed a multi-centre, retrospective cohort study at IVFMD (My Duc Hospital) and IVFMD Phu Nhuan (My Duc Phu Nhuan Hospital) from January 2019 to September 2019. We studied couples treated by intracytoplasmic sperm injection (ICSI), using fresh, ejaculated semen and undergoing day-5 embryo transfer. Couples who underwent in vitro maturation (IVM) or oocyte donation cycles or couples where the woman had uterine abnormalities were excluded. From January 2019 to May 2019, we used Mineral oil (LiteOil, LifeGlobal) while Paraffin oil (Liquid Paraffin, Origio) was used from June 2019 to September 2019. The primary outcome was live birth rate after the first transfer, either from a fresh transfer or frozen embryo transfer.
Results: Between 1st January 2019 to 30th September 2019, there were 2,312 couples undergoing ART in both centres, of which 762 (377 in the Paraffin group and 385 in the Mineral group) eligible couples were included in the study. Baseline characteristics of couples were comparable between the two groups, with mean female age 31.5 ± 4.3 versus 31.9 ± 4.7 in the Paraffin and Mineral group. Live birth after the first transfer occurred in 153 (40.6%) couples in the Paraffin group, compared to 152 (39.5%) couples in the Mineral group (risk ratio 1.02, 95% confidence interval 0.91 – 1.14). Other secondary outcomes were comparable between the two groups.
Conclusion: In day-5 embryo culture, Paraffin and Mineral oil resulted in a comparable live birth rate.

Introduction

Culture system plays a vital role in assisted reproductive technology (ART) programs, influencing the developmental and implantation capacity of embryos. Oil has been widely used as an overlay in microdrop culture systems to prevent medium evaporation, maintain appropriate pH, temperature, osmotic conditions and protect embryos from microbial contamination [1–3]. Therefore, it can limit the adverse effects on the metabolism of embryos as well as the variation in DNA and protein. However, it has been shown that oil could also cause some detrimental effects on embryos due to the peroxidation process [4,5]. The high level of peroxidation in culture overlay is supposed to be negative to fertilisation and embryo development due to the toxic contamination and deterioration of oil quality [6].

Several studies have investigated the effect of overlaying oil on embryo culture. It has long been shown in mammalians that the quality of oil could significantly influence the ability of embryos to reach the blastocyst stage, with an improvement of their freezability, following in vitro development [7,8]. In humans, there have been so far only two studies comparing the effectiveness of different commercial Paraffin oils [3] and Paraffin versus Mineral oil [9]. The results showed that there was no difference between the groups in terms of fertilisation, day-2 and day-3 embryo formation rate. However, both these two studies involved only cleavage embryos and used embryo quality as the primary outcome. Live birth was found to be no difference between the Paraffin and Mineral groups in only one study [9]. However, this data was

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obtained from a limited number of patients (68 cycles), in which day-3 embryo transfer was used.

The aim of this study was to compare the effectiveness of Paraffin oil (Liquid Paraffin, Origio) versus Mineral oil (LiteOil®, LifeGlobal) for day-5 embryo culture in couples undergoing intracytoplasmic sperm injection (ICSI).

Materials and methods

Population, study design and setting

We performed a multi-centre, retrospective cohort study, at IVFMD (My Duc Hospital) and IVFMD Phu Nhuan (My Duc Phu Nhuan Hospital). Both centres locate in Ho Chi Minh City, with constant warm temperatures throughout the year. Data were extracted from the database of each centre. All patients undergoing assisted reproductive technology (ART) from 1st January 2019 to 30th September 2019 were evaluated for inclusion. Patients treated by ICSI, using fresh, ejaculated semen and undergoing day-5 transfer were included. The ART indications included male factor, tubal factor, ovulation disorder, unexplained, diminished ovarian reserve and others. The diminished ovarian reserve was defined as women having antral follicle count (AFC) <5 or anti-Mullerian hormone level <1.2 ng/mL [10]. Exclusion criteria were women who underwent in vitro maturation (IVM) or oocyte donation cycles, or those who had uterine abnormalities. The study was approved by the Institutional Ethics Committee at My Duc Hospital, Ho Chi Minh City, Viet Nam (16/19/ĐĐ-BVMĐ) on 30th September 2019.

