Scientific and Clinical Advances Advisory Committee (SCAAC) – Matters arising

Monday 02 October 2023

Date	Action	Responsibility	Due date	Progress to date
31/01/2022	Assess whether further outputs are required on the topic of the impact of the microbiome, and whether it needs to be considered as a treatment add-on.	Mina Mincheva, Policy Manager	Ongoing	The topic 'Impact of microbiome on fertility treatment outcomes' is brought as an agenda item for the October 2023 SCAAC meeting. The committee will assess if an add-ons application on this topic should come back to the SCAAC for consideration.
06/06/2022	The Executive will amend the treatment add-ons application form decision and tree in line with the updated treatment add-ons rating system. SCAAC can then reconsider the application for Androgen supplementation as a treatment add-on.	Dina Halai, Head of Policy	Ongoing	The Executive are in the process of amending the treatment add-ons application form and decision tree. The application for Androgen supplementation as a treatment add-on will then be brought to a future meeting of the SCAAC for reconsideration.
03/10/2022	Consider a framework for assessing AI technologies which fall within the regulatory remit of the HFEA. Publish a Clinic Focus article for the sector on developments in the regulation of AI.	Mina Mincheva, Policy Manager	Ongoing	AI will be next discussed by the SCAAC in February 2024. In the interim, the Executive will develop a report detailing the uses of AI in clinics that fall within HFEA's regulatory remit and publish a clinic focus as part of that work.
25/07/2023	Executive to update the website information for patients about treatment add-ons to reflect new ratings recommended by SCAAC members in July 2023.	Dina Halai, Head of Policy	Ongoing	The anticipated go live date for the updated website add-ons information is early October 2023 and will be accompanied by a media campaign.

Health outcomes in children conceived by ART (incl. the impact of culture media)

Details about this paper

Area(s) of strategy this paper relates to:	The best care
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	5
Paper number:	HFEA (02/10/2023) 005
Meeting date:	02 October 2023
Author:	Mina Mincheva, Policy Manager
Annexes	N/A

Output from this paper

For information or advice?	For information
Recommendation:	Members are asked to: • Advise the executive if they are aware of any other recent developments; and • Review whether any outputs from the HFEA are required addressing health outcomes of children conceived from ART.
Resource implications:	N/A
Implementation date:	N/A
Communication(s):	None
Organisational risk:	Low

1. Introduction

1.1. Assisted reproductive technology (ART) includes techniques such as egg freezing, in vitro fertilisation (IVF) and intra cytoplasmic sperm injection (ICSI). There is a possibility that children born from ART may be at risk of birth defects or developing longer-term health issues, though this could be due to underlying infertility rather than the ART procedure. Culture media used in IVF systems acts as a surrogate for maternal nutrition for the first few days, therefore it is important to optimise the culture environment of embryos during IVF treatment. The components of embryo culture media, therefore, require scrutiny to ensure that risks are minimised, embryo stress is avoided, and embryo health is maintained.

1.2. SCAAC last discussed health outcomes following ART in **February 2020** with an an invited expert speaker. It was noted that there has been an increase in the number of studies investigating the effects of fresh and frozen embryo transfer on perinatal outcomes. However, as with the paper considered by SCAAC in June 2017, studies looking at long-term developmental outcomes still appear to generally have small sample sizes compared to studies that focus on birth outcomes. The committee **concluded** that the research shows reassuring data on health outcomes, and the overall trajectory of IVF is getting better, not worse.

1.3. The HFEA's Code of Practice (section 4.8 and 4.9) currently requires clinics to provide certain information about the treatment and risk of treatment. It specifically states that before treatment is offered, the centre should give the woman seeking treatment and her partner, if applicable, information about:

Section 4.8

(a) the likely outcomes of the proposed treatment (data provided should include the national live birth rate and clinical pregnancy rate and the centre's most recent live birth rate and clinical pregnancy rate; centres are encouraged to provide data per embryo transferred where relevant)

Section 4.9

a) the potential immediate and longer-term risks of the treatment and any treatment add-ons used, including the risks to the patient and the possibility of any children conceived having developmental and birth defects

(d) the nature and potential risks (immediate and longer term) of using emerging or unproven treatments, including reference to the centre's experience and wider evidence base

1.4. 'Risk of fertility treatment' section on the HFEA's **website** provides some information about the risks of birth defects associated with ART and states that research in this area is ongoing and the HFEA will continue to review and update the information as more evidence becomes available.

1.5. 'Impact of embryo culture media' was last discussed in **February 2021**. It was highlighted that it is not within the HFEA legal remit to regulate the composition, quality and safety of culture media, but under that of the MHRA and associated UK Approved Bodies. It was noted that it is up to the discretion of SCAAC to consider long-term health outcomes of children born after ART and to make recommendations since neither MHRA, nor the European Commission track those outcomes.

1.6. Concerns about the effect of culture media on birthweight, imprinting disorders, pregnancy rate and live birth rate (LBR) have been previously discussed by the SCAAC, and in 2021 the SCAAC concluded that though research in this area has continued to progress, the impact of changes in culture media composition for early embryo development and the long-term health effects of children conceived by ART remains unclear.

1.7. At the **January 2022 SCAAC** meeting the Executive suggested that the monitoring of embryo culture media is incorporated into the 'Health outcomes in children conceived by ART' topic as culture media is evaluated only in the context of its safety for the embryo and the health of any child born as a result of fertility treatment.

1.8. The research highlighted in this paper has been published between January 2020 and September 2023.

2. Research

Studies comparing different assisted reproductive technology (ART) methodologies

2.1. A retrospective study by (Liu et al., 2021) evaluated the association between serum oestradiol (E2) levels on the day of human chorionic gonadotrophin (hCG) administration and neonatal birthweight after IVF embryo transfer (ET) cycles (n=3659 patients). Patients were categorized by serum E2 levels (pg/ml) into 6 groups: group 1 (≤ 1000, n= 230), group 2 (1001 – 2000, n= 524), group 3 (2001 - 3000, n= 783), group 4 (3001 - 4000, n= 721), group 5 (4001 - 5000, n= 548), and group 6 (> 5000, n= 852). There was no difference in low birthweight (LBW) rates among the groups. There was no association between peak E2 level during ovarian stimulation (OS) and neonatal birthweight after IVF-ET, or between higher E2 levels and higher LBW risk.

2.2. A registry-based study by (Luke et al., 2021a) evaluated the associations between excess embryos transferred, excess foetal heartbeats (FHB) and adverse perinatal outcomes (n= 138,435 children). In singletons with [2 ET, FHB =1] and [≥3 ET, FHB=1], risks [adjusted odds ratio (aOR) and 95% confidence interval (CI)] were increased, respectively, for major non-chromosomal birth defects [1.13 (1.00–1.27) and 1.18 (1.00–1.38)], small for gestational age (SGA) [1.10 (1.03–1.17) and 1.15 (1.05–1.26)], LBW [1.09 (1.02–1.13) and 1.17 (1.07–1.27)], and preterm birth [1.06 (1.00–1.12) and 1.14 (1.06–1.23)]. With excess embryos transferred and excess FHB, risks of all adverse outcomes except for major non-chromosomal birth defects increased further for both singletons and twins.

2.3. A retrospective study by (Li et al., 2021a) analysed pregnancy outcomes in frozen embryo transfer (FET) (n=12,950) performed using different endometrial preparation protocols. FET cycles were categorised into three groups: natural cycles, hormone replacement therapy (HRT) and OS protocols. The live birth rate (LBR) was slightly lower for HRT cycles than for natural cycle (HRT vs. NC: 28.15% vs. 31.16%, $p < 0.001$). The pregnancy loss rate was significantly higher in OS or HRT cycles than in natural cycle (HRT vs. NC: 17.14% vs. 10.89%, $p < 0.001$; OS vs. NC: 16.44% vs. 10.89%). Preparing the endometrium using OS or HRT protocols

increased the risk of preeclampsia (PE) in both singleton and multiple deliveries. OS and HRT protocols increased the risk of LBW and SGA in both singletons and multiples after FET.

2.4. A retrospective cohort study by (Shats et al., 2021) compared obstetrical, perinatal and neonatal outcomes of ART cycles with laser assisted hatching (AH) technique (n=120) with control cases (n=113). No significant differences were observed between AH and control group in rates and risks of all obstetrical and perinatal outcomes rates, as well as those for congenital malformations [5.8 vs. 4.4%, respectively, odds ratio (OR) 1.33, 95% CI 0.41–4.34] and developmental delay (19.2 vs. 12.8%, respectively, OR 1.62, 95% CI 0.74–3.52).

2.5. A prospective cohort study by (Wessel et al., 2021) investigated the effect of ovarian stimulation and laboratory procedures on perinatal outcomes of ART-conceived singleton pregnancies (n=472) in couples with unexplained infertility and compared them with outcomes from naturally conceived singleton pregnancies (n=125). Singletons born after intrauterine insemination (IUI) with ovarian stimulation (IUI-OS) had a lower birthweight (adjusted difference - 157.7 g, 95% CI -277.4 to -38.0), while singletons born after IVF had comparable birthweights (adjusted difference 20.9 g, 95% CI -110.8 to 152.6).

2.6. A retrospective cohort study by (Xu et al., 2022) investigated the association between embryo cryopreservation duration and pregnancy-related complications and birth weight after FET cycles (12,158 cycles with 3864 deliveries). Women undergoing FET after a cryopreservation time longer than 3 months did not show any increased risk of gestational diabetes mellitus (GDM), gestational hypertension, PE, meconium staining of the amniotic fluid, or preterm birth. The risk of lower birthweight, macrosomia, SGA or large for gestational age (LGA) was not affected by long-term cryopreservation.

2.7. A study by (Zhang et al., 2021a) compared pregnancy outcomes of two regimens of FET (letrozole-FET and HRT-FET) in women with ovulation disorders undergoing IVF/ICSI FET cycle (n=2782 cycles). Neonatal outcomes were similar between the two groups. The LBR was higher in the letrozole-FET group (aOR 1.30, 95% CI 1.06-1.58). The rate of miscarriage was lower in the letrozole-FET group (aOR 0.63, 95% CI 0.44-0.90).

2.8. A retrospective study by (Chen et al., 2021) investigated the impact of laser-assisted selection of viable but immotile spermatozoa in testicular sperm aspiration (TESA) ICSI cycles on the obstetric and neonatal outcomes. In the test group (25 fresh ET and 8 FET) oocytes were injected with immotile spermatozoa selected by laser. The control group included 75 fresh ET and 24 FET. No significant differences were found in the pregnancy, implantation, miscarriage, LBR and cumulative LBR in the test group in either fresh ET or FET cycles. There were no differences in the average gestational age, premature birth rate, neonatal birth weight, and the malformation rate between the test and control groups.

2.9. A study by (Lloyd et al., 2022) examined the effect of ovulation drugs and ART on methylation status of differentially methylated CpGs in nine imprint control regions (ICRs) from cord blood in ART-exposed (n = 27), clomiphene-only-exposed (n = 22), and non-exposed (n = 516) women. After stratifying for offspring sex, an aberrant methylation pattern was observed in several of the nine ICRs in males and males born to ART- clomiphene-only-exposed mothers.

2.10. A retrospective cohort study by (Xie et al., 2022) compared perinatal outcomes of singletons after blastocyst (n = 3364) versus cleavage-stage (n=1471) embryo transfer (ET) in FET cycles.

Compared to infants born after cleavage-stage embryo transfer, those born after blastocyst transfer had a higher risk of preterm birth (PTB) (aOR: 1.480; 95% CI: 1.213–1.807), LGA (aOR: 1.329, 95%CI: 1.149–1.536) and very large for gestational age (VLGA) (aOR: 1.317, 95%CI: 1.092–1.590). The proportion of LGA was higher for boys born after blastocyst-stage ET between gestational week (GW) 37 to 41 (aOR 1.301, 95%CI 1.051–1.609) and for girls born after blastocyst-transfer between GW 32 to 41 (aOR 2.690, 95%CI 1.154–6.271, aOR 1.377, 95%CI 1.094–1.733), compared to boys and girls born after cleavage-stage ET.

2.11. A prospective cohort study by (Legro et al., 2020) investigated the development from birth to age 3 of children conceived from non-IVF infertility treatments (n=185) of women with polycystic ovary syndrome (PCOS) or unexplained infertility diagnosis who were treated with letrozole, clomiphene or gonadotropins. There were no significant differences among the three groups at any time point with respect to scores on the Scores from Ages and Stages Developmental Questionnaire (ASQ). On the MacArthur-Bates Communicative Development Inventory (MCDI) Words and Gestures, the letrozole Z group scored higher than the gonadotropin group for phrases, early gestures, later gestures, and total gestures. Those in the clomiphene group scored higher than the gonadotropin group for the later gestures and total gestures items.

2.12. A meta-analysis (n=7 studies) by (Strowitzki et al., 2021) assessed obstetric and neonatal outcomes in children conceived after in-vitro culture of immature oocytes (IVM). They compared the outcomes to those of children conceived after OS in relation to maternal indications and the type of IVM protocols with or without ovulation trigger. Birthweights of IVM children was comparable to OS controls. IVM children had a comparable birthweight to OS children, irrespective of whether an ovulation trigger was used in IVM cycles or not. There was no difference in the PTB rate of singleton pregnancies between IVM and COS. The malformation rate in IVM children did not differ in COS children versus children after natural conception. At the age of 2 years, IVM singletons showed similar anthropometric and mental development compared to COS children or children from natural conception.

Outcome after fresh versus frozen embryo transfer

2.13. A study by (Conforti et al., 2021) performed meta-analysis (n=20) to compare perinatal and obstetric outcomes between cryopreserved transfer and fresh blastocyst embryo transfer. The risk of both PTB (OR: 0.89, 95% CI: 0.80–0.99, I2= 39%) and LBW (OR: 0.82, 95% CI: 0.68–0.99, I2=68%) was significantly lower after cryopreserved blastocyst transfer than after fresh blastocyst transfer. The rate of LGA births was significantly higher (OR: 1.68, 95% CI: 1.55–1.82, I^2=23%) and the rate of SGA births significantly lower (OR 0.59: 95% CI: 0.54–0.65, I2=10%) after cryopreserved blastocyst transfer. Transfer of cryopreserved blastocysts was associated with a lower risk of placental abruption (OR: 0.58, 95% CI 0.40–0.83, I2=0%) but a higher risk of Caesarean section (OR: 1.21, 95% CI: 1.01–1.43, I2=77%).

2.14. A retrospective register-based case–control study by (Hallamaa et al., 2021) compared pregnancy and perinatal outcomes of transfers using embryos cryopreserved twice (case group, n = 89) and once (control group, n = 304). The rates of live birth (27.0% versus 31.9%, aOR: 0.70, 95% CI 0.40–1.22), clinical pregnancy (31.5% versus 36.8%, adjusted OR 0.71, 95% CI 0.42–1.21) and miscarriage (4.5% versus 3.9%, adjusted OR 1.10, 95% CI 0.33–3.60) in the case and the control groups were comparable. No difference was seen in the preterm delivery rate

(cases 4.2% versus controls 10.3%). No difference in birthweight was detected between the groups and there were no LGA foetuses or congenital malformations in the case group.

2.15. A study by (Jin et al., 2021) performed meta-analysis (n=4) to compare pregnancy outcomes between frozen and fresh embryo transfers in ovulatory women and women with non-PCOS. There was no difference in LBR, singleton birth weight, clinical pregnancy, ongoing pregnancy, gestational diabetes, and gestational hypertension between frozen and fresh embryos. In frozen embryos, the relative risk of moderate or severe ovarian hyperstimulation syndrome (OHSS) was lower, the incidence rate of PE higher, and the standardized mean difference of twin birth weight higher than in fresh embryos.

2.16. A retrospective single centre study by (Permadi et al., 2021) compared LBR and neonatal outcomes between fresh (n=239) and frozen (n=112) IVF cycles. Among patients aged ≤30, LBR was higher in the fresh-IVF cycle group [27 (47.4%) vs 2 (12.5%)]. Among those with ovulatory disorder, LBR was significantly higher with frozen compared to fresh IVF cycle [7 (43.8%) vs 10 (17.5%)]. Neonates from singleton pregnancies from frozen IVF cycles had a higher birth length compared to those resulting from fresh IVF cycle.

2.17. A retrospective study by (Terho et al., 2021a) compared birthweight of singletons born after FET (n=17,500) to those born after fresh ET (n=69 510) and natural conception (n=3 311 588). Mean birth weights were significantly higher after FET compared to fresh ET starting from GW 33 (range 75-228 g by week) for boys and starting from GW 34 (range 90-236 g by week) for girls. All singletons born after FET had a higher risk of LGA compared to singletons born after fresh ET (aOR 1.87, 95% CI 1.76–1.98) and singletons born after natural conception (aOR 1.28, 95% CI 1.22–1.35).

2.18. An observational cohort study by (Terho et al., 2021b) compared the growth of singletons born after FET (n = 110) and fresh ET (n = 181) and their matched natural conception controls (n = 543). Childhood growth did not differ between term singletons born after FET, fresh embryo transfer and natural conception, correcting for exact age at measurement and adjusting for maternal body mass index (BMI) and paternal height.

2.19. A retrospective study by (Zhang et al., 2021c) compared perinatal outcomes of 1663 FET and 3964 fresh ET cycles in women ≥35 years old. FET-conceived singletons had significantly higher mean birth weight (3388.78 ± 538.47 vs. 3316.19 ± 549.08) and had an increased risk of macrosomia (13.5 % vs. 10.4 %, aOR: 1.35, 95% CI: 1.07-1.71) and decreased risk of LBW (3.6 % vs. 5.3 %, aOR: 0.67, 95% CI: 0.45-1.00).

2.20. A nested case-control study by (Gao et al., 2022) the growth characteristics of children born to women who underwent FET using embryos developed from frozen gametes (DFT) (n=745) and compared them to controls, born to women who underwent FET with fresh gametes (n=2,980) or fresh ET (n=2980). The rate of LGA babies in the DFT and FET group was higher than that for the fresh ET group (30.9% vs. 24.8%; 29.4% vs. 24.8%, respectively). The height z-score of children born after DFT and FET was higher than that for children born after fresh ET (β = 0.21, 95% CI 0.07–0.35; β = 0.17, 95% CI 0.05–0.28, respectively). However, childhood growth measurements were not significantly different among the three groups.

2.21. A cohort study by (Mizrak et al., 2022) investigated arterial stiffness and other characteristics of cardiovascular function in 8- to 9-year-old singletons conceived FET (n=50) or fresh ET (n=50)

and compared it to that of naturally conceived children (n=50). There was non-significant tendency of lowered aortic ascendens distensibility in children born after FET compared to fresh ET (β estimate (95% CI): −0.99 10−3 mmHg−1 (−2.20; 0.21)) and naturally conceived children [β estimate (95% CI): −0.77 10−3 mmHg−1 (−1.98; 0.44)].

2.22. A retrospective cohort study by (Asserhøj et al., 2023) assessed the effect of FET vs fresh ET on BMI of singletons aged 7–10 years (n = 200 and n = 203, respectively) and compared it to naturally conceived peers (n = 203). FET-conceived children had higher birthweight compared to both fresh ET and naturally conceived children [mean difference (MD) 0.42, 95% CI 0.21 – 0.62 and MD 0.35, 95% CI 0.14 – 0.57]. At 7–10 years, no differences were found in BMI comparing FET to fresh ET, FET to naturally conceived, and fresh ET to naturally conceived. Similar results were also found regarding the secondary outcomes weight, height, sitting height, waist circumference, hip circumference, fat, and fat percentage.

2.23. A registry-based cohort study by (Landsverk et al., 2023) compared birthweight, foetal growth and placental efficiency between singletons born after FET (n=3093), fresh ET (n=15,510) naturally conceived singletons (n=1,125366). Within sibships, FET-conceived children born had longer mean length and head circumference at birth, but a similar ponderal index compared to naturally conceived, while fresh ET-conceived children had a shorter length and head circumference, and lower ponderal index.

2.24. A population-based cohort study by (Westvik-Johari et al., 2023) investigated whether the risk of stillbirth and neonatal death differ after fresh ET (n=78,642) and FET (n= 18,084) compared with spontaneously conceived singletons. The odds of stillbirth were similar after fresh ET and FET, whereas the odds of neonatal death were high after fresh ET (OR: 1.69; 95% CI 1.46–1.95) and FET (OR: 1.51; 95% CI 1.08–2.10). Within gestational age categories, risk of stillbirth and neonatal death was similar for all conception methods, except that fresh ET singletons had a higher risk of stillbirth during GW 22–27 (OR: 1.85; 95% CI, 1.51–2.26).

