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

Monday 9th June 2025

Date	Action	Responsibility	Due date	Progress to date
03/02/2025	The Executive to update the topic prioritisation list and the Committee workplan for 2025/26.	Dharmi Deugi, Scientific Policy Officer	09/06/2025	The topics prioritisation list and committee workplan for 2025/26 have been updated (refer to Annex A).
03/02/2025	The Executive to continue to monitor polar body transfer under the horizon scanning topic of mitochondrial donation.	Molly Davies, Policy Manager	09/06/2025	Search terms for polar body transfer have been incorporated and relevant literature will be included for when this topic is next discussed.
03/02/2025	The Executive to publish a statement on the website highlighting the absence of conclusive evidence as to the impact of stress on treatment outcomes.	Rebecca Taylor, Scientific Policy Manager	09/06/2025	Text has been uploaded to the website on the following pages. 1. Getting emotional support | HFEA 2. Complementary and alternative therapies | HFEA 3. In vitro fertilisation (IVF) | HFEA
03/02/2025	The Executive to draft and publish treatment add-ons information on androgen supplementation to the website.	Rebecca Taylor, Scientific Policy Manager	09/06/2025	Text was drafted and reviewed by some members of the SCAAC and the Patient Engagement Forum (PEF). The Chair has approved the text and will be uploaded to the website.

Annex A: Revised Committee Workplan 2025/26

The below table presents the agreed workplan of the SCAAC for 2025/26.

Priority topic	Item	Possible speaker(s)	Last discussed	Meeting
Impact of the microbiome on fertility treatment outcomes	Literature review	Internal	October 2023	June 2025
Application for treatment add-on: Platelet-Rich Plasma (PRP)	Add-ons application review	Internal	N/A	June 2025
Health outcomes for ART patients (including gestational surrogates, egg donors, and the impact of treatment using donated eggs)	Literature review	Academic	N/A – new topic	June 2025
Artificial intelligence, robotics and automation in fertility treatment	Literature review	Internal	February 2024	October 2025
Reproductive organoids	Literature review	Academic	N/A – new topic	October 2025
Testicular transplantation to restore fertility in males	Literature review	Academic	N/A – new topic	October 2025
Horizon scanning and agreeing workplan for 2026/27	Workplan review	Internal	February 2025	February 2026
Impact of long-term cryopreservation	Literature review	Internal	February 2024	February 2026
Emerging technologies in embryo and gamete testing	Literature review	Internal	June 2024	February 2026
Germline/heritable genome editing	Literature review	Academic	February 2024	June 2026
Alternative methods to derive embryonic and embryonic-like stem cells	Literature review	Internal	June 2024	June 2026

Application to include platelet-rich plasma as a new add-on in the HFEA's rated list

Details about this paper

Area(s) of strategy this paper relates to:	Supporting scientific and medical innovation
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	5
Paper number:	HFEA (09/06/2025) 005
Meeting date:	09 June 2025
Author:	Molly Davies, Policy Manager (HFEA) Veronique Berman, External Advisor to the SCAAC (HFEA) & Scientific Advisor (Chana)
Annexes	Annex A: Platelet-rich plasma add-ons application form Annex B: SCAAC treatment add-on application decision tree

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to: • advise whether platelet rich plasma meets the criteria set out by the treatment add-ons decision tree to be eligible for a HFEA rating. Note: we are not asking the committee to make a recommendation at this meeting on the rating itself.
Resource implications:	Medium
Implementation date:	To be determined
Communication(s):	To be determined
Organisational risk:	Low

1. Introduction

1.1. Treatment add-ons are optional non-essential treatments that may be offered in fertility clinics in addition to routine proven treatment with the claim that they can improve treatment outcomes. As with all new treatments or technologies being introduced into reproductive medicine, we expect the introduction of treatment add-ons into clinics to be preceded by good quality scientific research into the effectiveness and safety of these interventions. However, some treatment add-ons are being offered to patients without this evidence base for effectiveness at increasing live birth rate, safety, or other treatment outcomes. They are frequently offered outside of a research setting and incur additional costs for the patient(s).

1.2. Medical professionals, academics, or patient organisations can propose that the HFEA reviews the evidence base for a treatment add-on if they are concerned that it is being offered to patients in a UK licensed clinic:

- with the claim that it will increase the live birth rate or improve other treatment outcomes;
- without conclusive evidence of its effectiveness at improving the live birth rate or other treatment outcomes;
- it is not already listed in the HFEA's rated list of add-ons;
- there is evidence that an add-on treatment may reduce treatment effectiveness or there are potential safety concerns.

1.3. The below application has been submitted by the HFEA Executive and SCAAC External Adviser, Veronique Berman, for the SCAAC to consider the eligibility of platelet-rich plasma (PRP) for intrauterine and intraovarian infusion/injection for a HFEA add-ons rating. The treatment add-on application decision tree can be found in Annex B.