Ovarian stimulation

All patients were treated with a gonadotropin-releasing hormone (GnRH) antagonist protocol [11]. Recombinant follicle-stimulating hormone (FSH) was given on day 2 or day 3 of the menstrual cycle for five days until the first check-up. The starting dose (150 IU to 300 IU) was individualised for each patient based on the Anti-Mullerian Hormone (AMH) level, with subsequent titrate based on the clinical judgment of the treating physician. Follicular development was monitored by ultrasound scanning and measurement of estradiol and progesterone levels, starting on day-5 of stimulation. Scanning and hormonal measurement were repeated every two to three days, depending on the size of the follicles. An antagonist was routinely used on day 5 until the day of triggering. Criteria for triggering, by human chorionic gonadotropin (hCG) was the presence of at least three leading follicles of 17 mm. In women with an excessive follicular response (≥15 follicles ≥12 mm), 0.2 mg Triptorelin was used when there were at least two leading follicles of 17 mm. Oocyte retrieval was performed 36 h after triggering [11].

Insemination and embryo culture

Insemination was performed by ICSI, at 39 to 41 h after triggering. Only metaphase II (MII) oocytes were used. Fertilisation check was performed at 16–18 h after insemination. Embryo evaluation was performed at fixed time points of 66 ±2 h and 116 ±2 h after ICSI, using the Istanbul consensus [12].

Embryos were cultured in groups (maximum of three embryos each) in a 30 μl microdrop of Global Total LP (LifeGlobal) at 37 °C in 5% CO2 and 5% Oxygen incubator G210 (K-System). On day 3, media were renewed and embryos were cultured to the blastocyst stage on day 5. All culture dishes were equilibrated overnight. In both hospitals, the culture droplets were overlaid by Mineral oil (LiteOil, LifeGlobal) from 1st January to 31st May 2019 and then by Paraffin oil (Liquid Paraffin, Origio) from 1st June to 30th September 2019. The rationale behind the changing of the oil manufacturers was driven by the high number of ART cycles in both centres. Our safety policy in risk management is that we have to maintain two suppliers for one product. This is to minimise any potential risk from supply chains. This change of oil manufacturers happened at the same time in both centres.

Embryo transfer

Embryo transfer (ET) was performed under ultrasound guidance. The number of embryos transferred, from one to a maximum of two day-5 embryos, was based on the quality of embryos and couples' preferences. The remaining good-quality day-5 embryos were vitrified (Cryotech). Luteal-phase support was done with exogenous estradiol and vaginal progesterone until the 7th week of gestation.

If there were contra-indications for fresh ET, a freeze-all strategy was applied. Indications for freeze-all included risk of ovarian hyperstimulation syndrome (OHSS), agonist trigger, premature progesterone rise (≥1.5 ng/ml), thin endometrium (<7 mm), fluid in the cavity on day of ET, endometrial polyp, hydrosalpinx that have not removed before oocyte retrieval or pateints' preference [11].

In the subsequent cycle, the endometrium was prepared using oral estradiol valerate 8 mg/day, starting from the second or third day of the menstrual cycle. Endometrial thickness was monitored from day six onwards, and vaginal progesterone was started when endometrial thickness reached 8 mm or more. A maximum of two embryos was thawed on the day of ET, five days after the start of progesterone. Two hours after thawing, surviving embryos were transferred into the uterus under ultrasound guidance.

A serum hCG will be measured two weeks after the embryo transfer, and if positive, an ultrasound scan of the uterus will be performed at gestational weeks 7 and 12 [11].

Outcomes

The primary outcome was live birth after the first transfer, either from the fresh or frozen transfer. Live birth was defined as the birth of at least one baby after 24 weeks' gestation that showed any sign of life (twins as a single count). Secondary outcomes were the number of (good-quality) blastocyst, number of frozen blastocyst and the rates of clinical pregnancy, ongoing pregnancy, implantation, multiple pregnancy and ectopic pregnancy.

Statistical analyses

Baseline data were presented using descriptive statistics (mean and standard deviation for normally distributed variables, or median and interquartile range for skewed variables). Categorical data were presented as number (%). The rate of live birth and the associated 95% confidence interval (CI) were estimated and compared between groups using the exact method for a binomial proportion. Differences between groups in secondary outcome variables were analysed by using the Student *t*-test for normally distributed data, Wilcoxon signed-rank test for skewed data, or Fisher's exact test for categorical variables, and were reported as relative risk (RR) with 95% CI.