2.25. A study by (Turner et al., 2020) assessed the growth trajectory of children born following fresh ET (n=576), FET (n=179) and children born after natural conception (n=65,683). The first trimester crown-rump length was significantly longer after fresh ET compared to the naturally conceived group. Second trimester head size was larger after fresh ET and after FET compared to naturally conceived group. Birth weight was lower after fresh ET conception compared to FET. At 5 years of age, children conceived by fresh ET and FET were no heavier than naturally conceived peers.

2.26. A study by (Vuong et al., 2020) investigated the longer-term development outcomes in children born after freeze-only (n=147) versus fresh ET (n=120). Height and weight were similar in the freeze-only and fresh ET groups. Scores for problem solving were significantly better in the freeze-only vs fresh ET group. Fine motor skills scores were numerically higher in freeze-only vs fresh ET group in the overall analysis, but not statistically significant.

Outcomes after embryo testing

2.27. A study (SITES et al., 2021) compared singleton births outcomes following autologous frozen-thawed single embryo transfers of cycles having embryo biopsy (n=585) to those having no biopsy as the reference (n=2,191). There were no differences between groups with respect to placental disorders (aOR=1.16, 95% CI: 0.60-2.24); preterm birth (aOR=1.22, 95% CI: 0.73-

2.03); low birthweight (aOR=1.12, 95% CI: 0.58-2.15); caesarean section delivery (aOR=1.04, 95% CI: 0.79-1.38); or GDM (aOR=0.83, 95% CI: 0.50-1.38).

2.28. A retrospective study by (Lewis et al., 2021) examined the health, well-being and development of school-aged children (5–8 years old) conceived after IVF with following preimplantation genetic testing (PGT) (PGT cohort n=155) and those conceived after IVF without PGT (IVF-only cohort, n=303). The two cohort did not diff with regards to maternal characteristics, birth defect frequency and pregnancy characteristics. While no significant differences between the PGT and IVF-only cohorts were found for most of the general health and psychological scales, there were differences when compared with population data. Children in the PGT-cohort appeared to have more positive outcomes in many of the measures.

Outcomes after the use of donor versus partner sperm

2.29. A study by (Allen et al., 2021) performed a meta-analysis (n=36 studies) of obstetric and perinatal outcomes of children conceived through IVF/ICSI or IUI using donor sperm compared with partner sperm. There was an increased risk of SGA [relative risk (RR): 1.42, 95% CI: 1.15–1.76] in pregnancies conceived with donor sperm versus partner sperm. There was no difference in the overall RR of preterm birth, high birthweight, LGA, stillbirth and neonatal death. Infants born following IUI or IVF/ICSI using donor sperm had higher mean birthweight compared to infants born following IUI or IVF/ICSI using partner sperm, respectively (MD: 80.53 g, 95% CI: 43.90–117.15 and MD: 57.88 g, 95% CI: 31.9083.86). When comparing donor vs partner sperm in IVF/ICSI, there was a reduced risk of low birthweight (RR: 0.91, 95% CI: 0.85–0.97) and an increased risk of a high birthweight (RR: 1.14, 95% CI: 1.04–1.25).

2.30. A study by (Pohjonen et al., 2022) performed meta-analyses analysis (n=17 studies) of obstetric and perinatal outcomes after use of donor versus partner sperm. IUI pregnancies resulting from donor sperm were at an increased risk for PE (pooled aOR: 1.77, 95% CI 1.26–2.48) and hypertensive disorders of pregnancy (pooled aOR 1.55, 95%, CI 1.20–2.00) compared to those with partner sperm. No increased risk was seen for low birthweight or PTB after the use of donor versus partner sperm in IUI. IVF with donor sperm had lower risk of low birthweight compared with IVF with partner sperm (pooled aOR 0.89, 95% CI 0.83–0.94). For hypertensive disorders of pregnancy, PE and preterm birth, no difference was found between IVF with donor sperm versus partner sperm.

Effect of male factor infertility on reproductive outcomes

2.31. A retrospective cohort study by (Ram et al., 2021) compared obstetrical outcomes from pregnancies (n=225), resulting from IVF/ICSI treatment for male factor infertility (n=94) and non-male factor infertility(n=131). Analysis of singleton pregnancies revealed a less likelihood of caesarean delivery, preterm birth, and male gender offspring in the male factor group ($p < 0.05$). These differences were not observed in the sub-analysis for twin pregnancies.

Studies on obstetric, perinatal, neonatal outcomes and growth

2.32. A study by (Li et al., 2021b) compared perinatal maternal and infant condition, physical development and complications of singleton infants at 6 months between women who conceived by IVF (n=145) and women who conceived naturally (n=160). There was no difference in the incidence of complications (very low birth weight, respiratory distress, neonatal haemolysis,

hyperbilirubinemia, hypoglycemia, infectious pneumonia and neonatal asphyxia) between the groups. Hypertension during pregnancy, placental previa, premature membrane rupture, gestational age <32 weeks and very low birthweight were found to be independent risk factors for perinatal complications in IVF-conceived pregnancies [OR (95% CI): 2.49 (1.670–3.731), 2.68 (2.011–3.579), 2.57 (1.911–3.469), 2.656 (1.689–4.178), 2.656 (1.689–4.178), 2.514 (1.286–4.915)].

2.33. A retrospective cohort study by (Libby et al., 2021) compared preterm birth rates and low birthweight rates from IVF pregnancies in couples with infertility (n=371,488) and in couples with prior tubal ligation as their sole indication for IVF n (n=8,478). Of the singletons born to parous women (n=26,463), the incidence of preterm birth was not significantly different in fertile, sterilized couples compared to infertile couples (13.7% vs. 12.0%). The incidence of low birthweight among term singletons was also not significantly different between fertile couples compared to infertile couples (3.5% vs. 3.2%).

2.34. A prospective population-based study by (Magnus et al., 2021) compared growth patterns of children conceived by ART to naturally conceived children. Authors assessed growth from foetal life to age 7 in 81,461 children participating in the Norwegian Mother, Father and Child Cohort Study (79,740 children conceived naturally and 1721 conceived by ART). Differences in body size at age 17 were explored using data for 544 113 adolescents mandatorily screened for military service. At birth, children conceived by ART were shorter (boys −0.3 cm; 95% CI, −0.5 to −0.1), girls −0.4 cm; 95% CI, −0.5 to −0.3) and lighter (boys −113 grams; 95% CI, −201 to −25, girls −107 grams; 95% CI, −197 to −17). ART-conceived children grew more rapidly, achieving both greater height and weight at age 3. They also had a greater height up to age 7 but did not have a greater height or weight by age 17.

2.35. A retrospective study by (Pontesilli et al., 2021) analysed the effect of parental and ART treatment characteristics on perinatal outcomes after IVF, ICSI and FET cycles (n= 36,683) from 13 Dutch IVF clinics. Data was linked to the Dutch Perinatal Registry (n = 2,548 977). The parental characteristic with the greatest effect size on birthweight was maternal diabetes (adjusted difference 283 g, 95% CI: 228–338). Among ART treatment characteristics, FET had the greatest effect size on birthweight (adjusted difference 100 g, 95% CI: 84–117) compared to fresh ET.

2.36. A retrospective cohort study by (Tsakiridis et al., 2022) assessed the impact of abnormal cord insertion on uterine artery pulsatility index (UtA PI), PE, and offspring's birthweight in 4453 pregnancies. Conception via ART was associated with increased risk of abnormal cord insertion (OR: 2.237; 95% CI: 1.561-3.206). Pregnancies with abnormal cord insertion were associated with higher mean UtA PI than those with central/eccentric cord insertion (z score: 0.29 vs 0.01; mean difference: -0.28; 95% CI: -0.399 to -0.165) and lower birth weight centile, but not with increased risk of PE.

2.37. A retrospective cohort study by (Wang et al., 2021c) investigated the effect of infertility aetiology on pregnancy outcomes following IVF-FET cycles (n=4211) and compared to spontaneously conceived pregnancy controls (n=8422). Infertility aetiology was found to affect maternal and neonatal outcomes among all births in the IVF-FET population and to be an additional risk factor for abnormal pregnancy outcomes besides the use of IVF techniques. Higher risk was found for ovulation disorders, and lower risk was found for male infertility.

2.38. A retrospective study by (Cristea et al., 2022) investigated the respiratory pathology in 112 late preterm infants, 9.8% of whom were conceived by IVF. Transient tachypnoea of the newborn was present in 18.1% of IVF-conceived newborns and 33.6% of non-IVF newborns. No IVF late preterm infant required hospitalization in neonatal intensive care for more than 3 days, compared to 19.8% of naturally conceived late preterm infants.

2.39. A study by (Xiong et al., 2022) compared neonatal outcomes between ART-conceived (n=1112) and spontaneous conception (n=4801) infants admitted to the neonatal intensive care unit (NICU). ART was independently associated with neonatal respiratory distress syndrome, NRDS (OR: 1.46; 95% CI: 1.11-1.93) and retinopathy of prematurity, ROP (OR: 1.79; 95% CI: 1.06-3.05) even after adjusting for confounders. ART was associated with neither birth defects (OR: 0.98; 95% CI: 0.77-1.25) nor mortality (OR: 0.98; 95% CI: 0.51-1.91).

2.40. A population-based retrospective cohort study by (Yu et al., 2022) assessed the risk of adverse birth outcomes in 9480 IVF-conceived singletons and 1,952,419 singletons from the general population accounting for infertility cause. Higher risk of preterm birth and low birthweight were observed among parents with PCOS, endometriosis, uterine and semen abnormalities. Compared to the general population, after excluding the influence of infertility causes, IVF-conceived singletons were at higher risk of macrosomia [standardised RR (SSR): 1.28, 95% CI 1.14–1.44] and LGA (SRR: 1.25, 95% CI 1.15–1.35).

2.41. A population-based retrospective cohort study by (Dimanlig-Cruz et al., 2023) investigated the effect of mode of conception on perinatal and paediatric outcomes in 177,901 births (spontaneous conception, ART - IVF and non-ART - ovulation induction, intra-uterine or vaginal insemination). There were increased risks (aRR [95% CI]) of preterm birth (ART: 2.06 [1.98-2.14]; non-ART: 1.85 [1.79-1.91]), very preterm birth (ART: 2.99 [2.75-3.25]; non-ART: 1.89 [1.67-2.13]), 5-min Apgar < 7 (ART: 1.28 [1.16-1.42]; non-ART: 1.62 [1.45-1.81]), and composite neonatal adverse outcome indicator (ART: 1.61 [1.55-1.68]; non-ART: 1.29 [1.25-1.34]).

2.42. A nationwide prospective study by (Rodriguez-Wallberg et al., 2020) assessed infant (<1 year) and childhood (1–18 years) mortality in singletons conceived through ART versus naturally conceived singleton controls (2,847,108 of whom were 43,506 ART-conceived). Higher infant mortality risks were seen in ART-conceived infants (aHR 1.45; 95% CI, 1.19–1.77), especially after transfer of cryopreserved embryos (aHR 2.30; 95% CI, 1.46–3.64). Early neonatal mortality risk was increased in children born after transfer of blastocysts (HR 2.40; 95% CI, 1.05–5.48). No increased mortality risk was observed between the ages of 1 and 18 years.

Studies on obstetric and neonatal outcomes in twin pregnancies

2.43. A retrospective study by (Li et al., 2022) investigated clinical and maternal–neonatal outcome data of women with twin pregnancies (n=698) based on whether women underwent ART or conceived naturally. The rate of twin pregnancy complicated by PE was 17.62% (123/698) and twin pregnancies conceived with ART (321/698) had a higher rate of PE (20.9% vs. 14.9%, $p < 0.05$). ART was a risk factor for developing PE in twin pregnancies (aOR: 1.868, 95% CI: 1.187–2.941). Mothers with PE who conceived with ART had a higher rate of delivery at GW < 34 (29.9% vs. 12.5%, $p < 0.05$) and asphyxia of the neonate at 5 min after delivery (13.4% vs. 1.8%, $p < 0.05$) than those who conceived without ART. There was no significant difference in low birthweight, very low birthweight, and neonatal malformations between the two groups.

2.44. A cross-sectional study by (Jiang et al., 2021) compared maternal and perinatal outcomes of 3270 twin pregnancies: 2003 conceived spontaneously and 1209 conceived by IVF/ICSI. The incidences of GDM (aOR: 1.42, 95% CI 1.10–1.83), preterm premature rupture of membranes (PPROM) [aOR: 1.65, 95% CI 1.21–2.25], placenta accreta spectrum (aOR: 2.12, 95% CI 1.42–3.17) and postpartum hemorrhage (aOR: 1.38, 95% CI 1.02–1.86) were significantly higher in the IVF/ICSI group than in the natural pregnancy group. Multivariate analysis also revealed that conception mode was not an independent risk factor for neonate outcomes.

2.45. A study by (Lin et al., 2021) compared PTB and perinatal outcomes between spontaneous twin pregnancies (n=213) and IVF/ICSI dichorionic-diamnionic (DCDA) twin pregnancies (n=1084). IVF/ICSI pregnancies were associated with a slight increase in PTB (aOR: 1.72; 95% CI: 1.24-2.39), iatrogenic preterm birth (aOR: 1.41; 95% CI: 1.00-1.97) and NICU admission (aOR: 1.34; 95% CI: 1.00-1.80), as well as with a decrease in PPROM (aOR: 0.64; 95% CI 0.42-0.99). There were no differences between IVF/ICSI and spontaneous DCDA pregnancies in terms of spontaneous PTB, GDM, pregnancy-induced hypertensive disorder, PE, intrahepatic cholestasis of pregnancy, placenta previa, birthweight discordance, SGA, neonatal respiratory distress syndrome, ventilator support, and perinatal death and/or severe morbidity.

2.46. A cohort study by (Kondowe et al., 2023) compared growth (data collected at birth, 6–8 weeks, and 4–7 years old) between twins conceived with ART (n=13,528) and naturally conceived twins (n= 10,180). The average birthweight of fresh ET twins was lower (–35 g; 95% CI: −53, −16g) than controls, while FET twins were heavier (71 g; 95% CI: 33, 110 g) than controls and heavier (106 g; 95% CI: 65, 146 g) than fresh ET twins. Growth rates did not differ for the three groups from birth to 6–8 weeks. However, FET twins grew faster from 6 to 8 weeks than controls (by 2.2 g/week) and fresh ET twins (by 2.1 g/week). By 4-7 years old, FET twins were heavier than naturally conceived and fresh ET twins, respectively.

Congenital malformations and birth defects

2.47. A study by (Dieamant et al., 2021) conducted meta-analysis (n=3) to compare rate of birth defects in children (n=3907) born after ICSI (n=2627) or intracytoplasmic morphologically selected sperm injection (IMSI) [n=1280]. The incidence of birth defects was statistically different, with 2.5% (32/1280) in IMSI and 4.5% (119/2627) in ICSI (RR=0.59; 95% CI=0.40-0.87). IMSI decreased the incidence of structural defects compared to ICSI (RR: 0.58; 95%CI=0.35- 0.96). No significant difference was observed in chromosomal abnormalities (Trisomy 13; 18; 21 and Triple X) between children conceived after IMSI (8/830) or ICSI (19/2049) (RR: 1.07; 95% CI 0.47-2.43).

2.48. A retrospective case control study by (Iacusso et al., 2021) analysed the relationship between ART and the development of anorectal malformation (ARM) [n=369 patients, 143 consecutive cases and 226 controls (infants admitted for bronchiolitis)]. Patients with ARM born after ART were compared with naturally conceived infants for disease complexity [association to vertebral, anal, cardiovascular, tracheoesophageal, renal, limb (VACTERL) or associations to genetic disorders]. Prevalence of ART was significantly higher in ARM patients (OR: 2.59; 95% CI: 0.98–0.68). Prevalence of VACTERL association was significantly higher in ART-born patients with ARM as compared to those naturally conceived (OR: 3.39; 95% CI, 0.96–0.12).

2.49. A population-based cohort study by (Luke et al., 2021b) investigated the risk of birth defects with conception by ART. Compared to naturally conceived children, ART singletons (conceived from autologous oocytes, fresh embryos without the use of ICSI) had increased risks of a major non-

chromosomal birth defect, cardiovascular defects, and any birth defect. Compared to naturally conceived children, ART singletons conceived with the use of ICSI had higher risks of a major non-chromosomal birth defect, blastogenesis defects, cardiovascular defects. Within ART singleton births conceived from autologous oocytes and fresh embryos, the use of ICSI was associated with an increased risks of a major non-chromosomal birth defect, blastogenesis defects, gastrointestinal defects and any defect.

2.50. A prospective cohort study by (Lv et al., 2021) investigated the prevalence of birth defects in 1,825 women with ART-pregnancy and 3,483 women with spontaneous pregnancy. The prevalence of any defects was significantly higher among ART-births than their non-ART counterparts at each follow-up, specifically at prenatal screening (2.2% vs. 1.2%), at delivery (4.9% vs. 2.9%), at 6 months (10.4% vs. 5.3%) and 1 year of age (13.9% vs. 7%). Among ART-births, GnRH antagonist regimen for ovulation induction was associated with an increased risk of birth defects (adjusted risk ratio: 1.47, CI: 1.04–2.07). Additionally, mediation through twinning accounted for 31.1% of the risk of ART-associated birth defects.

2.51. A study by (Talebi et al., 2022) performed systematic review (n=56 studies) to assess the association between ART and the risk of congenital heart defects (CHDs). Children conceived by IVF/ICSI manifested an increased risk of CHDs compared with spontaneously conceived children.

2.52. CHDA prospective birth cohort study by (Wang et al., 2021a) performed a whole-genome sequencing of 1137 individuals from 160 families conceived through ART and 205 families conceived spontaneously to investigate the effect of ART-related elements on germline de novo mutations (gDNMs) and their associations with CHD. ART-conceived children carried 4.59 more gDNMs than those conceived spontaneously, including 3.32 paternal and 1.26 maternal DNMs. The accumulation of non-coding functional mutations was independently associated with CHD and 87.9% of the mutations were with paternal origin.

2.53. A population-based study by (Zhang et al., 2021b) compared birth defects between ART-conceived (n=2699) and naturally conceived children (n=191 368). ART use was associated with an increased risk of any birth defect (5.4% vs 3.5% in ART and non-ART aRR: 1.43, 95% CI: 1.08 to 1.90), especially for chromosomal abnormalities (0.5% vs 0.2% in ART and non-ART group, aRR: 3.11, 95% CI: 1.28 to 7.58), in singleton births to mothers < 35 years old. These associations were not detected in multiple births or mothers ≥35 years.

2.54. A cohort study by (Henningsen et al., 2023) compared the risk of congenital malformations in singletons conceived after fresh- or cryo-ICSI [with fresh (n=32,484) and with cryopreserved embryos (n=7200)], those conceived after fresh IVF (n=47,178) and without medical assistance (n=4,804 844) [aOR 1.07; 95% CI 1.01-1.14 in ICSI vs. IVF; and aOR 1.28; 95% CI, 1.23-1.35 in ICSI vs. no medical assistance; and aOR 1.11; 95%CI 0.99-1.26 in ICSI fresh vs cryo-ICSI]. When grouping malformations by organ systems, ICSI-conceived children had a higher risk of respiratory and chromosomal malformations vs their IVF-conceived peers. When categorizing ICSI-conceived children according to treatment indication, there was a higher risk of hypospadias only after performed for male-factor infertility (aOR 1.85 95%CI 1.03-3.32).

2.55. A cross-sectional study by (Huang et al., 2023) investigated the incidence and type of molecular defects among ART-conceived neonates who are in NICUs with suspected genetic conditions (n=535) and compared them to naturally conceived neonates (n=1316). The overall diagnostic

yield was comparable between the ART group and the control group (10.1% vs 13.2%; OR 0.74; 95% CI 0.53-1.02), as was the proportion of single-nucleotide variant, SNVs (63.0% vs 69.0%; OR 0.68; 95% CI 0.46-1.00) and copy number variation, CNVs (37.0% vs 31.0%; OR 0.91; 95% CI 0.54-1.53) detected by sequencing. The proportions of de novo variants in the ART group and the control group were similar (OR 0.89; 95% CI 0.62-1.30).

2.56. A study by (Yin et al., 2023) investigated the association between IVF and hearing loss in twin neonates. IVF conception and preterm birth conferred a higher risk of hearing loss (IVF aOR 2.82; 95% CI 1.17–6.80 and preterm birth aOR 6.14; 95% CI 2.30–16.40) than naturally conceived peers. More IVF-conceived twins failed in dual-step hearing screening (3.26%) and were diagnosed with hearing loss (2.21%) than those conceived naturally.