All tests were two-tailed tests, p-values <0.05 were considered to be statistically significant. Univariable and multivariable logistic regression analyses were performed to identify factors associated with live birth, the primary outcome. All variables with a p-value of <0.25 in the univariate analysis were included in the multivariable analysis. All analyses were performed using the R statistical programme (R version 4.1.0; ©2021 The R Foundation for Statistical Computing).

Result

Between 1st January 2019 and 30th September 2019, there were 2,312 couples undergoing ART treatment at both centres, of which 1,550 couples were excluded (102 couples undergoing conventional IVF cycles, 130 treated with oocyte donation cycles, 336 cycles using sperm from retrieval surgery or sperm bank, 97 undergoing IVM cycles, 18

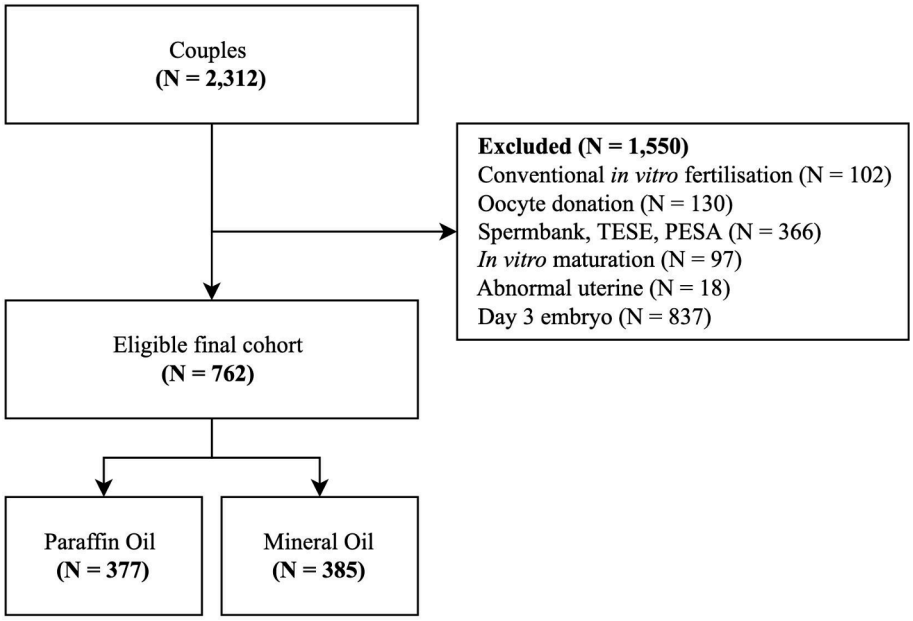

Fig. 1. Flowchart of the study. TESE: Testicular Sperm Extraction, PESA: Percutaneous Epididymal Sperm Aspiration.

Table 1
Baseline characteristics of the two groups.

Characteristics	Paraffin oil (n = 377)	Mineral oil (n = 385)	p-value
Age (year)	31.5 ± 4.3	31.8 ± 4.7	0.25
Body mass index (kg/m^2)	21.8 ± 2.6	21.5 ± 2.4	0.11
Anti Mullerian Hormone (ng/mL)	4.2 [2.7, 6.8]	4.0 [2.5, 6.1]	0.24
Antral Follicle Count	19.0 [13.0, 26.0]	18.0 [12.0, 24.0]	0.43
Type of infertility causes – no. (%)			0.29
Primary	204 (54.1)	224 (58.2)	
Secondary	173 (45.9)	161 (41.8)	
ART indication – no. (%)			0.13
Diminished ovarian reserve	12 (3.2)	8 (2.1)	
Tubal factor	58 (15.4)	40 (10.4)	
Male factor	146 (38.7)	177 (46.0)	
Ovulation Disorder	47 (12.5)	45 (11.7)	
Unexplained	53 (14.1)	44 (11.4)	
Others	61 (16.2)	71 (18.4)	

Values are mean ±standard deviation, median [quartile 1; quartile 3], or number (%). ART: assisted reproductive technology.

Table 2
Treatment cycles' characteristics.