2.57. A national registry data study by (Fauque et al., 2021) assessed the risk of congenital anomalies in 3,501 495 singleton children born after natural conception (n=3,417 089), IUI (n=20,218), fresh-ET (n=45,303) and FET (n=18,885). Children born after fresh ET and FET had a higher prevalence of malformations (aOR 1.15; 95% CI 1.10–1.20 and aOR 1.13; 95% CI 1.05–1.21). The overall risk of congenital malformations, and the risk by subtype, was similar in the IUI group and the natural conception group (aOR 1.01 95% CI 0.94–1.08). There was an overall independent increase in the risk of congenital defects when the mothers were diagnosed with endometriosis (aOR 1.16; 95% CI 1.10–1.22), PCOS (aOR 1.20; 95% CI 1.08–1.34) or primary ovarian insufficiency (POI) [aOR 1.52; 95% CI 1.23–1.88]. Chromosomal, cardiac and neurological anomalies were more common in the three maternal infertility groups.

Studies on neonatal outcomes and risk of cancer

2.58. A retrospective cohort study by (Borgmann-Staudt et al., 2022) compared perinatal outcomes, malformations, heart defects and risk of cancer between ART-conceived (n=74) and spontaneously conceived children (n=1585) born to childhood cancer survivors. Multiple-sibling births ($p < 0.001$, 29.7% vs. 2.5%), low birth weight ($p < 0.001$; OR: 3.035, 95%-CI: 1.615–5.706), and preterm birth ($p < 0.001$; OR: 2.499, 95% CI: 1.401–4.459) occurred significantly more often in cancer survivors' offspring following ART utilisation than in spontaneously conceived children. ART did not increase the prevalence of childhood cancer, congenital malformations, or heart defects.

2.59. (Gulrajani et al., 2023) performed a cross-sectional survey of 1701 parents of children with cancer to investigate the association between ART use and childhood cancer subtype. ART use was highest among children with osteosarcoma relative to children with other cancer types, and this association was significant in multivariable models (OR: 4.4; 95% CI: 1.7–11.3). Multiple gestation was associated with a 2.7-fold increased risk of hepatoblastoma (OR: 2.71; 95% CI: 1.14–6.42) and a 1.6-fold increased risk of neuroblastoma (OR: 1.62; 95% CI: 1.03–2.54), but these associations were not retained in multivariable models.

2.60. A population-based cohort study by (Luke et al., 2023) evaluated the risk of childhood cancer as a function of birth defect status and method of conception. The risk of any cancer was increased among ART autologous-fresh and non-ART siblings (hazard ratios [HRs]: 1.31 and 1.34, respectively). The presence of chromosomal defects was strongly associated with cancer risk (HRs: 8.70 for all cancers and 21.90 for leukaemia), and this was further increased in the presence of both chromosomal and non-chromosomal defects (HRs: 21.29 for all cancers, 64.83 for leukaemia, and 4.71 for embryonal tumours).

2.61. A nationwide population-based cohort study by (Weng et al., 2022) investigated the association between different modes of conception and childhood cancers in 2, 308 016 parents-child triads. ART conception was associated with an increased risk of any type of childhood cancers compared with natural conception (HR; 1.58; 95% CI: 1.17-2.12) and subfertility with non-ART conception (HR: 1.42; 95% CI: 1.04-1.95) and was mainly owing to leukemia and hepatic tumor. The elevated cancer risk associated with ART conception was not mediated by preterm birth or low birth weight.

2.62. A multi-institutional retrospective study by (Nemes et al., 2023) compared genetic differences between children with rhabdoid tumours (RT) conceived after ART (n=14) and those with RT conceived without ART (n=211). The incidence of pathogenic germline variants was not different between groups. The 5-year overall survival and event free survival rates of RT-ART patients were comparable to the control (42.9 ± 13.2% and 21.4 ± 11% vs 41.1 ± 3.5% and 32.1 ± 3.3). DNA methylation analyses of tumours and blood samples showed no evidence of a general DNA methylation difference or underlying imprinting defects between groups.

Other health outcomes in ART-conceived children

2.63. A cohort study by (Cui et al., 2021) compared cardiovascular health of 764 ART-conceived and 382 naturally conceived children. ART-conceived children had significantly increased blood pressure, left ventricular systolic dysfunction, and diastolic dysfunction. They also had significantly increased parameters of left ventricular structure, increased prevalence of left ventricular hypertrophy, high relative wall thickness and left ventricle remodelling patterns.

2.64. A prospective longitudinal follow-up cohort study by (Wang et al., 2022a) investigated the association between mode of conception, ART and leukocyte telomere length (LTL), an indicator of age-related phenotypes in later life in offspring. Authors estimated the LTLs of 1,137 individuals from 365 families, including 202 ART-conceived and 205 spontaneously conceived children. One-year-old ART-conceived children had shorter LTLs than those conceived spontaneously (beta, −0.36; P = 1.29 × 10–3) after adjusting for plurality, sex and other potential confounding factors. Blastocyst-stage embryo transfer was associated with shorter LTL (beta, −0.54, P = 2.69 × 10–3) in ART-conceived children. The association was validated in 586 children conceived by ART using different LTL quantification methods.

2.65. A retrospective study by (Cristea et al., 2022) investigated the respiratory pathology in 112 late preterm infants, 9.8% of whom were conceived by IVF. Transient tachypnoea of the newborn was present in 18.1% of IVF-conceived newborns and 33.6% of non-IVF newborns. No IVF late preterm infant required hospitalization in neonatal intensive care for more than 3 days, compared to 19.8% of naturally conceived late preterm infants.

2.66. A multicohort study by (Elhakeem et al., 2022) investigated the association of ART conception with offspring growth and adiposity from infancy to early adulthood (a total of 158 066 offspring with 4329 conceived by ART). Compared to naturally conceived, ART-conceived offspring were shorter, lighter, and thinner from infancy to early adolescence, with differences largest at the youngest ages and attenuating with older child age. Smaller offspring size was limited to individuals conceived by fresh ET but not FET compared to naturally conceived. There was imprecise evidence that offspring conceived by ART developed greater adiposity by early adulthood (ART vs controls difference in fat mass index at age >17 years: 0.23 [95% CI, −0.04 to 0.50] SD units).

2.67. A study by (Penova-Veselinovic et al., 2022) investigated the long-term health of offspring conceived by ART (n=303) by comparing health parameters assessed at age 14, 17 and 20 to those of offspring conceived without ART. There were no significant differences in the DNA methylation profiles between the cohorts, a trend towards lower body size, less subcutaneous adipose tissue, and an increase in visceral and preperitoneal adipose tissue. At 14- and 20-year follow-up, the mean thyroid function was in the normal range for both groups. At 14-year follow-up, there were no differences in the prevalence of asthma, but slightly altered lung function, and a higher prevalence of all allergies assessed in the ART vs. non-ART cohort.

2.68. A study by (Jiang et al., 2022) looked at cardiovascular function in IVF-conceived children (n=482) and compared it to that of naturally conceived offspring (n=421). At 2 years of age systolic blood pressure was higher in IVF born offspring compared to controls. Authors investigated potential mechanism behind high risk of hypertension by analysing expression of maternally expressed gene 3 (MEG3) and endothelium-derived genes in human umbilical vein endothelial cells (HUVEC) cells isolated from umbilical cord of IVF- and naturally conceived children. MEG3 expression was higher in HUVEC cells from IVF-conceived offspring and was significantly related to blood pressure. Endothelium-derived genes in HUVEC cells from IVF-conceived offspring had aberrant expression.

2.69. A study by (Mitter et al., 2022) investigated the risk of upper or lower respiratory tract infections (URTI, LRTI) in ART- conceived children (n=7334) and naturally conceived children of sub-fertile parents (n=1901). Only at age 19–36 months, ART children had increased risk of any LRTI (aRR 1.16; 95% CI 1.01–1.33), increased frequency of LRTIs [incidence rate ratios (IRR) 1.22; 95% CI 1.02–1.47], a higher risk of hospitalization for LRTI (aRR 1.35; 95% CI 1.01–1.80) and an increased frequency of URTIs [adjusted IRR (aIRR) 1.19; 95% CI 1.07–1.33].

2.70. A cross-sectional register-based study by (Pettersson et al., 2022) investigated morbidity up to five years of age, in the children of older, single, and/or ART-treated mothers (n= 23 772). Children born to single mothers and ART-conceived children had significantly more outpatient visits for specialist care and significantly more diagnoses compared to children with married/cohabiting mothers, and spontaneously conceived children. Children born to mothers of advanced maternal age (≥40) experienced significantly more morbidity in the neonatal period.

2.71. A study by (Polinski et al., 2022) investigated the risk of developing asthma and atopic condition in children conceived after infertility treatment (ART and ovulation induction (OI) with or without IUI). Compared to children conceived without treatment, children conceived with any infertility treatment had an increased risk of persistent wheeze by age 3 years (RR: 1.66; 95% CI: 1.17, 2.33) with adjustments for parental atopy among other risk factors. At 7–9 years, children conceived with treatment were more likely to have current asthma (RR: 1.30; 95% CI: 0.98, 1.71), eczema (RR: 1.77; 95% CI: 1.25, 2.49) or receive allergy-related medications (RR: 1.45; 95% CI: 1.06, 1.99). Similar effect sizes were found when examining associations by treatment type (ART versus OI/IUI).

2.72. A retrospective study by (Sanders et al., 2022) looked at associations between fertility treatment and preterm birth (PTB), compared to no treatment in sub-fertile women. After adjustment for confounders, the OR for PTB were 2.17 (95% CI 0.99, 4.75) for births conceived using ovulation drugs, 3.17 (95% CI 1.4, 7.19) for IUI-conceived and 4.24 (95% CI 2.05, 8.77) for IVF-conceived neonates, compared to women with subfertility with no treatment. Female factor infertility

increased the aOR of having a PTB (2.99; 95% CI 1.5, 5.97). Odds ratio was not significant for any type of treatment after restricting analyses to singleton gestation.

2.73. A population-based record-linkage study by (Sutcliffe et al., 2023) compared postnatal general health outcomes between ART-conceived children (n=63,877), their naturally conceived siblings (n=11,343), and matched naturally conceived population controls (n=127,544). ART-conceived children had increased risk of any hospital admission compared with naturally conceived population controls (HR: 1.08; 95% CI 1.05 – 1.10) but not naturally conceived siblings (HR, 1.01; 95% CI 0.94 – 1.09). They also had an increased risk of diagnoses related to neoplasms and diseases of the respiratory, musculoskeletal, digestive, and genitourinary systems, and a lower risk of injury, poisoning, and consequences of external causes. ICSI-conceived children had a lower risk of hospital admission compared with those born after IVF (HR: 0.95; 95% CI, 0.92 – 0.97).

2.74. A population-based cohort study (14,370 920 mother-newborn pairs) by (Wang et al., 2022c) examined the association between fertility treatment and preterm singleton births (122,944 ART-conceived and 71,176 non-ART conceived neonates). Compared with neonates who were naturally conceived, ART- (aOR: 1.49, 95% CI: 1.46-1.52) and non-ART treatment (aOR: 1.35, 95% CI: 1.31-1.38) had significantly higher risk for PTB after full adjustment.

2.75. A study by (Wijs et al., 2022d) performed meta-analysis (n=14 studies) to assess the risk of atopic disorders in offspring conceived with ART compared to those conceived without ART. ART-conceived offspring had increased risk of asthma (RR: 1.28, 95% CI: 1.08–1.51). The allergy rates were not increased in ART-conceived offspring in 9 of 12 studies (aOR range 0.60–1.30).

2.76. A prospective cohort study by (Wijs et al., 2022a) compared the cardiometabolic health of adolescents conceived through ART (n=163) to that of non-ART conceived peers (n=1457). Waist circumference was lower in ART-conceived adolescents. Compared to controls serum triglycerides were lower in ART-conceived female adolescents, while ART-conceived males had higher serum high-density lipoprotein cholesterol (HDLC) and a lower total cholesterol/HDLC ratio compared to controls. Also, IVF-conceived females had less subcutaneous adipose tissue, while ART-conceived males had greater visceral adipose thickness. Pulse wave velocity was lower in ART-conceived males and heart rate corrected augmentation index was lower in ART-conceived females.

2.77. A prospective cohort study by (Wijs et al., 2022c) investigated the prevalence of asthma and allergies in adolescents (14-year-old) conceived with ART (n=152) compared with non-ART conceived counterparts (n=1845). There were no differences in the prevalence of current asthma (aOR: 0.82 95% CI 0.44–1.52), while lung volumes were larger in the ART adolescents. Current allergic rhinoconjunctivitis (ARC) rates were significantly higher in the ART cohort (aOR 1.52, 95% CI 1.03–2.26), with no cohort differences in atopic dermatitis. Food allergies were more prevalent in the ART cohort (aOR 1.89 95% CI 1.17–3.06).

2.78. A study by (Yeung et al., 2022) assessed cardio-metabolic risk in children born after assisted ART or ovulation induction (OI) and compared it to that of children not conceived by treatment (n=559). Singletons conceived with or without ART did not differ in blood pressure, heart rate or pulse wave velocity. OI-conceived singletons were smaller than controls, but the average BMI of the latter was higher than national norms. OI-conceived twins had higher arterial stiffness (0.59 m/s; 95% CI, 0.03-1.15), which was attenuated after accounting for maternal blood pressure (0.29

m/s; 95% CI, -0.03 to 0.46). Twins did not differ in fat measures across the groups. The mode of conception was not associated with the levels of lipids, C-reactive protein, or glycosylated haemoglobin.

2.79. A prospective cohort study by (Boutet et al., 2023) compared foetal cardiac remodelling in singleton pregnancies: spontaneously conceived (SC) from fertile couples (n=96), SC from sub-fertile couples (n=97), and from IVF after fresh ET (n=96). While both fertile and sub-fertile SC groups presented similar foetal cardiac results, IVF foetuses showed larger atria, more globular ventricles and thicker myocardial walls. SC foetuses from fertile and sub-fertile couples had preserved cardiac function, IVF foetuses showed signs of suboptimal systolic and diastolic function.

2.80. A study by (Romanowska et al., 2023) used mother-father-newborn trios from the Norwegian Mother, Father and Child Cohort Study (MoBA) of ART (n= 982) and non-ART (natural) conceptions (n=963) to investigate sex-specific DNA methylation (DNAm) differences on the X chromosome. There were more differentially methylated CpGs and differentially methylated regions (DMRs) in girls than boys and associations persisted after controlling for confounders. Many of the significant CpGs and DMRs were in gene-promoter regions, and several of the genes linked to these CpGs are expressed in tissues relevant for both ART and sex (testis, placenta, and fallopian tube). Observed associations could not be explained with parental DNAm-dependent features.

2.81. A multi-cohort study by (Elhakeem et al., 2023) investigated the association of ART conception (vs. natural conception) with offspring cardiometabolic health outcomes (n=35, 938 offspring of which 654 conceived after ART). There was no statistical difference (ART minus natural conception) in heart rate (0.02 beat/min; -0.91 to 0.94) or systolic/diastolic blood pressure, and for triglycerides, glucose, insulin, and glycated haemoglobin. Total cholesterol (2.59%; 0.10-5.07), high density lipoprotein (HDL) cholesterol (4.16%; 2.52-5.81), low density lipoprotein (LDL) cholesterol (4.95%; 0.47-9.43) were significantly higher in ART-conceived offspring.

2.82. A register-based cohort study by (Henningsen et al., 2020) compared OR of imprinting disorders between children conceived by ART (form Denmark, n = 45,393 and in Finland, n = 29,244) and naturally conceived children (full background populations born during the same time periods). The overall adjusted OR for four imprinting disorders in ART children compared with naturally conceived children was 1.35 [95% CI: 0.80–2.29]. Adjusted OR and absolute risk for Beckwith–Wiedemann syndrome in ART children was 2.84 [95% CI: 1.34–6.01] and 10.7 out of 100,000 newborns, respectively. The risks of Prader–Willi syndrome, Silver–Russell syndrome and Angelman syndrome were not increased in children conceived after ART.

2.83. A population study by (Norrman et al., 2020b) investigated the risk of developing type I diabetes in children born after ART (n=47 938) and compared to those born after spontaneous conception (SC) [n=3,090 602]. The risk of type I diabetes in ART vs SC children was not increased after adjustment for calendar year of birth, sex, maternal age, country of birth, educational level, smoking and parental diabetes (aHR 1.07; 95% CI, 0.93–1.23). There was an association between FET and type I diabetes (FET vs fresh ET: aHR 1.52; 95% CI, 1.08–2.14 and FET vs SC: 1.41; 95% CI, 1.05–1.89). No difference was found when comparing ICSI vs IVF (HR 1.08; 95% CI 0.77–1.51).

Studies on mental, social, cognitive development

2.84. A study by (Andreadou et al., 2021) performed a meta-analysis (n=15 studies) to investigate the risk of bearing a child with autism spectrum disorder (ASD) after being conceived by ART. Analysis of a subset of studies (n=9) that examined all offspring and controlled for main common confounder factors revealed that the use of ART is associated with a higher risk of ASD (RR: 1.11, 95% CI: 1.03–1.19, I2=0% after removal of two studies to eliminate the source of heterogeneity), while in the case of studies that focused on singletons (n=4), a there was no statistically significant association between ART and ASD (RR: 0.96, 95% CI: 0.82–1.13, I2=0%).

2.85. A study by (Cheung et al., 2021) compared developmental and behavioural outcomes between IVF- and ICSI-conceived children at 3 years of age (IVF families n=451, ICSI families n=1914). IVF-conceived children had impaired developmental characteristics compared with the ICSI-conceived cohort (aOR: 0.72, 95% CI: 0.5-0.9). Though the length of embryo culture did not seem to influence child development, the abnormal behaviour rate was significantly higher in children from the day 3 embryo transfer cohort (aOR:0.4; 95% CI: 0.05-0.34). Children conceived via ICSI with ejaculated spermatozoa displayed impaired developmental and behavioural characteristics compared to those conceived from surgically retrieved specimens (aOR: 4.9; 95% CI: 1.2-20.7).

2.86. A study by (Cozzani et al., 2021) investigated the cognitive development from early infancy to mid-adolescence of offspring conceived after medically assisted reproduction (MAR) who were born LBW, compared with naturally conceived children who were born LBW and non-LBW (NLBW). MAR LBW children [age 3: β=0.021, 95% CI: -0.198, 0.241; age 5: β=0.21, 95% CI: 0.009, 0.418; age 7: β=0.163, 95% CI: -0.148, 0.474; age 11: β=0.003, 95% CI: -0.318, 0.325; age 14: β=0.156, 95% CI: -0.205, 0.517], on average performed similarly in cognitive ability relative to naturally conceived NLBW at all ages, and displayed higher cognitive scores than naturally conceived LBW children until age 7. When accounted for family characteristics, differences were largely attenuated and became close to zero at age 14.

2.87. A systematic review (n=35) by (Perros et al., 2022) assessed neurodevelopmental outcomes of newborns born after ART compared to those conceived naturally. Most studies showed no effect on the neurodevelopmental outcomes of the offspring. When such an effect was observed, it could be attributed to confounding factors such as subfertility, multiple pregnancies and gestational age at delivery. The increase in the prevalence of neurodevelopmental disorders after ART, as described in studies with statistically significant results, was predominantly marginal, and given the low incidence of neurodevelopmental disorders in the general population, its clinical significance is debatable.

2.88. A retrospective cohort study by (Roychoudhury et al., 2021) compared neurodevelopmental outcomes of ART-conceived preterm infants born at <29 weeks' gestation at 18 to 24 months' corrected age (n=651) and those who were conceived naturally (n=3318). After adjusting for confounders, ART-conceived infants had lower odds of neurodevelopmental impairment (aOR: 0.67; 95% CI: 0.52–0.86) and the composite of death or neurodevelopmental impairment (aOR: 0.67; 95% CI: 0.54–0.84).

2.89. A prospective cohort study by (Vo et al., 2021) compared psychomotor development of 426 ICSI/IVF children with 509 naturally conceived children (control) using Brunet-Lézine scale to assess their Developmental Quotient (DQ). There was no difference in single DQs as motor posture, sociability and global scores between the two cohort groups. However, ICSI/IVF group's

low-score proportion for coordination was 2.16 times that of control group [95 % CI: 1.11–4.21] and its low-score proportion for language was 2.15 times that of control group [95 % CI: 1.15–4.01].

2.90. A population-based cohort study by (Wang et al., 2021b) analysed neurodevelopmental outcomes in 732 ICSI-conceived and 2046 naturally conceived children, sub-categorising them in singleton and multiple-birth groups. The risk of neurodevelopmental disorders was not increased for ICSI children in both groups [single birth (aHR: 0.70, 95% CI: 0.39–1.27); multiple-birth group (aHR: 0.77, 95% CI: 0.43–1.35)]. In the single-birth group, male sex (aHR: 1.81, 95% CI: 1.29–2.54), and intensive care unit (ICU) admission (aHR: 3.10, 95% CI: 1.64–5.86) were risk factors for neurodevelopmental disorders.

2.91. A hospital-based cohort study by (Aoki et al., 2022) assessed developmental outcomes in ART-conceived (n=189) and naturally conceived children (n=536) by covariance analysis of parent-rated questionnaire in nine domains. At 48 months, there was no significant difference between ART- and naturally conceived children, except for the development of receptive language [$F(1,718) = 4.869$, $p = 0.028$], which was found only for boys. At 60 months, no significant difference was found between ART- and naturally conceived children in all domains.

2.92. A study by (Noda et al., 2022) investigated the association between infertility treatment and neurodevelopment in children at 2 and 3.5 years of age. Of 9655 mother–child pairs assessed, 273 were conceived through ovulation induction (OI) or artificial insemination with husband's sperm (AIH) (OI/AIH), and 487 were conceived via ART. The odds of having developmental delays at 2 years of age were higher in OI/AIH- and ART-conceived children (OR: 1.36; 95% CI 1.00 to 1.85 and OR: 1.36; 95% CI 1.07 to 1.72, respectively) than in those conceived naturally. Also, OI/AIH and ART were significantly associated with communication (OR, 1.93; 95% CI 1.25 to 2.98) and gross motor (OR, 1.50; 95% CI 1.08 to 2.09) delays, respectively.

2.93. A follow-up birth cohort study by (Wang et al., 2022b) investigated the risk of psychiatric disorders in adolescents and young adults conceived with ART. After adjustment for parental characteristics ART-conceived adolescents did not have an elevated risk of obsessive-compulsive disorder (OCD) when compared with all others (aHR: 1.10 95% CI: 0.98-1.24). ART-conceived adolescents were not at elevated risk of depression or suicidal behaviour (irrespective of parental infertility). Compared with non–ART-conceived children of couples with infertility, fresh, but not frozen, embryo transfer was associated with a lower risk of mood disorders (aHR: 0.90, 95% CI: 0.83-0.97).

2.94. A prospective cohort study by (Wijs et al., 2022b) compared the mental health and behaviour of adolescents (at age 14, 17 and 20 years) conceived through ART (n=303) with that of counterparts conceived without ART (n=2868). The study reported less externalizing behaviour (delinquent/aggressive), more parent-reported internalizing behaviour, and more (clinical) depression at age 14 years in ART-conceived adolescents compared to their non-ART peers. Comparing outcomes between ART-conceived adolescents and non-ART controls born to sub-fertile parents, as well as between those born to sub-fertile versus fertile parents within the control, did not alter results from parent and adolescent-filled questionnaires.

2.95. A matched cohort study by (Zhang et al., 2022) investigated the effect of embryo grading on the physical, metabolic, and cognitive development of offspring conceived through cleavage-stage ET IVF/ICSI (48 and 96 singletons conceived with poor-quality vs good-quality ET, respectively).

There was no difference in the full-scale intelligence quotient based on the Wechsler Preschool and Primary Scale of Intelligence (109.96±12.42 vs 109.60±14.46) or the general adaptive index based on the Adaptive Behaviour Assessment System (108.26±11.70 vs 108.08±13.44) between the 2 groups. These findings remained after linear regression analysis.

2.96. A study by (Djuwantono et al., 2023) performed meta-analysis (n=34 studies) to compare intelligence, motor skills, and behavioural problems IVF/ICSI – conceived and naturally conceived children. IVF toddlers scored significantly higher while ICSI toddlers sored significantly lower in verbal intelligence score compared to naturally conceived toddlers. Naturally conceived toddlers had higher externalizing behaviour scores compared to ART toddlers, and preschool and primary school IVF children had significantly lower externalizing behaviour score compared to naturally conceived children. There was no difference in all domains of intelligence between ART and naturally conceived children in preschool and primary school. ART adolescents scored higher academically than their NC counterparts.

2.97. A population-based study by (Fallesen, 2023) compared cognitive outcomes of children conceived after assisted conception (ART or IUI) and naturally conceived children (total births were 259608, including a subset of 13,566 births conceived with ART or IUI). After adjusting for confounders, ART-conceived children performed worse than the naturally conceived peers, while IUI-conceived children performed equally well as naturally conceived and better than ART-conceived children.

2.98. A study by (Lefebvre et al., 2023) investigated whether the effect of ART on neurodevelopmental outcomes at 4 years in preterm infants born <34 GW (n=166 ART) and compared to preterm naturally conceived infants (n=679). ART-conceived infants were at lower risk of having at least two domains in difficulty (aOR 0.34, 95% CI 0.13–0.88). The factors associated with non-optimal neurodevelopment at 4 years were male gender, low socio-economic level and 25–30 GW at birth. The need for therapy services was similar between groups.

2.99. A prospective cohort study by (Li et al., 2023) used Gesell developmental scale scores neurodevelopmental outcome in 12-month-old offspring born to infertile women undergoing ART (n=488) and women with natural conception (n=1,397). Gross motor, adaptive behaviour, language and total development quotient scores were comparable between the groups. Following multivariate linear regression and inverse probability of treatment weighting (IPTW), social behaviour development quotient scores were higher in the ART group than the control (adjusted β 0.99; 95% CI 0.26 – 1.71).

2.100. A cross-sectional study by (Eisemann et al., 2023) compared psychosocial health (PH) and quality of life, QoL (using KINDL questionnaire) between ICSI-conceived adolescents (n=545) and unmatched singleton controls (n=427) aged 14-18 years. Both ICSI and control adolescents had high PH and QoL. Significant differences occurred for 'impact of behavioural problems', the 'total KINDL' score and the dimensions 'physical wellbeing' and 'school' (p = 0.005), but all differences were far below minimal importance.

2.101. A population-wide cohort study by (Kennedy et al., 2023) compared with outcomes following natural conception school-age developmental and educational outcomes between IVF conceived and naturally conceived children (n= 412,713). At school-entry, there was no causal effect of IVF-conception on the risk of developmental vulnerability compared with spontaneously conceived peers [adjusted risk difference −0.3%; 95% CI −3.7% to 3.1% and adjusted risk ratio 0.97; 95%

CI 0.77 to 1.25]. At age 7 to 9 years, there was no causal effect of IVF-conception on overall z-score across 5 domains of literacy and numeracy between IVF- and naturally conceived children (adjusted MD: 0.030; 95% CI −0.018 to 0.077).

2.102. A population-based study by (Luke et al., 2020) compared differences in standardized testing in reading and mathematics between IVF- and naturally conceived children at age 8-9 years. IVF children scored significantly higher in reading and mathematics. Children of mothers ≥ 30 years of age scored consistently higher than children of mothers 18–29 years of age. The differences were of similar magnitude between IVF and control children for older ages, but not significant for IVF.

2.103. A register-based Swedish cohort study by (Norrman et al., 2020a) compared school performance of singleton children born after ICSI (n = 6953), IVF (n = 11,713) or spontaneous conception (SC) (n = 2,022 995). There was no significant difference between ICSI and IVF children for total score (aORs 1.03; 95% CI −0.22 to 2.28), specific subjects, qualifying for secondary school (aOR 1.02; 95% CI 0.82–1.26) or poor school performance (aOR 0.92; 95% CI 0.75−1.14). In the third grade, children born after ICSI had a significantly lower chance of passing all of the subtests in Mathematics (aOR 0.89; 0.83–0.96) and Swedish (aOR 0.92; 0.85–0.99) compared to children born after SC.

2.104. A study by (Takeshige et al., 2021) analysed developmental characteristics of children from birth to 6 years of age who were born after ICSI with vitrified-warmed oocytes and compared it to nationally reported average data serving reference. The results suggested that the physical and mental development of babies born from vitrified oocytes is comparable to that of naturally conceived babies.

Metabolic and reproductive function of ART-conceived men

2.105. A cohort study by (Arendt et al., 2022) evaluated reproductive function (semen analysis and reproductive hormones) of 1058 born to sub-fertile couples. Sons conceived after IVF or ICSI had a higher semen concentration (adjusted relative difference, aRD: 29%; 95% CI, –7%–79%) and a higher percentage of sperm with normal morphology (aRD: 20%; 95% CI: –8%–56%), but with 95% CI overlapping the null. They also had slightly higher estradiol levels (aRD: 30%; 95% CI: 7%–57%).

2.106. A cohort study by (Catford et al., 2022a) compared the reproductive function between IVF/ICSI-conceived males (n=120) and non-ART conceived males (n=356). IVF/ICSI-conceived men had lower mean total and progressive sperm motility with higher mean normal morphology. Differences in progressive motility (ß, -9.9; 95% CI: -16.7 to -3.0), normal morphology (ß, 4.3; 95% CI: 3.0–5.7), and proportion with abnormal morphology (aOR: 0.1; 95% CI: 0.04–0.5) remained significant after adjusting for confounders. IVF/ICSI-conceived men had lower mean follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels, and higher mean testosterone levels than controls (3.3 IU/L, 3.9 IU/L, 19.1 nmol/L vs 4.2 IU/L, 11.0 IU/L, 16.8 nmol/L, respectively).

2.107. A study by (Catford et al., 2022b) compared the cardiovascular and metabolic profiles of ICSI-conceived men (n=121) with controls [IVF-conceived (n=74) and spontaneously conceived (SC) men (n=688)]. Compared to SC controls, 121 ICSI-conceived men had higher diastolic blood pressure (β 4.9, 95% CI 1.1–8.7), lower fasting glucose (β −0.7, 95% CI −0.9 to −0.5), higher

fasting insulin (ratio 2.2, 95% CI 1.6–3.0), higher HDL-C, HDLC (β 0.2, 95% CI 0.07–0.3) and lower highly sensitive C-reactive protein, hsCRP (ratio 0.4, 95% CI 0.2–0.7) levels. Compared to IVF-conceived men, only glucose differed in the ICSI-conceived men (β −0.4, 95% CI −0.7 to −0.1).

Animal studies on the effect of ART on offspring health outcomes

2.108. A study by (Beilby *et al*., 2023) performed meta-analysis (n=61 studies) to compare postnatal outcomes between in vitro-conceived (IVF, ICSI or IVM) and in vivo-conceived mammalian animal models (bovine, equine, murine, ovine, and non-human primate). Despite considerable heterogeneity, these studies suggest that the use of IVF or maturation results in offspring with higher birthweights and a longer length of gestation, with most of this evidence coming from studies in cattle. These techniques may also impair glucose and lipid metabolism in male mice. The findings on cardiovascular outcomes and behaviour outcomes were inconsistent across studies.

3. Culture media and ART

Imprinting

3.1. Body text (select ‘A single-centre cohort study by (Barberet *et al.*, 2021) the epigenetic profiles of children according to the mode of conception (ART vs natural conception), type of embryo culture medium used [global medium (LifeGlobal) and single step medium (Irvine Scientific)] and the mode of in vitro fertilization (i.e. IVF vs ICSI). The DNA methylation profiles of four DMRs of imprinted genes (IGs) and two transposable elements (TEs) were assessed in buccal smears. Changes in the IGs' DNA methylation levels were found in ART children compared to controls. However, no differences were found between the culture media. After observing these targeted modifications, analyses were performed at wider scale. No differences were detected according to the culture media, but imprinted-related DMRs overlapping promoter region near the genes major for the development were detected between the ART and controls.

Birthweight

3.2. A systematic review by (Lena et al., 2021) compared G5 series (Vitrolife, Sweden) culture media to other common culture media in terms of primary (LBR and birthweight) and secondary (fertilization rate, implantation rate, biochemical pregnancy rate, clinical pregnancy rate, miscarriage rate, multiple pregnancy rate and congenital malformations) outcomes. Authors conclude that Vitrolife G5 series was found to have a trend towards higher live birth rates, but not significant compared to other common culture media. This result was comprised of only one randomised controlled trial (RCT). Birthweight had equivocal results with three out of six studies showing significantly lower (2)/higher (1) birthweights, whereas the others were non-significant. Overall, no significant differences were found concerning the secondary outcomes.

Embryo quality

3.3. A study by (Lee et al., 2021) investigated the relationship between concentrations of different cytokines in preimplantation embryo culture media, embryo quality and ART outcomes (n=72

samples obtained from 39 infertile couples undergoing IVF/ICSI). Each embryo was cultured separately, and the embryo culture medium was collected 72 h after fertilization. Before embryo transfer on day 3, a morphological evaluation of each embryo was performed. Among the nine cytokines that were quantified Chemokine (C-C motif) ligand 15 (CCL15), C-C motif chemokine ligand 27 (CCL27), and C-X-C motif chemokine 12 (CXCL-12) were significantly elevated in the top-quality embryo group as compared to non-top-quality embryo group.

Animal studies

3.4. A study by (Canovas et al., 2021) analysed the effect of different in vitro production protocols on whole-genome DNA methylation profiles of bovine blastocysts and compared them to in vivo produced controls. Blastocysts were produced by IVF cultured with three different sources of protein (foetal calf serum, bovine serum albumin or reproductive fluids). In vitro produced serum group had high levels of methylation similar to controls, while reproductive fluids or albumin in vitro groups showed less global methylation. The serum group was the most affected during the repetitive element analysis. Sex determination produced a stronger bias in DNA methylation than embryo origin, with in vivo blastocysts showing a lesser distance between male and female blastocysts.

3.5. An experimental study by (Moreau et al., 2021) used mouse embryos to investigate the effect of polycarbonate (PC)—bisphenol A (BPA)—releasing strippers used for embryo manipulation on embryo development. All embryos were cultured in Global Total medium (CooperSurgical). PC strippers-manipulated embryos developed significantly more often to the blastocyst stage compared to controls (embryos manipulated with glass strippers) (55 vs 46%; $P< 0.05$). Embryos pre-exposed to 0.5 ng/ml BPA before being handled in a glass stripper showed no difference in blastocyst rate when compared glass strippers-handled controls (43 vs 46%). Gene expression in blastocysts between the three groups did not differ significantly.

3.6. A study by (Zacchini et al., 2021) investigated the cumulative effect of embryo transfer (ET), in vitro culture and blastomere biopsy (BB) in the onset of metabolic disorders in ART-conceived mouse offspring. ART offspring had deregulated expression of proteins involved in lipid, carbohydrate, energy metabolisms and cellular processes and increased body weight, while i) insulin resistance in BB male ii) females glucose intolerance and high level of triglycerides and cholesterol in BB females and iii) low levels of interleukin-6 in BB, in vitro culture and ET males.

4. Conclusions

4.1. Since SCAAC last considered health outcomes in children conceived by ART, there has been an increase in the number of follow-up studies on developmental outcomes during childhood and adolescence, particularly on cardio-metabolic health, mental, social, and cognitive development. Many of these studies use cohort or nationwide population-based studies.

5. Recommendations

5.1. Members are asked to:

- Advise the executive if they are aware of any other recent developments; and
- Review whether any outputs from the HFEA are required addressing health outcomes of children conceived from ART.

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In vitro derived gametes

Details about this paper

Area(s) of strategy:	The right information
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item	6
Paper number	HFEA (02/10/2023) 006
Meeting date	2 October 2023
Author	Ana Hallgarten, Public Policy Manager
Annexes	None

Output from this paper

For information or recommendation?	For recommendation
Recommendations	Members are asked to: • consider the progress in research (since June 2020) into in vitro derived gametes; and • advise the executive if they are aware of any other recent developments; and • review whether any outputs from the HFEA are required addressing the use of in vitro derived gametes.
Resource implications	N/A
Implementation date	N/A
Communication(s)	N/A
Organisational risk	☒ Low ☐ Medium ☐ High

1. Introduction

1.1. Human eggs and sperm (germ cells) are derived from a type of cell called primordial germ cells (PGCs). They are produced in ovaries or testes by a process called gametogenesis. Researchers are investigating whether it is possible to carry out gametogenesis in the laboratory using immature gametes that have achieved final maturation in vitro, PGCs, embryonic stem cells (ESCs) or other human cells. Eggs and sperm derived from such cells in the laboratory are called in vitro derived gametes.

1.2. The legislation in the UK (the Human Fertilisation and Embryology Act 1990, as amended) prohibits the use of in vitro derived gametes in treatment. Section 3ZA requires that eggs or sperm permitted for treatment are "produced by or extracted from the ovaries of a woman/testes of a man".

1.3. Whilst in vitro derived gametes cannot be used in treatment in the UK, they can be used in research, for example, research into germ cell development and cell differentiation. Researchers in the UK need an HFEA research licence if they wish to investigate whether human eggs and sperm derived in vitro could undergo fertilisation and early stages of embryo development. It is therefore important that the HFEA is aware of the progress of research in this area.

1.4. The committee last reviewed research on in vitro derived gametes in June 2020. The committee considered when in vitro derived gametes may be clinically relevant in human applications in the near future, and existing limitations to research and clinical applications under the HFE Act.

1.5. At the June 2021 SCAAC meeting, it was noted that there have been significant changes in the field over the last 5 years. In the most recent horizon scanning process in February 2023, this topic was identified as a medium priority area for the SCAAC.

1.6. This topic was also discussed at the Horizon Scanning meeting at the European Society of Human Reproduction and Embryology 2023 conference. Attendees noted the increased treatment options in vitro derived gametes may eventually create and the current legal restrictions in the UK and USA.

1.7. The International Society for Stem Cell Research guidelines were updated in 2021. These guidelines set out several recommendations related to in vitro derived gametes. The guidelines place human in vitro gametes (that have not been fertilised or generated into an embryo) within Category 1B research. This category is research that is 'reportable to the entity or body responsible for the specialized scientific and ethics oversight process, but not normally subject to further or ongoing review, at the discretion of the entity responsible for the oversight process and subject to regulations and policies in the jurisdiction'. A paper by Clark et al., (2021) summarises the discussion made by the working group ahead of the ISSCR revisions. This includes considerations related to human embryo research, in vitro gametogenesis, and stem cell-derived embryo models.

1.8. This review highlights developments in animal and human studies since June 2020.

2. Applications of in vitro derived gametes

2.1. Notini, L et al in 2020 reviewed the potential clinical applications of in vitro gametogenesis (IVG) which include opposite-sex reproduction, same-sex reproduction and solo reproduction, each of these with varying support. This paper examines the ethical principles that can be used to help draw the lines and distinguish between ethically desirable and undesirable uses of IVG. It concludes and suggests that these principles generate strong reasons for IVG use in opposite-sex and same-sex reproduction, but not for solo reproduction.

2.2. The benefits of IVG for fertility preservation and restoration are highlighted in Wesevich et al., 2023.They note the limited fertility preservation possibilities for cancer survivor patients and note the benefits that IVG could provide despite existing scientific and ethical issues. A publication by Oqani et al., 2023, examined the applications of artificial oocytes in both research and medicine, providing an overview of oocyte development and progress into the research of oogenesis in vivo.

2.3. A piece by Frost and Filchrist, 2023, considers the translation of IVG research to human applications, in vitro culture procedures, and reviews publications in mice, non-human primates, and humans. The review notes the commercial interest in human IVG and the broader implications of its development for human use.

2.4. In 2021, Galea K considered views regarding IVG and its potential to be used as an assisted reproductive technology ART. This article separates ethical and safety concerns and argues that there is no justifiable in-principle ethical objection to the use of IVG as an ART for those who cannot have offspring with whom they share genetic material by any other means.

2.5. Several papers have considered public attitudes and views towards the development and use of in vitro derived gametes. These include Japan (Akatsuka et al., 2021) and Belgium (Mertes et al., 2022). In 2023, Serour et al. discussed the ethical dimensions of IVG applications in human fertility treatment from a Sunni Islamic perspective. It was concluded that once its safety and efficacy is guaranteed, it would be acceptable for use in treating primary infertility, age-related infertility and preventing genetic diseases.

2.6. A paper by Rolfes et al., 2022, examined the ethical benefits and risks related to the development of IVG for use in assisted reproduction. Benefits included the reduction in psychological and physical burden that exists in oocyte retrieval, social benefits of women having greater decision regarding when to have children, and that IVG would address ethical issues raised by gamete donation. Horer et al., 2023, considered the ethical implications of the use of IVG in humans, highlighting a need for urgent ethical debate.