Characteristics	Paraffin oil (n = 377)	Mineral oil (n = 385)	p-value
Duration of stimulation (day)	9.0 [8.0, 9.0]	9.0 [8.0, 10.0]	0.83
Total FSH dosage (IU)	2025 [1800, 2400]	2400 [1894, 2700]	<0.01
Estradiol level on triggering day (pg/ml)	4957 [2533, 12650]	5408 [2768, 11250]	0.72
Progesterone level on triggering day (ng/ml)	0.9 [0.6, 1.3]	1.0 [0.7, 1.5]	0.05
No. of oocytes retrieved	18.0 ± 8.1	17.5 ± 7.9	0.44
No. of metaphase-II oocytes	14.4 ± 7.3	14.4 ± 6.8	0.87
No. of normal fertilised oocytes	10.7 ± 6.2	10.6 ± 5.8	0.85
Two-pronuclear fertilised oocytes formation rate	6.2 ± 3.7	6.1 ± 3.3	0.54
No. of blastocyst	2.8 ± 2.2	2.7 ± 2.3	0.35
No. of good quality blastocyst	18.0 ± 8.1	17.5 ± 7.9	0.44
No. of frozen blastocyst	10.7 ± 6.2	10.6 ± 5.8	0.85
Fresh embryo transfer group	3.0 ± 2.2	2.6 ± 1.7	0.48
Freeze-all group	5.2 ± 2.8	4.9 ± 2.5	0.20
Types of transfer – no. (%)			0.18
Fresh embryo transfer	21 (5.6)	32 (8.3)	
Frozen embryo transfer	356 (94.4)	353 (91.7)	
No. of blastocyst transferred	1.2 ± 0.4	1.2 ± 0.4	0.23
No. of good quality blastocyst transferred	0.9 ± 0.4	0.9 ± 0.5	0.13

Values are mean ±standard deviation, median [quartile 1; quartile 3], or number (%). FSH: follicle-stimulating hormone.

couples with uterine abnormalities and 837 couples undergoing day-3 transfer; Fig. 1). Therefore, a total of 762 (377 in the Paraffin group and 385 in the Mineral group) couples were included in the study.

Comparison of patients' baseline characteristics

The mean female age was 31.5 ±4.3 in the Paraffin group versus 31.8 ±4.7 in the Mineral group (p =0.25). The AMH levels in the Paraffin and Mineral groups were 4.2 ng/mL and 4.0 ng/mL, respectively (p =0.24). More than half of the patients were categorised as having primary infertility in both groups. Other baseline characteristics, including body mass index and antral follicle count, were not statistically significant differences between the two groups (Table 1).

Comparison the treatment cycles' characteristics

Total FSH doses were 2025 IU and 2400 IU in the Paraffin group and Mineral group, respectively (p <0.01). However, the mean number of oocytes retrieved, metaphase-II oocytes and the mean number of normal fertilised oocytes were not significantly different between the two groups (p >0.05). The mean number of blastocysts in the Paraffin oil group was 6.2 ±3.7 compared to 6.1 ±3.3 in the Mineral oil group (p =0.54) (Table 2). The number of good quality blastocyst and frozen blastocyst were also comparable between the two groups. Most of the transfers were frozen embryo transfers, with reasons for undergoing freeze-all provided in Appendix Table 1. Single embryo transfer has been performed in more than 75% of couples (Appendix Table 2).

Comparison of clinical outcomes

Live birth after the first ET occurred in 153/377 (40.6%) couples in

Table 3
Clinical outcomes.

Outcomes	Paraffin oil (N = 377)	Mineral oil (N = 385)	Between-group difference (95% CI)	Risk ratio (95% CI)	P-value
Live birth – no. (%)	153 (40.6)	152 (39.5)	1.1 (-6.12, 8.32)	1.02 (0.91, 1.14)	0.81
Singleton	148 (96.7)	137 (90.1)	–	–	0.04
Twins	5 (3.3)	15 (9.9)	–	–	
Ongoing pregnancy – no. (%)	174 (46.2)	175 (45.5)	0.7 (-6.64, 8.04)	1.02 (0.87,1.12)	0.90
Clinical pregnancy - no. (%)	208 (55.2)	207 (53.8)	1.4 (-5.93, 8.74)	1.03 (0.83, 1.13)	0.90
Positive pregnancy test – no. (%)	226 (59.9)	223 (57.9)	2.0 (-9.27, 5.22)	1.03 (0.92, 1.17)	0.62
Multiple pregnancy – no. (%)	19 (5.0)	29 (7.5)	−2.5 (-1.21, 6.19)	0.67 (0.38, 1.17)	0.75
Miscarriage (7–12 week) – no. (%)	34 (9.0)	30 (7.8)	1.2 (-5.43, 2.98)	1.15 (0.72, 1.85)	0.63
Miscarriage (12–24 week) – no. (%)	21 (5.6)	23 (6.0)	−0.4 (-3.17, 3.98)	0.93 (0.6–1.9)	0.93
Ectopic pregnancy – no. (%)	0	2 (0.5)	–	–	–
Implantation rate* (%)	226/446 (50.7)	237/469 (50.5)	0.2 (-6.76, 6.48)	1.00 (0.9–1.17)	0.75
Birthweight (grams)					
Singleton	3212.8 ± 441.7	3121.8 ± 442.5	91 (-200.80, 18.87)	–	0.10
Twins	2490.0 ± 466.9	2571.4 ± 432.7	−81.4 (-489.28, 652.14)	–	0.74