2.7. As regards animal uses, a review by Goszczynski et al., 2023 presents advances in IVG relevant to livestock science, considering its possible application to improve elite genetics in cattle. A review by Botigelli discusses an overview of advances in the developments in IVG in rodents and livestock, and the impact that the development of IVG would have for animal agriculture.

2.8. Further applications of IVG in animals are considered in Golkar-Narenji et al., 2023. This study considers how in vitro culture of PGCs could be developed as an efficient protocol for the in vitro culture of primordial germ cells to preserve endangered species.

2.9. Similarly, a publication by Hayashi et al., 2022, considers the application of IVG for animals at risk of extinction. The study successfully induced primordial germ cell-like cells from pluripotent stem cells of the northern white rhinoceros. An additional paper by Saragusty et al., 2020, suggests combining in vitro gametogenesis with inner cell mass transfer to support critically endangered species.

2.10. Nishie et al., 2023, studied the effect of nonylphenol on in vitro spermatogenesis in the endangered cyprinid Gnathopogon caerulescens. An in vitro differentiation system was used on spermatogonia in a suspension culture to identify the effect on the resultant number of germ cells. The analysis revealed that low concentrations of nonylphenol reduced the number of germ cells which was attributed to haploids, however the number of spermatogonia and spermatocytes was not affected by nonylphenol treatment.

2.11. An article by Iwasaki-Takahashi et al in 2020 described a novel method for the in vitro expansion of rainbow trout germline stem cells (GSCs) for future applications in aiding rescuing fishes on the verge of extinction due to their small gonads limiting other methods of preserving their genetic material. This method includes using a feeder layer derived from Sertoli cells and a culture medium containing trout plasma. A transplantation assay demonstrated that the in-vitro expanded GSCs from this method exhibited stem cell activity and potency to produce functional eggs, sperm and eventually healthy offspring.

3. Animal studies

3.1. A key study by Murakami et al., 2023, successfully converted the XY chromosome set to XX without an additional Y chromosome in mouse pluripotent stem (PS) cells. In this study, pluripotent stem cells were successfully induced into fully potent oocytes which were successfully fertilised. This was a significant breakthrough in the creation of gametes from stem cells.

3.2. A review by Jiang et al., 2023, examines advances in oogenesis in vivo and in vitro in mammals, with a focus on the regulation mechanisms of oocyte maturation. The review also examines advances in single-cell mRNA sequencing technology, providing a theoretical basis for subsequent research into oocyte maturation.

3.3. A study by Wang et al., 2023, established a culture system that was able to create high-quality oocytes. This was achieved through creating a three-dimensional organoid culture system that induced oocytes from premeiotic murine female germ cells that were similar to in vivo oocytes.

3.4. A study by Severino et al., 2020, investigated the reprogramming that the mammalian germline undergoes during oogenesis. The study explored X-inactivation and reactivation dynamics using a tailor-made in vitro system of primordial germ cell-like cell differentiation from mouse embryonic stem cells. The study established the importance of X-chromosome remodelling during the development of female germ cells towards meiosis and oogenesis.

3.5. Delessard et al., 2022, invested the impact of chemotherapies on in vitro spermatogenesis in experimental models. The development of in vitro spermatogenesis may be groundbreaking for prepubertal boys who have undergone chemotherapy prior to preserving any testicular tissue.

The study investigated the different effects of mono-chemotherapy and poly-chemotherapy on the first wave of in vitro spermatogenesis in prepubertal mice. The study established that the differentiation in vitro into sperm was possible following mono-chemotherapy and poly-chemotherapy, however poly-chemotherapy resulted in higher numbers of sperm with morphological abnormalities and fragmented DNA.

3.6. A study by Oblette et al., 2021, investigated the fertilizing ability of in vitro-produced spermatozoa in mice. The study showed that the use of in vitro generated spermatozoa altered the DNA methylation and demethylation dynamics in 4-cell embryos and morula.

3.7. A study by Feng et al., 2023. investigated seminiferous tubule tissue of double-transgenic mice, to monitor spermatogenic progression. The study found that spermatogenesis can be induced up to the elongating spermatid stage when the seminiferous tubule was cut into short segments and cultured in isolation. However, this method had a lower efficiency than tissue mass culture.

3.8. A study by Kan et al., 2022, successfully established a C. nasus gonadal somatic cell line capable of sperm induction in vitro. A gonadic stem cell line was isolated and cultured over a year and RT-PCR analysis showed that the stem cell line expressed some somatic cell markers.

3.9. A study by Dumont et al., 2023, investigated the impact of in vitro culture and freezing procedures on mice, as cryopreservation of pre-pubertal tissue is important research within fertility preservation and restoration. Pre-pubertal mice testicular tissue was directly cultured for various days or cryopreserved by controlled slow freezing and then cultured in vitro. The results showed that cryopreservation had a minimal effect on gene expression in testicular tissue, either directly after thawing or after 30 days in culture.

3.10. A piece by Gao et al., 2023, researched how high temperatures inhibit spermatogonial stem cells (SSC) differentiation in mice. SSCs are a type of germline stem cells that maintain spermatogenesis. A study by Sojoudi et al., 2023, supports the analysis of germ cell development in vivo and in vitro. In the study, mouse SSCs had mRNA expression levels compared with mouse somatic cells to examine the molecular mechanism and to study 'clump cells', highly compact colonies that were observed near SSCs.

3.11. Matsumura et al., 2023, were able to demonstrate in vitro production of functional haploid cells that yielded offspring in rats. Culture media was tailored to the metabolic dynamics of transgenic rat testis tissue, leading to successful induction of spermatogenesis up to haploid production and the formation of elongating spermatids. Use of the in vitro derived spermatids in microinsemination led to healthy offspring in rats.

3.12. A study by Ishikura et al., 2021, showed in vitro reconstitution of whole male germ-cell development from pluripotent stem cells. Mouse embryonic stem cells were induced into primordial germ cell-like cells, these then differentiated into spermatogonium-like cells and expanded into germline stem cell-like cells. These cells showed spermatogenesis both in vivo and in vitro, with the resulting spermatids contributing to fertile offspring.

3.13. An experimental study by Amirkhani et al., 2020, evaluated the progression of mouse spermatogenesis after testicular tissue culture in mini-perfusion bioreactor. After 8 weeks, histological analysis showed that there was successful maintenance of spermatogenesis in tissues grown in the bioreactor, which did not occur on agarose gel. The study demonstrated that

a dynamic culture system of a bioreactor can be used to support the induction of spermatogenesis.

3.14. Work by Khampang et al., 2021 demonstrated that in vitro-derived round spermatid-like cells generated in vitro from primate pluripotent stem cells, were able to mimic many of the capabilities of in vivo round spermatids. Approximately 12% of the stem cells developed into expanded blastocysts.

3.15. A paper by Zhang et al., 2022, studied the in vitro production of functional gametes with view to accelerate genetic breeding in aquaculture. Pre-meiotic spermatogonia in marine four-eyed sleeper fish were isolated and characterised and then induced into flagellated sperm in a 3D culture system. These sperm successfully fertilised mature oocytes in artificial insemination.

3.16. A study by Saulnier et al., 2022, investigated the underlying molecular mechanisms of meiotic arrest during in vitro spermatogenesis in rodent prepubertal testicular tissue. Using RNA-sequencing analyses, the study identified up to 600 differentially expressed genes between in vitro and in vivo conditions, noting that that gene deregulation could be at the origin of meiotic arrest. Lei et al., 2023, further reviewed the importance of key meiotic checkpoints in in vitro spermatogenesis.

3.17. The objective of a study by Saulnier et al., 2023, was to determine whether sequential two-step culture protocols can improve the efficiency of rat in vitro spermatogenesis. Although the two-step culture protocol led to complete in vitro meiosis and the beginning of the elongation phase of spermiogenesis, the protocols showed low efficiency requiring further work in the field.

3.18. A study by Oikawa et al., 2022, demonstrated the induction of functional primordial germ cell-like cells from rat pluripotent stem cells. The transplantation of these functional primordial germ cell-like cells into transgenic rats demonstrated that spermatogenesis in vivo was taking place. This method provides key information on the mechanisms required for in vitro gametogenesis research, as rat models are more similar to humans than mouse models.

3.19. A study by Iwatsuki et al., 2023, developed optimal culture conditions for the propagation of post-implantation epiblast derived pluripotent stem cells in mice. The study demonstrated that under optimum culture conditions these cells could be used to produce primordial germ cell-like cells that successfully underwent spermatogenesis that led to offspring.

3.20. A study by Önen et al., 2022, researched how a co-culture setup supported by syngeneic bone marrow derived mesenchymal stem cells (BM-MSC) could lead to survival, expansion and differentiation of mice spermatogonial stem/progenitor cells in vitro. The study noted the benefits of developing the technology for restoring fertility to prepubertal cancer survivors.

3.21. A study by Cooke et al., 2023 researched possible new sources of primordial germ cell-like cells. Primordial germ cells (PGCs) are precursors to sperm and egg cells, and therefore the development of new sources is beneficial to research primordial germ cell development, and for in vitro derived gamete research. The study identified that mouse gastruloids contain gastruloid-derived PGC-like cells that resemble early PGCs in vivo.

3.22. Given the scarcity of spermatogonial stem cells for research and developments of in vitro gametogenesis, Segunda et al., 2023, conducted a comparative analysis into how mesenchymal

stem cells could be used for germ cell derivation. The study compared the germ cell differentiation potentials from bull spermatogonial stem cells and bull peripheral blood-derived mesenchymal stem cells. The study established that in a co-culture with Sertoli cells, bull spermatogonial stem cells showed a greater germ cell differentiation potential than bull peripheral blood-derived mesenchymal stem cells.

3.23. Umair et al., 2023 examined the influence of in vitro produced equine embryos and in vivo derived equine embryos on the expression of cell lineage markers. They found that in vitro produced embryos showed a poorly compacted inner cell mass with intermingled epiblast and primitive endoderm cells.

3.24. A study by Yao et al., 2022, discusses in vitro germline specification as a way to provide a modelling platform to investigate gametogenesis. Fertile sperm and oocytes can be obtained from mouse embryonic stem cells (ESCs) through a primordial germ cell (PGC)-based method. Since human PGC-like cells can be derived from a similar strategy used for the same purpose in mice, this review describes the reconstitution of PGCs and the subsequent meiosis as well as the signalling pathways and factors involved in these processes.

4. Human and human & animal studies

4.1. A review by Coxir et al., 2023, surveyed the key molecular and cellular aspects of human female gametogenesis, considering main advances and methodologies for the development of in vitro derived egg cells. A study by Yu et al., 2023, explored a method to initiate meiosis in the process of inducing completed oocyte differentiation from human stem cells in vitro.

4.2. A study by Martin et al., 2021, investigated the effects of culture media and substrates on male human fetal germ cells. Both the culture media and substrate had significant impacts on male human fetal germ cells, demonstrating the importance of optimising culture conditions for gametogenesis in vitro.

4.3. A study by Wang et al., 2022, focused on whether the development and support of spermatogonia and somatic cells would be possible through organotypic culture of testicular tissue. This was conducted by culturing testicular tissue from infant boys with bilateral cryptorchidism in different culture media and evaluating the effects after 60 days. The study found that the culture conditions created supported the initiation of spermatocytes and enhanced maturation of Sertoli cells in the tissue.

4.4. A study by Jabari et al., 2023, evaluated the potential of co-culturing Sertoli cells and spermatogonial stem cells in a hybrid hydrogel of agarose and laminin. The study found that the hybrid gel showed all three phases of spermatogenesis, as well as morphological spermatozoa.

4.5. A mini-review by Kurek et al., 2020, summarises the literature regarding in vitro derivation of human male germ cells from human pluripotent stem cells (hPSCs), keeping a particular focus on the culture methods, growth factors, and cell lines used to achieve the diverse strategies and outcomes so far in this field.

4.6. A review by Zhao et al, 2021, focussed on the in vitro differentiation of human induced pluripotent stem cells (hiPSCs) into male germ cells with view to provide mechanistic insights into the regulation of spermatogenesis and provide opportunity for families with infertility.

4.7. A review by Diao et al., 2020, investigated the regulatory network of spermatogonial stem cells (SSCs) in animals and humans to better establish the mechanisms of SSC function in the creation of sperm, and possible developments leading to male infertility. This review summarised research on the applications of SSCs in in vivo and in vitro spermatogenesis as well research into the mechanisms of SSCs.

4.8. A review by Sanou et al., (2022), discusses spermatogonial stem cell-based therapies. These include the transplantation of spermatogonial stem cells, grafting of testicular tissue and in vitro and ex vivo spermatogenesis approaches.

4.9. Research by Overeem et al., 2021, examined the signalling pathways during the sex-specific transition from primordial germ cells (PGCs) to premeiotic oogonia or prospermatogonia. This work is essential in research into the reconstitution of human in vitro gametogenesis.

4.10. A study by Mishra et al., provided insights for the optimization of in vitro gametogenesis in humans. The study used surface markers to isolate and male and female foetal germ cell and human primordial germ cell-like cells derived from embryonic stem cells. The study demonstrated that sex differences were shown in the isolation and propagation of foetal germ cells and human primordial germ cell-like cells.

4.11. A review by Cui, Y et al., 2023 explores the effective methods of obtaining functional male gametes in order to restore male fertility in humans and animals. It examined the generation of male germ cells from stem cells including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), spermatogonial stem cells (SSCs) and mesenchymal stem cells (MSCs) and addresses current advances from different studies, highlighting the perspectives and potential applications of stem cell-derived male gametes in reproductive medicine. An earlier review by Tran et al., 2020, considered the current options for preservation and restoration of fertility in prepubertal patients undergoing gonadotoxic therapies, considering experimental options in in vitro spermatogenesis.

4.12. A paper by Ramsoomair et al., 2023 summarises the use of human testis-on-a-chip models for in vitro spermatogenesis and examines possible improvements to such models for research. A review by Shuchat et al., 2022, considered the types of fluid flows and incorporation in organ/organoid-on-a-chip models. These models are used to replicate in vivo environments in vitro, with the review considering the benefits of the use of active microfluidic flow for in vitro spermatogenesis and perfusion-based testis-on-chip models. Additionally, a review by Robinson et al., 2023, summarises advances in human in vitro spermatogenesis. This includes summaries into organotypic culture systems, 3D scaffolds, 3D organoids and 3D bioprinted systems, and culture media.

4.13. Research by Alves-Lopes et al., 2023, investigated the broad spectrum of human PGC-like cells (hPGCLC) precursors and how these different precursors impact hPGCLC development. This research supports the development of efficient in vitro models of human gametogenesis.

4.14. Chang et al., 2021, investigated the effect of X chromosome inactivation on the differentiation efficiency of human pluripotent stem cells during embryoid body formation. This work contributes to the understanding of differentiation methods for higher numbers of human pluripotent stem cells, that can be used within in vitro gametogenesis research.

4.15. A further study by Kiani et al., 2023, examined a new protocol to differentiate primed human induced pluripotent stem cells into primordial germ cell-like cells due to existing protocols showing low efficiency.

4.16. A review by Makar and Sasaki, 2020, assessed the mechanisms leading to germ cell development in mammals, and their applicability to human germ cell development. The review also surveys induction methods for recapitulating germ cell development in vitro, focussing on in vitro gametogenesis from pluripotent stem cells.

4.17. In 2020, Bharti et al. reviewed current strategies related to stem cell-based in vitro oocyte-like cell (OLC) development in animals and humans used for pathological complications including female reproductive disorders, with special focus on the usage of mesenchymal stem cells (MSGs), induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). It was found that the differentiation abilities of both the ovarian and non-ovarian stem cell sources under different conditions have different effects after in vivo transplantations. The attainment of oocyte-like morphology, size expansion and meiosis initiation were found to be major obstacles during in vitro gametogenesis, and whilst many valuable insights have been made, there is still a long way to go before stem cell-derived OLCs are fully functional.

4.18. A study by Mizutra et al., 2022, showed the process of reconstitution foetal oocyte development in humans and cynomolgus monkeys. The study established key mechanisms and characteristics of oocyte development in humans and primates.

4.19. A review by Saitou and Hayashi, 2021, surveyed IVG in rodents, primates, rabbits, and pigs. This review noted the potential for a range of future applications, however that the genetic and epigenetic integrities of gametes generated appear substantially lower than those created in vivo. Kulibin and Malolina, 2023, reviewed the current issues of complete reconstruction of spermatogenesis in vitro, reviewing advances in the field in mice, rats, and humans. The review examines organ culture methods, different 3-D cultures, and conventional adherent cell cultures. An additional review by Li et al., 2022, summarises advances in the field of in vitro sperm cell induction, examining research in mice and humans.

4.20. Strange and Alberio's review, 2023, considers the key technical challenges raised by in vitro gametogenesis. The review summarises research into the development of primordial germ cells, their specification, and the transcription factor network of primordial germ cell specification in non-rodent mammals. The review then goes on to describe the advances in in vitro generation of primordial germ cell-like cells from pluripotent stem cells across a range of animals and humans, as well as possibilities in in vitro gametogenesis of both male and female gametes. The review notes possible applications of in vitro derived gametes including in livestock, the rescue of endangered species, and within infertility research.

4.21. Hong et al., 2021, reviewed advances in in vitro male and female germ cell derivation in humans and mice from pluripotent stem cells (PSCs). This technique was reviewed to understand the

biological mechanism of gamete development and to gain insight into its application in infertility. A further article by Zhang et al., 2020, provided a review of research into artificial gamete and embryo creation in animal and human studies. The review by Tahmasbpour Marzouni et al., 2022 examines the development of in vitro generation of gametes to restore fertility to humans with reproductive failure by considering work in animals and humans. Further publications, such as Yoshimatsu et al. 2022, have summarised studies on the derivation of oocytes and sperm from pluripotent stem cells in mice, as well as in further animal and human models.

5. Conclusions

5.1. SCAAC last considered in vitro derived gametes in June 2020. Since the committee last discussed in vitro derived gametes, research in this area has made some promising progress. However, there are still many fundamental technical challenges that need to be overcome before in vitro gametogenesis could be considered for used in clinical practice. Additionally, there are serious ethical and safety concerns that make research in humans challenging and necessitates the use of animal models.

6. Recommendations

6.1. Members are asked to:

- consider the progress in research since into in vitro derived gametes; and
- advise the Executive if they are aware of any other recent developments; and
- review whether any outputs from the HFEA are required addressing the use of in vitro derived gametes.

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Impact of the microbiome on fertility and fertility treatment outcomes

Details about this paper

Area(s) of strategy this paper relates to:	The best care
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	7
Paper number:	HFEA (02/10/2023) 007
Meeting date:	02 October 2023
Author:	Bethany Lockwood, Scientific Policy Manager
Annexes	N/A

Output from this paper

For information or advice?	For information
Recommendation:	Members are asked to: • Advise the Executive if they are aware of any other recent developments; • Review whether any outputs from the HFEA are required, addressing the impact of the microbiome on fertility treatment outcomes; and • Consider the potential for companies to market supplements that claim to improve fertility based on the microbiome and if a treatment add-ons application for microbiome tests should be brought back to a future SCAAC meeting.
Resource implications:	N/A
Implementation date:	N/A

Communication(s):	None
Organisational risk:	Low

1. Introduction

1.1. The microbiome refers to the microorganisms which inhabit a particular environment, for example, the body or part of the body. Our understanding of the microbiome has developed rapidly in recent years, along with our understanding of its role in human health and disease.

1.2. Researchers have long been interested in the possible interactions between the male and female reproductive tract and its microbiome. If the composition of the microbiome is shown to be related to fertility, or indeed, fertility treatment outcomes, there may be potential for development of interventions aimed at altering the microbiome to improve outcomes for patients.

1.3. This topic was last discussed at the Horizon Scanning meeting during the ESHRE 39th Annual Meeting in June 2023. Current research suggests that there may be some relationship between the microbiome of the urogenital tract and successful treatment. It was concluded that it was essential to firstly characterise the distinction between a healthy microbiome compared to an unhealthy microbiome before interventions could be developed. It was also agreed that patients need to be advised that unproven treatments are not harmless, and further study is required to establish how widespread microbiome-related issues are for patients experiencing recurrent implantation failure.

1.4. In the most recent horizon scanning process presented to SCAAC in February 2023, the possible effect of the microbiome on fertility and fertility treatment outcomes was identified as a medium priority area and the SCAAC commented that although the topic is classed as a medium priority, we should be aware of the potential for companies to market supplements that claim to improve fertility based on the microbiome.

1.5. The committee last reviewed research on the impact of the microbiome in fertility and fertility treatment outcomes in February 2019. It was noted that many studies were descriptive and that the field is not yet at a stage to be translated into clinical significance. There appears to be a potential role for the microbiome in fertility and fertility treatment outcomes but there was no proof of causalities and there was no current role for consideration around the microbiome to be taken into account in the management of infertile couples.

1.6. This paper looks at studies investigating the possible relationship between the human microbiome and fertility or fertility treatment outcomes, from February 2019 to September 2023.

2. Research

Investigating infertility

2.1. A proof-of-concept case study by Wrønding et al. 2023, demonstrated the resolution of severe vaginal dysbiosis in a 30-year-old woman with recurrent pregnancy loss by the administration of an antibiotic-free vaginal microbiota transplant (VMT). A successful pregnancy and live birth were achieved following the VMT.

2.2. A systematic review and meta-analysis by Hong et al., 2020, explored the association between the vaginal microbiota and infertility in 15 studies. A negative correlation was identified between

high-*Lactobacillus* vaginal microbiota samples and female infertility. However due to limitations, specific mechanisms could not be established and further high-quality studies are required.

2.3. A review by Garcia-Velasco et al., 2020, provides an overview of the current and future research into the reproductive microbiome in relation to fertility, and shares clinical practice recommendations for physicians to benefit patients.

2.4. A review by Muzii et al., 2022, examines the role of the microbiota in human reproduction and its contribution to infertility, while also investigating the role of endometrial bacteria in recurrent implantation failure.

2.5. A meta-analysis by Li et al., 2023, conducted among 24 cohorts (18,340 participants), investigated causal relationships between gut microbiota composition and infertility. It identified specific bacterial taxa associated with both male and female infertility, suggesting a causal link between gut microbiota and infertility.

2.6. A study by Chen et al., 2021, examined the vaginal microbial profiles of women with tubal infertility who were *Chlamydia-trachomonas* negative and those who were *C-trachomonas* positive, pre and post antibiotic treatment. Women with tubal infertility and C. trachomitis infection presented a unique *Lactobacillus iners*-dominated vaginal microbiota rather than one dominated by *Lactobacillus crispatus*, along differing microbiota and cytokine levels. This altered vaginal microbiota could be restored with varying degrees after standard treatment for C. trachomatis. This was the first study to provide data on the association of vaginal microbiota with C. trachomatis infection among women with tubal infertility and highlights potential opportunities to predict C. trachomatis infection.

2.7. A study by Liu et al., 2022, reported that the microbiota of the lower and upper reproductive tracts from patients with recurrent spontaneous abortion (RSA) showed no significant differences in α-diversity compared to that of controls. However, β-diversity was significantly higher and dramatic decreases in gamma interferon and interleukin-6 cytokine levels were observed in the RSA group.

2.8. A study by Sezer et al., 2022, examined vaginal and endometrial samples obtained in the mid-menstrual cycle from 26 women with unexplained infertility. The proportion of *Lactobacilli*-impaired microbiota was significantly higher in the vaginal samples of the unexplained infertility patients compared to the 26 age-matched fertile controls. Those with impaired microbiota had a 9x increased risk for infertility compared to those with normal *Lactobacilli* microbiota.

2.9. A study by Liu et al., 2021, highlighted that patients with early pregnancy miscarriage had a significantly different vaginal microbiota compared to those who hadn't had an early pregnancy miscarriage.

2.10. A study by Yao et al., 2022, aimed to explore the relationship between five main whole blood trace elements and two enterotypes in 651 Chinese women with infertility. It was inferred that the two enterotypes may have an effect on the iron metabolism in patients with infertility, highlighting the importance of further research into the interaction between enterotypes and trace elements in reproductive function.

2.11. A review by Ravel et al., 2020, described the current evidence for the associations of BV, pelvic inflammatory disease (PID) and endometritis, and the impact of untreated BV and PID on

infertility. Although a relationship was found between BV, endometritis, PID and infertility, additional large, prospective, longitudinal studies are required to confirm the findings.

2.12. A review by Ding et al., 2021, focuses on the normal vaginal floral, bacterial vaginosis and its effects on reproduction, as well as different treatments for BV. It was highlighted that patients with symptomatic BV should be actively treated to improve reproductive outcome and prevent endometritis, pelvic inflammation and other reproductive disorders.

2.13. A study by Masucci et al., 2023 evaluated the endometrial and vaginal microbiota of patients with celiac disease (CD) and recurrent pregnancy loss (RPL) compared to healthy pregnant women. Two RPL subgroups - HLA-DQ2/DQ8 (CD genetic components) positive and negative, showed a different endometrial and vaginal composition in the Lactobacillaceae family compared to controls. Evidence appeared to show a predisposition to altered vaginal environment of HLA-DQ2/DQ8 positive RPL-women. However, a larger sample size is required.

2.14. A study by Mori et al., 2023, compared the vaginal and cervical microbiomes between 88 patients with unexplained RPL and 17 healthy women with no history of miscarriage. The abundance of certain bacterial species was significantly higher in patients who had subsequently miscarried compared to those who gave birth.

2.15. A study by Manzoor et al., 2021 characterised the gut and genital tract microbiomes from several married Pakistani couples. Several fluctuations in the diversity and composition of the gut and genital microbiota among the 31 fertile and 35 infertile individuals were identified.

2.16. A review by Grobeisen-Duque et al., 2023, explored the significance of a healthy microbiota in successful implantation and pregnancy development, including infertility. The establishment of bacterial vaginosis, infertility, still birth and preterm birth was associated with imbalances in the microbiota.

2.17. A study by Patel et al., 2022, examined the gut-vaginal microbiota axis in 20 infertile women, 10 of whom had RIF and 10 with unexplained infertility. Compared with controls, α-diversity and β-diversity of the gut bacteria among the infertile groups differed significantly ($p < 0.05$). Vaginal microbiota was dominated by the genus *Lactobacillus* across the groups.

2.18. A review by Inversettie et al., 2023, aimed to summarise the evidence on the molecular mechanisms by which the endometrial microbiota may interact with the immune system. The review also looked at the link between dysbiosis and reproductive disorders such as infertility, RPL and preterm birth.

2.19. A review by Lebedeva et al., 2023, presents new data on the impact of the vaginal and uterine microbiome on the local immunity and its association with miscarriages. The majority of studies demonstrated that the dominant species of the vaginal and uterine microbiome in patients with early miscarriages are non-*Lactobacillus* bacteria.

2.20. A nested case-control study by Peuranpää et al., 2022, analysed endometrial and vaginal samples from 37 women with two or more consecutive pregnancy losses and 39 healthy control women without history of pregnancy loss. *L.crispatus* was less abundant in the endometrial samples of women with RPL compared with controls. *Gardnerella vaginalis* was more abundant in the RPL group than in controls in both endometrial and vaginal samples.

2.21. A prospective pilot study by Lundy et al., 2021, examined the genitourinary and gastrointestinal microbiota in the pathogenesis of male infertility in 25 men with primary idiopathic infertility. This represents the first comprehensive investigation into the microbiome in male infertility. Infertile men harboured different bacterial species and *Prevotella* abundance was inversely associated with sperm concentration, and *Pseudomonas* was directly associated with total motile sperm count.

2.22. A comprehensive review by Venneri et al., 2022, examined the effects of bacterial dysbiosis in both sexes and how partners might influence each other to allow for better personalisation of infertility management.

2.23. A review by Bardos et al., 2020 highlights the various microbiome found during the periconceptual period, the microbiomes interaction with immunological responses around the time of implantation, its effect on implantation, placentation and ultimately maternal and neonatal outcomes.

2.24. A study by Tapilskaya et al., 2023, assessed the vaginal and endometrial microbiota composition and its relation to the levels of cytokines produced by the endometrium in reproductive-aged women with unknown secondary infertility. A reliable decline in endometrial TGFB1 and bFGF2 and an increase in DEFa1 was demonstrated in women with idiopathic infertility compared to fertile patients. However, their expression correlated reliably only in the presence of *Peptostreptococcus spp.* and HPV in the uterine cavity.

2.25. A review by Magill 2023, explored the relationship between the microbiome and male infertility. It was identified that certain microbial species are associated with higher levels of oxidative stress, with a link to abnormal semen parameters in infertile men. However, it was noted that there is a need for larger studies on this topic.

2.26. A study by Ncib et al., 2022, assessed vaginal flora in 65 women with unexplained RPL, and investigated the pathogenic properties of the microbiota associated with aerobic vaginitis (AV). The prevalence of AV was five-fold higher in the RPL group than in the controls (64.6% vs 12.0%) The most prevalent isolates in the case group were *Enterococcus spp. (*52%) and *Staphylococcus spp.* (26%), which all tolerate low pH.

2.27. A review by Chen et al., 2023, discussed the microbiological changes associated with male infertility and how microorganisms can affect the normal function of the male reproductive system through immune mechanisms.

2.28. A review by Tomaiuolo et al., 2020, looked at the state of the art regarding the role of the female reproductive system microbiota in women's health and human reproduction, highlighting its contribution to infertility.

2.29. A review by Tomaiuolo et al., 2020 looked at the state of the art regarding the male reproductive system microbiome and its alterations in infertility.

2.30. A review by Hashem et al., 2022, aims to summarise current knowledge on the biodiversity of the microbiota of the reproductive tract, and the possible relationships between eubiosis or dysbiosis and reproductive health and function in both females and males.

2.31. An observational, exploratory, preliminary study by Azpiroz et al., 2021, compared rectal and vaginal microbiota samples between fertile and unexplained infertile women and its correlation with miRNA expression levels. Infertile patients showed a lower bacterial richness and increased Firmicutes/Bacteroidetes ratio at rectal level and increased *Lactobacillus brevis/iners* ratio in vaginal samples compared to the fertile group. Additionally, miR-21-5p, associated with tight junction disruption and yeast overgrowth, was upregulated and miR-155-5p associated with inflammation was overexpressed in the infertile group.

2.32. A systematic review of 9 studies by Brandão et al., 2021, investigated the effect of seminal microbiota studied by NGS on sperm quality and male fertility. It appears that the microbiota may play a role in seminal quality and male fertility but the way this effect is modulated is still unknown. *Lactobacillus spp.* seemed to play a beneficial role in semen quality.

2.33. A study by Hong et al., 2022, collected pre-pregnancy vaginal swabs from 478 women across two phases to assess the impact of the vaginal microbiome on the fecundability of women planning pregnancy. The pre-pregnancy vaginal microbial community structures of the pregnant and non-pregnant group were significantly different, with *Lactobacillus* abundance, particularly *Lactobacillus gasseri* being higher in the pregnancy group and *Gardnerella* abundance being higher in the non-pregnant group at follow-up.

2.34. A review by Zhu et al. 2023, explored the association between the gut microbiome and RPL. It discusses potential mechanisms through which the gut microbiome may influence RPL, including inflammatory responses and hormonal imbalances.

2.35. A review by Toson et al., 2022, examines the current knowledge regarding the uterine microbiota and how it relates to human conception.

2.36. A review by Alqawasmeh et al., 2022 discusses the past and current literature surrounding the testicular and semen microbiome in correlation with male infertility and highlights the potential benefits of probiotics as an alternative therapeutic option for male infertility.

2.37. A review by Wang et al., 2022, discusses the relationship between the microbiome and male infertility, and explores the role of the microbiome in male infertility. It was identified that more high-quality studies are required.

2.38. A systematic review by Vitale et al., 2021 aimed to identify and appraise studies investigating the correlation of genital microbiomes to infertility. Regardless of the anatomical site under investigation, the *Lactobacillus*-dominated flora seems to play a pivotal role in determining fertility, in particular *L.crispatus*.

2.39. A review by Salliss et al., 2021, examined the potential relationship between the microbiome and endometriosis, infertility and chronic pelvic pain. In the 28 clinical and 6 animal studies, bacteria were enriched in the endometriosis groups, although there was no clear consensus on the microbial composition. However bacterial vaginosis-associated bacteria and *Lactobacillus* depletion in the cervicovaginal microbiome was associated with endometriosis and infertility in the majority of studies. The review highlights demand for more rigorous and transparent methodology and controls, and consistency across the field.

2.40. A systematic review of 55 observational studies by Farahani et al., 2020, compared the semen microbiome of infertile and fertile men (51,299 subjects). Several bacterial species were found to negatively impact on semen parameters, whereas *Lactobacillus* appeared to protect sperm quality.

2.41. A review by Osadchiy et al., 2020, examined studies in the last decade that looked at the seminal microbiome and its relationship to male factor infertility. Lactobacillus was consistently associated with normal semen analysis parameters and fertility.

2.42. A prospective cohort study by Shi et al., 2022, evaluated whether uterine endometrium microbiota was associated with pregnancy outcome in 67 women with RPL. The study demonstrated that increases in *Ureaplasma* species in UEM of women with RPL was associated with increased risk of miscarriage and preterm delivery.

2.43. A systematic review by Souza et al., 2023, sought to understand the influence of *Lactobacillus spp.* and female fertility. 18 articles were analysed with total of 2,011 women included in the study. Results demonstrated a higher likelihood of *Lactobacillus spp.* dominance in fertile women, while infertile women showed a dysbiotic profile.

2.44. A review by Garg et al. 2023, summarises the evidence on the vaginal microbiome in obese women and the impact on reproductive outcomes including conception rates, early pregnancy and preterm birth. The evidence indicated that obese women are more likely to have adverse vaginal microbiomes characterised by less *Lactobacillus*-dominance or less *L. crispatus*, and more likely to have increased diversity and bacterial vaginosis.

2.45. A study by van der Tweel et al., 2022, investigated the association between pH and BV diagnosed by qPCR in an asymptomatic sub-fertile population and examined the usefulness of pH level. pH was strongly associated with BV as diagnosed by qPCR.

2.46. A study by Ersahin et al., 2022, investigated the characteristics of the endometrial microbiota according to serum AMH levels in 45 women (20-30 years old) with two or more implantation failures. A negative correlation was detected between low AMH levels and the microbiome detection rates in endometrial cultures. Low serum AMH level increased the rate of positive endometrial microbiome in culture and decreased clinical pregnancy rates.

2.47. A review by Morawiec et al., 2022, looked at whether the seminal microbiome can impact on fertility and prevent pathological conditions that affect seminal parameters. Reviewed recent studies on the seminal microbiome revealed there is great diversity of microorganisms in the male genital tract but there is no clear definition of the importance of individual groups and the proportion of bacteria in infertility.

2.48. A retrospective narrative mini-review of 33 studies by Doroftei et al., 2022, summarised existing literature regarding the composition of the seminal flora in infertile men. Findings indicated the seminal microflora is decisive and able to modulate psychological and physiological responses.

2.49. A study by Cheong et al., 2023, profiled the cervical microbial composition of a population of 70 reproductive-age Malaysian women, among which 40 had genital C.trachomitis infection, and 30 did not have C.trachomitis infection. A distinct compositional difference between the two subjects

was observed, with significant elevations of mostly strict and facultative anaerobes seen in the cervical microbiota of C.trachomitis-positive women.

2.50. A cohort study by Hong et al., 2022, examined pre-pregnancy vaginal swabs 478 women who took part in a free pre-pregnancy project. The cohort study demonstrated an association between the pre-pregnancy vaginal microbiome and female fecundability, with a higher abundance of *L. iners* and a lower abundance of *L. crispatus* and *L. gasseri* appearing to be associated with a lower fecundability.

2.51. A prospective cohort study by Lokken et al., 2022, examined the cultivable vaginal microbiota in 458 Kenyan women. Cultivable Lactobacillus was not associated with fecundability, although Lactobacillus morphotypes detected on Gram stain were somewhat associated with increased fecundability.

2.52. A review by Miko et al., 2023 summarises the current knowledge of the role of hydrogen-peroxide produced by the vaginal microbiota in female reproductive health, and the possible role of probiotic treatments.

2.53. A review by Nakama et al., 2022 discusses the current understanding regarding the compositional characterisations and temporal dynamics of various microbial niches along the female reproductive tract. The review discusses how recurrent disruptions in the lower reproductive tract microbiome can lead to dysfunction in the upper reproductive tract, which could be associated with infertility.

2.54. A study by Puerta Suárez et al., 2022, evaluated the impact of the semen microbiome on semen parameters in 11 fertile donors and 10 volunteers with prostatitis-like symptoms. No statistically significant differences were observed in seminal parameters, cytokine measurement, antioxidant capacity, nitric oxide concentration and ROR-γT, FoxP3, T-bet.

2.55. A review by Sehring et al., 2022, discusses the role of the vaginal and endometrial microbiome in implantation.

Impact on fertility treatment outcomes

2.56. A pilot study by Evans et al., 2023, investigated the microbiome in vaginal samples, and urine samples of men. It was found that some microbial species affected implantation, and samples from women at the time of embryo transfer who did not achieve implantation had a significantly higher percentage of samples that were positive for *Prevotella bivia* and *Staphylococcus aureus* compared to women who did achieve implantation.

2.57. A study by Tsai et al., 2023, analysed the microbial composition of cervical mucus from 29 women who had undergone frozen embryo transfer. Samples characterised by significantly increased *Gardneralla* and loss of Lactobacilli were correlated to a significantly adverse effect on ongoing pregnancy outcomes.

2.58. A nested case-control study by Hao et al., 2021, collected cervical swabs on the day of embryo transfer (ET) from 100 patients and analysed the clinical pregnancy outcomes. Analysis suggested that the composition of the cervical microbiota on the day of ET was associated with clinical pregnancy rates in fresh IVF-ET cycles.

2.59. A study by Carosso et al., 2020, investigated whether the vaginal and endometrial microbiota of women undergoing IVF could be modified by controlled ovarian stimulation and progesterone luteal supplementation in 15 women. Findings suggested that COS and P supplementation can significantly change the composition of vaginal and endometrial microbiota by reducing *Lactobacillus* abundance and increasing pathogenic species abundance. This could impact on endometrial receptivity and placentation, and indicates the freeze-all strategy may benefit fertility patients.

2.60. A study by Ge et al., 2023, investigated whether the joint detection of 22 vaginal microbes correlated with assisted reproductive outcomes in 107 vaginal secretion samples. The clinical pregnancy rate in women with normal vaginal microecology, indicated by the 22 vaginal microbes was higher than women with abnormal vaginal microecology.

2.61. A study by Lüll et al., 2022, assessed the microbial community of endometrial tissue and fluid samples in 25 women (28-42 years) receiving IVF. The findings show that *Lactobacillus* dominance is an important factor for influencing the microbial composition of embryo transfer and fluid samples.

2.62. A study by Amato et al., 2019, aimed to correlate the vaginal and seminal microbiome of 23 couples with idiopathic infertility to the clinical pregnancy rate after IUI. A predominance of *Lactobacillus crispatus* was a marker of a healthy vaginal ecosystem and *L.crispatus* dominance was the only factor strongly associated with IUI success.

2.63. A study by Keburiya et al., 2022, evaluated the influence of the uterine microbiota on embryo implantation success in 130 women undergoing ART for infertility. The study showed the uterine cavity is not free of microorganisms, and opportunistic microorganisms identified in the uterine cavity and cervical canal did not affect the pregnancy rate.

2.64. A prospective observational study by Koedooder et a., 2021, analysed the microbial composition from midstream urine samples from 85 women receiving IVF. The data primarily showed that clinical pregnancy results in significant changes in the abundance and diversity of urinary microbiota, and that the urinary microbiome composition before IVF/ICSI could potentially be used to predict clinical pregnancy.

2.65. A cohort study by Bernabeu et al., 2019, investigated the vaginal microbiome in 31 patients undergoing ART. The profiles dominated by *Lactobacillus* were associated with the achievement of pregnancy and there was a relationship between the stability of the vaginal microbiome and the achievement of pregnancy.

2.66. This study by Tong et al. 2023, explores the correlation between the vaginal microbiota and pregnancy outcomes in 19 women from Northern China who received IVF, compared to 6 women who conceived naturally. Several bacterial species showed potential in predicting pregnancy outcomes in women undergoing IVF. Additionally, the vaginal microbiota was found to be more stable in women who conceived naturally and those who carried pregnancy to term.

2.67. A study by Miyagi et al. 2023, examined the impact of the vaccinal microbiome on the pregnancy outcomes of 35 women undergoing ART. Pregnant women presented with significantly higher levels of *Lactobacillus spp.* abundance and low pathological bacteria, whereas the opposite was seen in women who did not become pregnant.