Values are mean ±standard deviation or number (%). CI: confidence interval.
*Total number of sacs / total number of embryos transferred.

Table 4
Multivariable logistic regression analysis of factors affecting live birth.

	Live birth (N = 305)	OR (95% CI), P-value
Type of oil		
Mineral oil	152 (49.8)	Ref.
Paraffin oil	153 (50.2)	0.99 [0.68, 1.45], 0.956
Trial centre		
Trial centre 1 (IVFMD)	238 (78.0)	Ref.
Trial centre 2 (IVFMD Phu Nhuan)	67 (22.0)	0.88 [0.54, 1.42], 0.602
Age (year)	31.1 ± 4.3	0.97 [0.93, 1.01], 0.201
Body mass index (kg/m^2)	21.7 (2.2)	–
Anti-Mullerian Hormone (ng/mL)	4.5 [2.8, 6.9]	1.00 [0.92, 1.09], 0.934
Antral Follicle Count	19.0 [14.0, 27.0]	1.02 [1.00, 1.05], 0.032
Type of infertility causes – no. (%)		
Primary	178 (58.4)	
Secondary	127 (41.6)	–
ART indications – no. (%)		
Diminished ovarian reserve	135 (44.3)	Ref.
Tubal factor	38 (12.5)	0.87 [0.50, 1.50], 0.622
Male factor	40 (13.1)	0.96 [0.56, 1.62], 0.87
Ovulation Disorder	44 (14.4)	0.62 [0.31, 1.22], 0.171
Unexplained	8 (2.6)	2.66 [0.85, 8.50], 0.091
Others	40 (13.1)	0.65 [0.37, 1.11], 0.121
Types of transfer – no. (%)		
Fresh embryo transfer	14 (4.6)	Ref.
Frozen embryo transfer	291 (95.4)	2.70 [1.20, 6.93], 0.025
No. of blastocyst transferred		
1	227 (74.4)	Ref.
2	78 (25.6)	1.57 [1.02, 2.43], 0.042
No. of good quality blastocyst transferred		
0	51 (16.7)	
1	229 (75.1)	–
2	25 (8.2)	–

Values are mean ±standard deviation, median [quartile 1; quartile 3], or number (%). ART: assisted reproductive technology. OR: odds ratio; CI: confidence interval.

the Paraffin oil group compared to 152/385 (39.5%) couples in the Mineral oil group (RR 1.02, 95% confidence interval [CI] 0.91 to 1.14%) (Table 3). Singleton delivery was recorded in almost cases in both groups. The average birth weight in singleton deliveries was 3212.8 g in the Paraffin group and 3121.8 g in the Mineral group. Other clinical outcomes such as ongoing pregnancy, clinical pregnancy, positive pregnancy, multiple pregnancy, miscarriage, ectopic pregnancy, and implantation were also comparable between the two groups (Table 3).

Predictive factors for live birth

The independent predictive factors for live birth after multivariate analysis were antral follicle count, types of transfer, and the number of embryos transferred. Neither the type of oil nor the different trial centres were a significant predictor of live birth (Table 4).

Discussion

In this multi-centre, retrospective cohort study on day-5 embryo culture, our data showed that Paraffin oil and Mineral oil resulted in a similar live birth rate and laboratory outcomes.

The success of an IVF/ICSI cycle, defined as a single healthy live-born baby, is highly dependent on the quality and stability of the embryo culture system, of which, oil overlaid plays a crucial role. Oil has been universally used, during the manipulation and culture of human gametes and embryos, to overlay embryo culture media [9]. An oil overlaid is supposed to have numerous advantages such as preventing and minimising fluctuations of culture conditions, including temperature, pH and osmolality evaporation, as well as reducing the chances of microbial contamination [1–3].