2.68. A retrospective cross-sectional study by Yao et al., 2023, examined the role of gut microbiota-derived short chain fatty acids in 147 patients undergoing IVF/ICSI-ET. Fecal propionate levels were significantly higher in the non-pregnant group than in the clinical pregnancy group ($p < 0.01$). Fecal acetate and butyrate levels were not significantly different in the two groups.

2.69. In a study by Chen et al., 2021, uterine fluids were collected, and microbial profiles were examined in 94 fertility patients with and without chronic endometritis (CE). The results demonstrated that patients with CE had significantly lower clinical pregnancy rates compared with non-CE patients and the relative abundances of Proteobacteria and Acidobacteria were higher in the non-CE group. CE may be a key factor in negative outcomes after IVF and uterine microbiota may serve as a biomarker to forecast IVF outcome success.

2.70. A study by Villani et al., 2022, examined the cervical swabs collected from 88 women undergoing ART. Statistically significant differences were identified at species level with several *Lactobacillus* species. Overall, the cervix was colonised by microorganisms which can play a role in ART outcomes, as seen by an overall decrease in embryo attachment rates and pregnancy rates in both fertile and infertile women.

2.71. A cross-sectional study by Guan et al., 2023, assessed the impact of the cervical microbiome on 120 women (aged 20-40 years) undergoing FET. The results indicated clustering of the cervical microbiome into 3 types. Types dominated by *L.crispatus* had a significantly higher biochemical pregnancy rate and clinical pregnancy rate than the group dominated by *L.iners* and the group dominated by other bacteria.

2.72. A study by Bednarska-Czerwinska et al., 2022, defined the microflora profile of the endometrium and uterine cervix in women qualified for IVF. Statistically significant relationships ($p < 0.05$) were found between several bacterial species including *Lactobacillus fermentum* and *Enterococcus faecalis*. Endometrial dysbiosis was not noted in patients qualified for IVF and the endometrium to a large extent appears to be colonised by lactic acid bacteria.

2.73. A study by Okwelogu et al., 2021 examined seminal fluid and vaginal swabs from 36 infertile couples to assess the impact on IVF outcomes. Semen samples with positive IVF outcomes were significantly colonised by *Lactobacillus jensenii* and *Faecalibacterium*, and significantly less colonised by *Proteobacteria*, *Prevotella* and lower Firmicutes/Bacteroidetes compared to semen samples with negative IVF outcomes. Vaginal samples with positive IVF outcomes were significantly colonised by *Lactobacillus gasseri* and less colonised by Bacteroides and *Lactobacillus iners*. This study has opened a window of possibility for *Lactobacillus* replenishments in men and women before IVF treatment.

2.74. A study by Kong et al., 2020, analysed the vaginal microbiota in 555 patients to identify the possible causes of IVF failure. The results indicated that a lower abundance of the probiotic *Lactobacillus* and a higher abundance of the pathogens *Gardnerella* and *Prevotella* were identified from non-pregnant patients. Therefore, a disordered microbiota may be a potential indicator for a higher IVF failure rate.

2.75. An exploratory study by Bui et al., 2023, analysed 141 infertile women with a failed first attempt at IVF/ICSI by comparing the microbiota profiles of women with and without a live birth within 12 months of follow-up. Women with a live birth had significantly higher *Lactobacillus crispatus*

relative abundance (RA). A small proportion of women in the male factor infertility group had ≤ 10% *L.crispatus* RA compared to women in the unexplained, and other infertility causes groups combined. Women with primary infertility compared to secondary infertility had significantly higher *L.crispatus* RA; lower proportions of them had ≤ 10% *L.crispatus* RA.

2.76. Findeklee et al., 2023, retrospectively investigated whether the results of microbiological vaginal swab results influenced the outcome of fertility treatment in 397 patients undergoing ART. Dysbiosis was associated with a worse outcome of fertility treatment. The pregnancy rate with a conspicuous swab was 8.6% vs 13.4% in an inconspicuous swab, but the association was not statistically significant. However, the absence of Lactobacilli was significantly associated with endometriosis.

2.77. A study by Farook Faisal et al., 2023, analysed vaginal samples on ovum pick-up from 46 asymptomatic infertile Iraqi women undergoing ICSI. The majority of patients had a genital tract infection and *Enterobacter spp.* had a substantial negative influence on the pregnancy rate. Conversely, Lactobacilli was highly related to positive outcomes in participating females.

2.78. A study by Wang et al., 2021 looked at the effect of the lower genital tract microbiota on pregnancy outcomes in 150 reproductive-aged women receiving embryo transfer. The microbial community of both vaginal and cervical microbiota between pregnant and non-pregnant groups were not statistically different. Although the microbial composition of the vagina and cervical canal was found to potentially influence the outcome of IVF-ET, more samples are needed to verify this conclusion.

2.79. A systematic review and meta-analysis by Singer et al., 2019, of 6 studies found a correlation between abnormal vaginal microbiota and lower rates of early pregnancy development after IVF treatment. An abnormal vaginal microbiota was associated with 1.4 times less chance of achieving successful early pregnancy development after IVF treatment compared to women with a normal microbiota.

2.80. A prospective cohort study by Koedooder et al., 2019, analysed the vaginal microbiome of 192 women undergoing fresh embryo transfer. Women with a low percentage of *Lactobacillus* in their vaginal sample were less likely to have successful embryo implantation and the degree of *L.crispatus* dominance was an important factor in predicting pregnancy. The failure rate was correctly predicted 94% of the time, indicating vaginal microbiome profiling could predict ART outcomes prior to the start of fertility treatment.

2.81. A study by Haahr et al., 2019, investigated whether a more detailed characterisation of the vaginal microbiota by 16S ribosomal RNA gene sequencing could improve predictions of poor reproductive outcomes in 120 patients undergoing IVF. The predictive value of 16S ribosomal RNA gene sequencing was not superior to the simpler and less expensive qPCR diagnostic approach in predicting the risk of a poor reproductive outcome in patients undergoing IVF. Additionally, no significant association between community state type and reproductive outcome could be demonstrated.

2.82. A prospective study by Vergaro et al., 2019, investigated the relationship between the vaginal microbiota profile at the time of embryo transfer and LBR in 150 Caucasian women undergoing IVF/ICSI with donated oocytes. The microbiota profiles of women who achieved a live birth compared to those who did not was similar. However, a higher proportion of samples dominated

by *L. crispatus* was found in women achieving a live birth and this correlation was also statistically significant for biochemical pregnant and clinical pregnancy. However, overall the results suggested that BV-like vaginal microbiota at the time of embryo transfer did not affect the live birth rate.

2.83. A study by Komiya et al., 2020, evaluated the effect of the prebiotic "partially hydrolysed guar gum" supplementation on gut dysbiosis in 18 infertile women undergoing ART. The present study showed differences in the abundance of gut microbiota between the two groups and prebiotic supplementation helped improve gut dysbiosis and the success of pregnancy in infertile women.

2.84. A study by Wu et al., 2023, sampled and analysed the microbiological profile of follicular fluid from 49 primary infertility and 52 secondary infertility patients. It was found that *Lactobacillus*, especially *L.crispatus* might have a positive effect on female pregnancy.

2.85. A prospective study by Kim et al., 2022, analysed follicular fluid and vaginal secretions on the day of ovum pick-up from 49 infertile females undergoing IVF/ICSI. Follicular fluid microorganisms were not associated with embryo quality or clinical pregnancy rates during IVF cycles. However, significantly decreased implantation rates and clinical pregnancy rates on embryo transfer day 5 were observed in the group that was positive for vaginal pathogens.

2.86. A review by Tsonis et al., 2020, summarised the current knowledge on the microbiota of the male and female reproductive tract and its impact on the success rate of ART in infertile couples.

2.87. A pilot study by Riganelli et al., 2020, analysed the vaginal and endometrial microbiome of 34 women undergoing personalised hormonal stimulation prior to oocyte pick-up. Analysis revealed a significant difference between vaginal and endometrial microbiota. The vaginal microbiota of pregnant women exhibited a *Lactobacillus*-dominant habitat compared to non-pregnant cases, while the endometrial bacterial colonisation was characterised by a polymicrobial ecosystem in which lactobacilli were exclusively detected in the group that displayed unsuccessful IVF.

2.88. A systematic review and meta-analysis by Skafte-Holm et al., 2021, investigated the impact of vaginal dysbiosis (VD) on the reproductive outcomes of 3534 patients undergoing IVF treatment across 17 studies. Across all methods used, VD was a significant risk factor for early pregnancy loss in IVF. A significant reduction in clinical pregnancy rate when compared to normal microbiota patients was also found, however VD did not significantly influence LBR. The quality of evidence was very low across all outcomes according to GRADE and thus more studies are required.

2.89. A review by Punzón-Jiménez et al., 2021 gathered available research focusing on the female genital tract (FGT) microbiome and its role in ART outcomes and gynaecological disorders. Current knowledge confirms predominance of *Lactobacillus* species, both in the vagina and endometrium, whereas higher variability of species is found in the fallopian tubes and ovaries. Broadly low variability of species and *Lactobacillus* abundance within the FGT is associated with better reproductive and ART outcomes.

2.90. A study by Dong et al., 2023, compared cervical, vaginal, urethral and rectal swabs from 22 infertile patients and controls, and examined follicular fluid from 22 infertile patients. *Lactobacillus* predominated in the female urogenital tract, but its abundance decreased in infertile patients, whereas the abundance of *Gardnerella* and *Atopobium* increased. Compared with healthy

controls, the cervical and rectal microbial biodiversity of infertile patients were significantly increased and decreased, respectively.

2.91. A review by Gao et al., 2022, reviewed the latest research on the female urogenital microbiome as a predictor of successful implantation. *Lactobacillus crispatus* appears to be a beneficial species in a healthy female genital tract and the vaginal microbiome is associated with ART outcome in terms of successful implantation and pregnancy.

2.92. A review by Simón et al., 2022, presents some of the current research around the clinical implications of endometrial factor testing for personalising reproductive care, including identification of commensal microbes colonising the endometrium lining, with reproductive outcomes in patients undergoing IVF.

2.93. A multicentre prospective observational study by Moreno et al., 2022, analysed endometrial fluid and biopsy samples before embryo transfer in a cohort of 342 infertile patients undergoing ART. A dysbiotic endometrial profile was associated with unsuccessful outcomes. In contrast *Lactobacillus* was consistently enriched in patients with live birth outcomes. Endometrial microbiota composition was proposed to be a useful biomarker to predict reproductive outcome before embryo transfer.

2.94. A review by Schoenmakers et al., 2022, presents an overview of the recent microbiome research and findings within the field of reproductive medicine and its relation with the outcome of ART. Results indicate that only the vaginal microbiome can be sampled without possible risk of contamination. Collection of the cervical microbiome has to be sampled with extreme caution and other sites have a high risk of cross-contamination. The vaginal composition prior to the start of hormonal treatment for ART seems to be predictive of IVF/ICSI outcome with a highly negative predictive value.

2.95. A study by Xu et al., 2020, utilised 16S rRNA sequencing of the vaginal microbiome in 85 women of reproductive age without vaginal infections of reproductive endocrine diseases. The vaginal pH, levels of basal E2, LH and FSH all had significant effects on the distributions of the vaginal microbiome. Several species were identified including *Escherichia coli* and *Prevotella intermedia* which could be used as biomarkers to reflect the pathological state of the reproductive, endocrine and genital tract.

2.96. A review by Mauries et al., 2021, examines literature on the association between the female genital microbiota and ART success, including diagnostic and therapeutic approaches in the management of patients with an altered microbiome.

2.97. A systematic review by Dube et al., 2022, examined the available evidence on the microbiota of the genital tract in women undergoing ART and studied the outcomes of IVF in different microbial compositions. Despite inconsistency in results, it was evident that the vaginal, cervical and endometrial microbiota might play a role in predicting ART outcomes.

2.98. A review by Cocomazzi et al., 2023, highlighted the impact of the vaginal microbiota in ART technique, the factors that influence the vaginal microbiota, the consequences of dysbiosis and potential interventions to restore a healthy female genital tract.

2.99. A systematic review of 14 studies by Brandão et al., 2020, analysed the effect of the female genital tract microbiota in fertility and ART outcomes. The results were diverse and incoherent, and although it seemed the microbiome may play a role in infertility, the influence on conception rates is unclear, due to paucity and inconsistency of the published data.

2.100. A randomised controlled trial by Gunderson et al., 2021 characterised the DNA virome in semen samples from 24 male partners of couples undergoing IVF. An association was found between virome diversity and pregnancy in couples undergoing IVF. However, no association was found with specific semen parameters or fertilisation rates, suggesting that viral exposure may negatively affect pregnancy after fertilisation.

2.101. A prospective cohort study by Karaer et al., 2021, examined the vaginal samples of 223 women undergoing ART treatment. Although relative abundance of Lactobacillus was lower in women who failed to become pregnant, the difference was not statistically significant. The abundance of Streptococcus was found to be statistically significant between the two study groups, with a relatively high abundance of Streptococcus in the vaginal microbiota being associated with a lower ART success rate.

2.102. A study by Reschini et al., 2022 analysed the microbiome in endometrial fluid samples from 53 women scheduled for IVF. Endometrial Lactobacillus-dominant cases were uncommon compared to previous evidence, being observed in only 8% of women. The comparison between women who did and did not subsequently become pregnant failed to identify any microorganism associated with the success of the procedure. However, the endometrial biodiversity resulted higher among pregnant women.

2.103. A study by Zhao et al., 2022, recruited 30 patients with secondary infertility undergoing IVF and analysed their vaginal microbiome composition using 16S rRNA gene sequencing. The results showed that women suffering from infertility exhibited a significant decrease in microbiome diversity and richness compared with healthy women during the nonovulation period, whereas the vaginal microbiome of healthy women revealed dramatic fluctuations during ovulation. Infertility patients showed no change of the vaginal microbiome under conditions of GnRH agonist and r-hCG induction and there were characteristic variations in the vaginal microbiome of infertile women.

2.104. A retrospective study by Zou et al., 2022, analysed the endometrial microbiota from 141 patients with RIF. In the endometrium of most RIF patients pathogenic bacteria could be found and administration of oral antibiotics over 14 days resulted in higher clinical pregnancy and ongoing pregnancy rates compared to the RIF group that did not receive antibiotic treatment.

Interventions (e.g., probiotics) and their role in fertility or fertility treatment outcomes

2.105. A study by Fernandez et al., 2021, investigated the ability of the probiotic *Ligilactobacillus salivarius* to increase pregnancy rates in women with reproductive failure. Lactobacilli were detected at higher frequency and concentration in fertile women compared to infertile women. Daily oral administration of *L.salivarius* over 6 months resulted in a successful pregnancy rate of 56% for the infertile women and it was concluded this probiotic could be a good candidate to improve success rates in these patients.

2.106. A drug intervention study by Haarh et al., 2020, described a protocol for an on-going double-blind placebo-controlled multicentre trial of IVF patients diagnosed with abnormal vaginal microbiota and randomised in three parallel groups 1:1:1. The interventions involved antibiotic and probiotic administration with clinical pregnancy rate per embryo transfer being the primary outcome.

2.107. A randomised, double-blinded, placebo-controlled study by Jepson et al., 2023 was conducted in 74 women (18-40 years), who had previously been diagnosed with an unfavourable vaginal microbiota prior to undergoing fertility treatment. The women were randomly assigned to receive probiotic capsules containing *L.gasseri* or *L.rhamnosus* or no active ingredient (placebo). The vaginal microbiota improved after intervention in 34.2% of all participants, with no significant difference in the improvement rate of the unfavourable vaginal microbiota between the Lactobacilli and placebo groups.

2.108. A randomised blinded controlled trial by Tanha et al., 2023, investigated the impact of using Lactovag, a probiotic product, to normalise the vaginal microbiome and improve pregnancy outcome in women undergoing FTET cycles. Although Lactovag showed higher pregnancy rates the findings were not significant. However, it was indicated that transferring grade A embryos would increase the odds of pregnancy if Lactovag was used.

2.109. A prospective cohort study by Iwami et al., 2023, analysed endometrial microbiota samples from 131 patients before embryo transfer (ET), while 64 patients proceeded to ET without analysis (control). Patients with an abnormal endometrial microbiota received targeted antibiotic therapy, followed by probiotic treatment. Following the intervention, the cumulative clinical pregnancy rate (study group: 64.5% vs. control group: 33.3%, $p = 0.005$) and the ongoing pregnancy rate (study group: 48.9% vs. control group: 32.8%, $p = 0.028$) were significantly increased in the study group compared to the control group.

2.110. A review by Blancafort 2022, assessed the capacity of probiotics as a single intervention to alter the female genital tract microbiota in non-symptomatic reproductive-aged women. Although research shows *Lactobacillus*-containing vaginal probiotics hold promise for dysbiosis, the scientific data is insufficient to recommend their use systematically for treating asymptomatic dysbiosis before IVF.

2.111. A study by Wang et al., 2022, discusses therapeutic options such as probiotics, prebiotics and fecal microbiota transplantation, as well as the challenges and opportunities behind ongoing research, while emphasising the need for additional research on the gut microbiome and male reproduction.

2.112. A prospective study by Kitaya et al., 2022, analysed vaginal secretions and endometrial fluid from RIF women and control infertile (non-RIF) women. Lactoferrin was administered to women with non-*Lactobacillus* dominance (NLDM). Lactoferrin supplementation improved NLDM in 43.2% of RIF women. Within the RIF group, the LBR in subsequent cycles was higher in women with improved microbiota than in those with unimproved microbiota.

2.113. A study by Hao et al., 2022, discovered that fecal microbiota transplantation of alginate oligosaccharide improved the gut microbiota and ameliorated decreased semen quality from a high-fat diet. FMT also benefited gut microbiota to improve liver function by adjusting lipid

metabolism, ameliorating HFD-impaired testicular microenvironment to rescue spermatogenesis and increase semen quality and fertility.

2.114. An intervention study by Iniesta et al., 2022, evaluated the effect of supplementation with *L.salivarius* PS11610 on the microbial composition of the urogenital tract in 17 couples undergoing ART with urogenital dysbiosis. Probiotic supplementation significantly modified the urogenital microbiome composition in male and female samples, resolving dysbiosis in 67% of couples. Pregnancy and delivery ratio were also improved.

2.115. A review by Lopez-Moreno et al., 2020, analysed results from studies which assessed the clinical impact of probiotics administered on several endocrine disorders' manifestations in women: mastitis; vaginal dysbiosis; pregnancy complication disorders; and polycystic ovary syndrome. In all cases the clinical modulation achieved by probiotics was evaluated positively through the improvement of specific disease outcomes with the exception of pregnancy disorder studies, where sample sizes were too small to be significant.

2.116. A review by Gholiof et al., 2022, examines the increasing evidence for the involvement of the female reproductive tract microbiotas and inflammation in gynaecological conditions, such as endometriosis and infertility. The use of antibiotics and probiotics to therapeutically alter the FRT microbiota is also discussed.

2.117. A systematic review and meta-analysis by Lopez-Moreno et al., 2021, analysed the protocols of vaginal probiotics that are commonly used to investigate the microbiome. Vaginal probiotic doses were found to be similar in dosage to oral probiotic protocols and moderate vaginal microbiota modulation was achieved, with Lactobacillus species increasing following probiotic application.

2.118. A study by Zhao et al., 2022, retrospectively assessed whether the fertility of 11 patients with IBD could benefit from washed microbiota transplantation (WMT compared to 10 controls without WBT). The pregnancy rate in WMT group was significantly higher than in those without WBT ($P = 0.047$).

2.119. A review by Hashem et al., 2022 summarises the current knowledge on the biodiversity of the microbiota in the reproductive tract, possible changes in the case of dysbiosis, and the use of probiotics for the management and improvement of reproductive eubiosis and function.

3. Conclusions

3.1. Since this topic was last discussed in February 2019 there has been a large increase in the number of studies identifying a possible interaction between the microbiome and fertility or fertility treatment outcomes.

3.2. In particular, the presence of *Lactobacillus* in the female reproductive tract, and particularly *Lactobacillus crispatus* appears to be the most heavily researched for its positive association in fertility. Conversely, an altered microbiome or dysbiosis, has been associated with poorer fertility treatment outcomes.

3.3. An increase in the number of studies utilising probiotics other therapeutic interventions to improve the gut microbiome has also been identified, with most identifying positive results in both fertility and fertility treatment outcomes.

3.4. However, the majority of studies call for larger sample sizes and more robust and standardised methodological processes to sample the microbiome before definitive conclusions can be drawn.