Several commercial oils for human IVF/ICSI are currently available on the market. However, these products are known to have different chemical properties [13]. It has been shown that the polycarbonate lipid tail in Mineral oil has more unsaturated bonds than in Paraffin oil [14]. Therefore, the mineral product tends to be more sensitive to photooxidation and peroxidation [15]. Moreover, it has been shown that the type of oil used could influence the in vitro development of mouse [16], bovine [7] and porcine embryos [17]. The reasons why different oil types might differently affect embryonic development remain unclear. However, two main mechanisms should be considered. First, the composition of the culture medium can be modified due to the interaction with lipophilic compounds. Second, embryo-toxic compounds in the oil may be transferred to the culture medium and affect the embryonic development [17]. In humans, Sifer et al. (2009) compared four different oil covering systems in different groups of patients. They showed that day-2 embryonic morphologies were not different between the four groups; however, more top-quality day-3 top embryos with Ovoil (Vitrolife) than with the other three systems (one being a mineral product from CryoBioSystem) were obtained [3]. However, these findings were not supported by a recent study, using sibling oocytes [9] and

Appendix Table 1
Reasons for undergoing freeze-all in the study.

	Paraffin oil (n = 356)	Mineral oil (n = 353)	P-value
Reasons for freeze-all			0.34
Agonist trigger	225 (63.2)	230 (65.2)	
Patients' preference	113 (31.7)	106 (30)	
Premature progesterone rise (≥1.5 ng/ml)	8 (2.2)	11 (3.1)	
Unfavorable endometrium	9 (2.5)	4 (1.1)	
Fluid in cavity	1 (0.3)	0 (0)	
Risk of OHSS	0 (0)	2 (0.6)	

Values are number (%). OHSS: Ovarian hyperstimulation syndrome.

Appendix Table 2
Number and type of blastocyst transfer.

	Paraffin oil (n = 377)	Mineral oil (n = 385)	P-value
No. of blastocyst transferred			0.23
1	308 (81.7)	301 (78.2)	
2	69 (18.3)	84 (21.8)	
Number of blastocyst transfer in the subgroup of the transfer type			0.25
Fresh transfer			
1	18 (4.8)	28 (7.3)	
2	3 (0.8)	4 (1.0)	
Frozen transfer			
1	290 (76.9)	273 (70.9)	
2	66 (17.5)	80 (20.8)	

Values are number (%).

by our data. Apart from the laboratory outcomes, we also showed that live birth was not statistically significant difference between the two groups. These contrary results could be partly due to the quality of the oil used in ART has been improved, through the exhaustive and specialised quality control testing of the companies [13]. In view of a 1.1% absolute difference in live birth between the two groups, the decision to which Paraffin oil or Mineral oil should be used should be solely based on the availability and the pricing of the product.

Our study is the first multi-centre trial to compare the effectiveness of Paraffin oil and Mineral oil on the blastocyst stage embryo development and to report the live birth with large sample size.

The retrospective analysis of this study is a limitation. However, this multi-centre design offered an easier approach, including faster data obtainment and reducing staff workload, than a prospective study or a randomised controlled trial. Although this might contribute to some bias, the large sample size might increase the precision of the treatment effect. The two centres were under the same management and used the same standard protocols. Although the total FSH dose was significantly different between the two groups, this difference was unlikely to have any effect on treatment outcomes, as shown in the results of laboratory outcomes and the multivariate regression analysis. In this trial, an ICSI-for-all strategy was applied. However, there is no data suggesting that the treatment response could be affected by fertilisation methods. Finally, continuous embryo culture media (Global Total LP, LifeGlobal) were used; however, the media were renewed on day 3. Therefore, data on the influence of continuously covering oils for 5 days culture was lacking.

In conclusion, a comparable live birth rate could be achieved with Paraffin oil or Mineral oil in day-5 embryo culture. Therefore, the choice of which one to use might be based on the availability and the product pricing.

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Declaration of Competing Interest

BWM has acted as a paid consultant to Merck, ObsEva and Guerbet, and is the recipient of money from an NHMRC Investigator Grant. All other authors declare no competing interests in the submitted work in the previous three years and no other relationships or activities that could appear to have influenced the submitted work.

Appendix

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