4. Recommendations

4.1. Members are asked to:

- Advise the Executive if they are aware of any other recent developments.
- Review whether any outputs from the HFEA are required addressing the impact of the microbiome on fertility treatment outcomes.
- Consider the potential for companies to market supplements that claim to improve fertility based on the microbiome and if a treatment add-ons application for microbiome tests should be brought back to a future SCAAC meeting.

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Review of artificial egg activation using calcium ionophore as an add-on

Details about this paper

Area(s) of strategy:	The right information
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item	8
Paper number	HFEA (02/10/2023) 008
Meeting date	2 October 2023
Author	Dina Halai, Head of Regulatory Policy
Annexes	Annex A: The experts review of the evidence base on the use of artificial egg activation using calcium ionophore Annex B: Definition of ratings for add-ons Annex C. Evidence decision tree for rating add-ons

Output from this paper

For information or recommendation?	For recommendation
Recommendations	To consider: • whether artificial egg activation using calcium ionophore should remain on the HFEA's treatment add-ons list following recent publication of professional guidelines providing best practice recommendations; and • if the committee agree that is should remain on the HFEA's treatment add-ons list, then to consider the quality of evidence for the use of artificial egg activation based on the findings from an independent assessor and recommend ratings for its use.
Resource implications	In budget
Implementation date	Recommendations will be implemented as soon as feasible

Communication(s)	Updates to the HFEA's website information on treatment add-ons and communication of updates to the sector, patients and public.		
Organisational risk	☒ Low	☐ Medium	☐ High

1. Introduction

1.1. Licensed clinics are authorised to use certain laboratory processes in order to carry out each licensed activity. Artificial egg activation using calcium ionophore is an authorised process for use only in suitable patients. When the SCAAC considered the use of calcium ionophore for egg activation as an authorised process, they highlighted the theoretical risks relating to embryo viability (eg premature activation and triploid embryos). Given the theoretical risks of using calcium ionophore, centres using it can only do so in selected patients, such as those with Phospholipase C zeta (PLCz) deficiency. Centres are expected to document their rationale for using calcium ionophore for individual cases. As with all treatments and processes, centres should ensure that patients are fully informed about the efficacy and potential risks and that validation is carried out.

1.2. Artificial egg activation using calcium ionophore was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee, which reflects the evidence base for increasing the chances of having a baby for patients who are eligible to undergo this treatment, as it is not authorised to be used in most fertility patients. An amber rating means that there is conflicting evidence from randomised controlled trials (RCTs) to show that it is effective at improving the outcome. No changes have been made to this traffic light rating since then.

1.3. At the July 2023 SCAAC meeting, it was agreed that the Executive would bring a discussion back after the professional guidelines providing best practice recommendations on the use of artificial egg activation have been published, so that the SCAAC could consider whether:

1.3.1. Artificial egg activation should remain on the HFEA's treatment add-ons list and therefore continue to be considered an add-on according the Authority's definition as agreed in July 2022; *Additional treatments (to the core treatment e.g. IVF or IUI) that are being offered to the general patient population in licensed fertility clinics in the UK.*

1.3.2. If the HFEA's information on artificial egg activation as a treatment add-on conflicts with the professional guidelines.

1.4. The Association of Reproductive and Clinical Scientists (ARCS) and British Fertility Society (BFS) have since published professional guidelines on best practice use of artificial egg activation, which states that:

1.4.1. *Artificial oocyte activation (AOA) should not be used routinely with ICSI as its safety, in terms of the potential developmental consequences and birth outcomes, has yet to be established.*

1.4.2. *ICSI with AOA may be used where two previous routine ICSI cycle(s) have resulted in <30% or no fertilisation.*

1.4.3. *Where AOA is used, patients should be advised that safety, in terms of the potential developmental consequences and birth outcomes, has not been established.*

1.4.4. *Patients should be provided with safety data relating to the specific AOA technique used.*

2. Recommendations

2.1. To consider whether artificial egg activation using calcium ionophore should remain on the HFEA's treatment add-ons list following recent publication of professional guidelines providing best practice recommendations.

2.1.1. In any case, we will update the information on the clinic portal about authorised processes to state that it can only be used in line with the professional guidelines.

2.1.2. If artificial egg activation using calcium ionophore is removed from the HFEA's treatment add-ons list, information for patients about its use can be placed elsewhere on our website.

2.2. If the committee agree that it should remain on the HFEA's treatment add-ons list, then to consider the quality of evidence for the use of artificial egg activation based on the findings from an independent assessor, as outlined in the remainder of this paper, and recommend ratings for its use.

2.2.1. Given that calcium ionophore is not authorised to be used in most fertility patients, and the professional guidelines recommend use in selected cases only, the committee does not need to allocate a rating for improving live birth rates for most fertility patients. The HFEA will include information on the add-ons page about this.

3. Expert review of the evidence

3.1. For the July 2023 meeting, the independent reviewer reassessed the rating for artificial egg activation using calcium ionophore in light of the new five-category rating system and additional studies identified and made the following recommendations based on 10 RCT's and 7 NRSI's:

Current rating	Expert review July 2023
Live birth rate for most fertility patients	• GREY for embryo formation and early development for most fertility patients • GREY for live birth rate for patients with failed fertilisation in previous ICSI treatments • GREY for embryo formation and early development for patients with failed fertilisation in previous ICSI treatments

3.2. The expert reviewers full report and results of analysis can be found within the July 2023 meeting papers, and their review of the evidence base on the use of artificial egg activation using calcium ionophore can be found at Annex A of this paper.

4. Annex A: The experts review of the evidence base on the use of artificial egg activation using calcium ionophore

The previous review in 2019 included four studies: two within-patient designs on sibling oocytes and two RCTs of patients that each suggested promise but studied quite different populations and were dogged by methodological issues. The current search identified a total of 37 primary research studies and four systematic reviews. Searching of these reviews identified one further randomised study for consideration.

Several studies randomised oocytes from each retrieval cycle to be subjected to artificial activation or not. I have referred to these as 'within-patient' studies. By design, these studies do not contribute to the information for clinical outcomes. In principle, they can provide valuable information on pre-transfer, developmental outcomes. For statistically valid inference, investigators need to have accounted for both the 'clustering' of multiple oocytes allocated to each intervention arm from each retrieval cycle and the 'pairing' of the two clusters from each retrieval cycle. In practice, such analyses were rare.

1 (i) General population

Nasr-Esfahani 2007 reported randomly assigning oocytes within retrieval cycles of 87 couples with severe male factor (teratozoospermia) and at least four mature oocytes. Oocytes were cultured in G1 medium with or without activation using 10μM ionomycin for 10 minutes. Assessment of fertilisation and embryo scoring were conducted blind to treatment allocation. There was no description of the randomisation process and the study design did not allow for comparison of clinical outcomes. Analysis of development outcomes failed to account for the paired design. Mean fertilisation rates were reportedly higher with activation, and the mean percentage of embryos considered high quality was similar.

Borges 2009 randomised 204 couples with severe male factor(azoospermia) to culture in G1-V3-Plus medium with or without activation using 5μM calcium ionophore A23187 for 30 mins. There was no description of the randomisation process and no discussion of blinding. Up to four embryos were transferred for each participant. The design was stratified by the method of sperm extraction into three groups: those with obstructive azoospermia undergoing percutaneous aspiration (PESA) or testicular aspiration (TESA) and those with a non-obstructive diagnosis undergoing TESA. Clinical pregnancy was reported only as a percentage. For those undergoing TESA it was possible to recalculate the number of participants achieving clinical pregnancy: OR=1.2 (0.51 to 3.0). Reported fertilisation rates were similar overall. The reported percentages of high quality embryos were similar for patients with non-obstructive azoospermia, whereas for those with an obstructive diagnosis rates were lower with activation in the TESA stratum and higher in the PESA stratum. It is unclear how these rates were calculated and analysed.

Liu 2011 conducted a non-clinical study with what appears to have been considered 'waste product'. From previous ICSI cycles they took oocytes that had failed to develop (germinal vesicle or metaphase I). These had been vitrified, thawed and then matured for 24-36 hours, with 204 oocytes maturing to be subject to ICSI using donor sperm. They then describe randomly assigning these to either standard cleavage medium or activation for 6 minutes in 7% ethanol prior to standard cleavage medium. There is no detail to assess the allocation but it appears to have been done regardless of sibling status. The number of women who provided the oocytes is not reported and there appears to have been no intention to transfer any resulting embryos. Reported fertilisation rates were similar between groups. Only the activated oocytes led to any high quality embryos (n=12 from 104), or high quality blastocysts (n=4).

Ebner 2012 prospectively recruited 66 couples undergoing ICSI with severe male factor and "sufficient" number of oocytes. All were treated with calcium ionophore immediately following ICSI. Unfortunately,

there are several methodological issues with this study that preclude statistical interpretation. The presentation and analysis do not account for the multiple cycles per participant. Comparison is made with multiple historic cycles of the same participants. Comparison also fails to account for the inherent matching and is almost guaranteed to show 'benefit' given that regression to the mean, Hawthorne and placebo biases all favour the intervention. The authors reported higher fertilisation and blastocyst formation in the studied cycles, with 26 (39%) participants achieving live birth.

Eftekhar 2013 randomised 38 couples with male factor (teratozoospermia) to culture in GIVF-Plus medium with or without activation using 5μM calcium ionophore A23187 for five minutes. There was no description of a concealment process and explicitly no blinding. All participants received the allocated intervention and analysis was presented by intention to treat. Ongoing pregnancy was higher in the activated arm: OR=2.5 (0.51 to 12) under a policy of transferring up to three embryos. Participants in the intervention arm had an average of 1.8 (0 to 3.5) more fertilised oocytes and 1.8 (-0 to 3.7) more embryos than controls. It should be noted however that they also had an average of 1.5 more oocytes retrieved prior to intervention differences.

Liu 2014 conducted a non-clinical study with what appears to have been considered 'waste product'. From 102 previous IVF and ICSI cycles they took 179 metaphase II oocytes with normal morphology that had failed to fertilise. They then describe randomly assigning these to activation with either 5μM calcium ionophore A23187 for five minutes or strontium chloride for 20 minutes. There is no detail to assess the allocation but it appears to have been done regardless of sibling status. Presentation and analysis also ignored all aspects of clustering. Activation was reported to be higher in the calcium ionophore arm (104 versus 66 oocytes) as was blastocyst formation (four versus one).

Caglar Aytac 2015 randomised 296 couples with diminished ovarian reserve but normal sperm parameters and no previous fertilisation failure. It appears that the allocation process was not concealed and there was no blinding. Transfer was more common in the activation group (68% vs 56%) and there were more pregnancies per transfer, leading to a higher ongoing pregnancy rate: OR=1.9 (0.80 to 4.4). Mean fertilisation rate was higher in the intervention arm: 5.3 (4.4 to 6.2) percentage points. Note that this figure was strongly statistically significant even if reported standard deviations were erroneously standard errors, yet the comparison was described only as 'not significant' in the paper. The mean number of high quality (Grade A or B) embryos per participant was almost identical.

Yang 2015: only available as an abstract so not assessed in this round.

Fawzy 2018 randomised 443 participants evenly between three groups: two active arms using either strontium chloride or calcymicin and a control. Participants had either a diagnosis of male factor infertility (61%) or at least two previous cycles with <30% fertilisation rate (6% total failure). Several methodological issues raise caution. In particular, early randomisation (day 21 of previous cycle) may have resulted in opportunity for selection bias. It is noteworthy that participants in the active arms had both more oocytes retrieved and more mature oocytes than those in the control arm. The trial also finished early following an interim analysis of the data but with no clear specification of any statistical stopping rule applied. The numbers of transfers and of embryos per transfer were similar across groups. The results however show clinical advantage for artificial activation in both active arms: live birth OR = 3.0 (1.6 to 4.5) and 2.2 (1.2 to 4.0) for strontium chloride and calcymicin respectively. Reported rates of fertilisation and of blastocyst formation from fertilised oocytes were higher in both activated arms.

Shebl 2021 presented a within-patient, sibling oocyte design in 78 couples undergoing ICSI with either a history of <50% fertilisation (n=47) or severe male factor (n=31). Activation was by ionophore (calcimycin) for 15 minutes within 10 minutes of ICSI. All embryos were then cultured in a time-lapse system to allow comparison of morphokinetics. Unfortunately, there was no description of how selection took place so major bias cannot be ruled out. Their analyses of embryo formation and early development did calculate

a value per person and then recognise the inherent pairing of the design. Fertilisation and utilisation rates were both significantly higher under activation. Time to appearance of two pronuclei (t2PNa) was reduced by 0.74 (0.28 to 1.25) hours. Other developmental times and occurrence of irregular cleavages did not differ between arms. Interpretation of clinical outcomes is unreliable as there was no description of how selection was undertaken between equal quality embryos in different treatment arms. However, 74 transfers took place using embryos from a single arm (all bar one were elective single embryo transfers) resulting in 22 live births from activated embryos and 11 from control embryos.

Yin 2022 presented a within-patient, sibling oocyte design in 140 couples identified through previous ICSI cycle failure due to either zero (n=66) or <30% (n=74) good quality embryo rate calculated for patients who had a normal fertilisation rate calculated from at least 5 mature metaphase II oocytes. Although the selection of embryos is described as 'random' this appears unlikely: no detail is provided and the 'spare' from an odd number was always allocated to the active arm. Activation was achieved by 10 minutes in ionomycin solution one hour following ICSI. Unfortunately, the inherent matching was ignored in both presentation and analysis of the data. Interpretation of clinical outcomes is unreliable as there was no description of how selection was undertaken between equal quality embryos in different treatment arms. However, 84 transfers took place using embryos from a single arm (all bar one were elective single embryo transfers) resulting in 32 live births. The mean numbers of fertilised oocytes and of day 3 good quality embryos were both higher in the activation arm.

Current rating amber.
Recommendation: GREY for all outcomes [No moderate/high quality study, no safety concerns]

1 (ii) Failed fertilisation in previous ICSI cycle

Meerschaut 2012 presented a within-patient design on sibling oocytes from 14 couples with normal sperm but failed or low fertilisation in a previous ICSI cycle. They did not specify allocation method so there is substantial scope for selection bias. Failure to present or analyse the data in a way that recognised the inherent matching precludes statistical interpretation. Ignoring matching, more embryos (74% vs 44%) were fertilised in the 'activation' arm. The nature of the sibling-oocyte design does not allow interpretation of the clinical outcomes.

Montag 2012 prospectively recruited 89 couples undergoing ICSI with previous failed fertilisation (Group 1); fertilisation between 1 and 29% (Group 2); or fertilisation between 30 and 50% (Group 3). All were treated with 10 µmol/l calcium ionophore A23187 for 15 minutes immediately following ICSI. This study was by the same team as Ebner 2012 (reviewed under 1(i) above) and unfortunately shared the same methodological issues. Live births were achieved by 19% of participants in Group 1, 37% in Group 2 and 25% in Group 3. The authors reported fertilisation rates ranging from 42% to 56% in each group. Although the comparison with previous failed cycle is clearly problematic, the uncontrolled cohort demonstrates that successful treatment is possible in this population.

Ebner 2015 largely repeated the study of Montag 2012 from the same team. They prospectively recruited 101 couples undergoing ICSI following previous fertilisation 'problems': failed fertilisation (n=15); fertilisation between 1 and 30% (n=52); fertilisation between 31 and 50% (n=34). All were treated with calcium ionophore A23187 for 15 minutes immediately following ICSI. Although analyses recognised the pairing of participants from index and previous cycle, the major methodological issues from Montag 2012 also apply to this study. There were 35 clinical pregnancies and 28 of these progressed to live birth, including seven twin deliveries. The authors reported substantially 48% fertilisation and 10% cultured to blastocyst from 884 metaphase II oocytes. Only one participant had total fertilisation failure and the remaining 100 all progressed to embryo transfer.

Darwish 2015 undertook a similar but far smaller 'preliminary' study. They prospectively recruited four couples whose previous ICSI cycle was incomplete due to 2PN arrest. The same statistical issues apply to interpretation of the data. All four participants progressed to embryo transfer with a total of eleven embryos transferred. Only one had a positive pregnancy test and this resulted in a healthy twin delivery at term from three transferred embryos. Fertilisation rate was 68% of metaphase II oocytes.

Aydinuraz 2016 presented a within-patient, sibling oocyte design in 21 couples with terato-zoospermia and a low fertilisation rate in the previous cycle. Unfortunately, their presentation and all analyses ignored the matching of the design, precluding statistical interpretation of their data. However, it is clear that only 13 of the 21 couples produced at least one top quality embryo from artificially activated oocytes, whereas 20 achieved this from conventionally cultured oocytes. There was a similar mean number of fertilised oocytes per participant (3.8 versus 3.4) but fewer Grade A embryos from the activated arm (1.3 versus 1.8).

Hao 2016 presented a within-patient design of eleven couples with low (<30%) previous fertilisation, no high quality day 3 embryo or round-headed sperm. Activation was by 10 µmol/l calcium ionophore A23187 for 15 minutes. There was no description of a concealment process and no discussion of blinding. From presented tables, it was possible to obtain the data for the six participants with low previous fertilisation and re-analyse respecting the paired design. Three of these participants had twin deliveries from double embryo transfers. The activated arm produced a mean of 1.8 (-0.8 to 4.4) more fertilised oocytes and 0.5 (-0.1 to 1.1) more good quality embryos.

Li 2019 presented a within-patient, sibling oocyte design in 50 couples identified through previous ICSI cycle failure (15 total fertilisation failure; 18 low fertilisation; 17 severe teratozoospermia). An independent embryologist divided oocytes into groups that were either activated using two 5-minute spells in ionomycin solution or subjected to 'simulated manipulation' by rinsing at comparable times. There is no suggestion that the selection process was randomised. If transferable embryos were achieved from both arms for a participant, the control embryos were preferentially selected. This design prevents interpretation of the clinical outcomes. Unfortunately the development arms were almost exclusively reported per oocyte rather than per participant and the inherent matching was ignored in both presentation and analysis of the data. The authors report higher proportions of transferable day 3 embryos (44% versus 27%) and of fertilisation (50% versus 34%) in the active arm.

Current rating amber.
Recommendation: GREY for all outcomes [No moderate/high quality studies, no safety concerns]

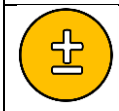
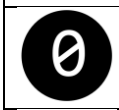
References of reviewed studies

Bold indicates studies added for the July 2023 update.

Adjunct	Study	DOI/reference
General	**Nasr-Esfahani 2007**	**10.1016/j.fertnstert.2007.10.047**
	Borges 2009	**10.1016/j.fertnstert.2008.04.046**
	Liu 2011	**10.1017/S0967199411000530**
	Ebner 2012	**10.1016/j.fertnstert.2012.07.1134**
	Liu 2014	**10.1089/cell.2013.0081**
	Eftiikhar 2013	**IRCT2012112610328N1**
	Caglar Aytac 2015	10.1016/j.fertnstert.2015.07.1163
	Fawzy 2018	10.1093/humrep/dey258
	Shebl 2021	**10.1007/s10815-021-02338-3**
	Yin 2022	**10.1007/s00404-021-06329-8**
Failed fertilisation	Meerschaut 2012	10.1093/humrep/des097

	Montag 2012	**10.1016/j.rbmo.2012.02.002**
	Ebner 2015	**10.1016/j.rbmo.2014.11.012**
	Darwish 2015	**10.1016/j.rbmo.2015.08.012**
	Aydinuraz 2016	10.1080/14647273.2016.1240374
	Hao 2016	**10.3760/cma.j.issn.0376-2491.2016.43.010**
	Li 2019	**10.1016/j.rbmo.2019.03.216**

5. Annex B: Definition of ratings for add-ons

	On balance, findings from high quality evidence shows this add-on is effective at improving the treatment outcome. An add-on can be rated green if at least one moderate/high quality RCT focuses on LBR.
	On balance, it is not clear whether this add-on is effective at improving the treatment outcomes. This is because there is conflicting moderate/high quality evidence. In some studies, the add-on has been found to be effective, but in other studies it has not.
	We cannot rate the effectiveness of this add-on at improving the treatment outcome as there is insufficient moderate/high quality evidence. If an insufficient number of publications can be identified as per the evidence decision tree, the intervention will be rated grey unless safety concerns have been identified in which case SCAAC may decide to rate the add-on red.
	On balance, the findings from moderate/high quality evidence shows that this add-on has no effect on the treatment outcome.
	There are potential safety concerns and/or, on balance, the findings from moderate/high quality evidence shows that this add-on may reduce treatment effectiveness.

6. Annex C. Evidence decision tree for rating add-ons