1.4. Note: we are not asking the committee to make a recommendation at this meeting on the rating itself. Should members consider platelet-rich plasma (PRP) meets the criteria for inclusion on our **rated list**, an expert literature review will be commissioned by the Executive and be bought for discussion to a future SCAAC meeting so that ratings can be allocated.

2. Recommendation

2.1. Members are asked to:

- Advise whether platelet rich plasma meets the criteria set out by the treatment add-ons decision tree (Annex B) to be eligible for a HFEA rating.

Annex A: Platelet-rich plasma add-ons application form

Proposed treatment add-on

What is the name of the treatment?

Platelet-rich plasma (PRP) for intrauterine and intraovarian infusion/injection[i].

Please provide some background about the treatment and include how the treatment is used and how it claims to improve live birth rate or other treatment outcome(s). (max. 600 words)

Obtained through centrifugation of a patient's blood, platelet-rich plasma (PRP) is a blood-derived concentrate of platelets which is rich in proteins, growth factors, and cytokines. Delivering a concentrated mix of these components, PRP therapy is believed to facilitate targeted tissue regeneration through several mechanisms characteristic of the wound healing response – including extracellular matrix remodelling, promotion of angiogenesis, induction of anti-inflammatory activities, and by directing cellular differentiation (1,2) – however, the cellular and molecular mechanisms are yet to be fully elucidated.

Application of PRP therapy has been described in other clinical fields, including cardiology, dentistry, ophthalmology, and physiotherapy, amongst other fields (3). The Medicines and Healthcare products Regulatory Agency (MHRA) regulate the use of medicinal products in the UK under the **Human Medicines Regulations (HMRs) 2012**. Use of PRP in fertility treatment must be compliant with MHRA requirements.

In the context of fertility treatment, PRP is administered through either:

(1) **intrauterine infusion** (or localised intrauterine injection) prior to embryo transfer, with the aim to improve endometrial thickness and receptivity to implantation, or

(2) **intraovarian injection**, with the intention of rejuvenating ovarian tissue, enhancing response to ovarian stimulation, and improving oocyte quality and quantity.

To date, the majority of studies have reported on the use of autologous PRP for both intrauterine and intraovarian treatment; however, application of commercially prepared PRP has also been reported (4,5).

Intrauterine infusion/injection

It is claimed that the use of intrauterine PRP infusion/injection will promote embryo implantation and sustained gestation (6,7) by improving endometrial thickness and receptivity. The hypothesised mechanism involves modulation of cytokine expression (including interleukin-1β (IL-1β), IL-6, and IL-8), increased expression of oestrogen and progesterone receptors, and enhanced proliferation of endometrial cells.

For intrauterine PRP, the primary treatment groups of interest include patients with endometrial thinness (refactory endometrium), recurrent implantation failure (RIF) or recurrent pregnancy loss (5, 8–27). Application of intrauterine PRP therapy has also been reported in patients with intrauterine adhesions (Asherman's syndrome) following adhesiolysis (5, 28–30) and endometriosis (31, 32) – conditions associated with recurrent pregnancy loss and/or infertility.

No standard protocol has been established for treatment with intrauterine PRP with both intrauterine infusion and local injection of PRP concentrate has been described in the published literature (5). Alongside variability in the methods of autologous plasma preparation, the dose, timing and mode of administration is heterogeneous, with studies reporting infusion or localised injection of between 0.5ml to 1.5ml of PRP one to two times prior to embryo transfer (2,8).

[i] Due to lack of availability, the use of PRP for the treatment of male factor infertility (including for improving sperm parameters and treating non-obstructive azoospermia) (65–68) is not currently being proposed for consideration. Research in this area will continue to be monitored and will be brought in a separate application if/when appropriate.

Intraovarian injection

As with intrauterine treatment (and PRP therapy more broadly), the mechanisms of action underlying intraovarian PRP therapy are speculative. The working hypothesis is that delivery of platelet-released cytokines directly into the ovary may stimulate de-novo oogenesis and/or follicular maturation by inducing proangiogenic and proliferative effects that support the immature follicle pool and/or oogonial stem cells (9,10).

When administered through intraovarian injection, PRP treatment is targeted to patients identified as having a diminished ovarian reserve (DOR), poor ovarian response (POR), or premature ovarian insufficiency (POI). For such populations, the aim of the treatment is to improve ovarian reserve parameters, resulting in increased oocyte yield, fertilisation rate and formation of good quality embryos (3,11–16).

In short, intraovarian protocols administer PRP concentrate through transvaginal injection into the ovarian cortex. Due to a lack of established protocol, there is variability in the volume of PRP administered during intraovarian treatment and divergence in the timing of injections. Reported volumes of PRP range from 0.7ml to 8ml per ovary, with a limited number of studies documenting the date of the procedure relative to the phase of the menstrual cycle (2).

Please demonstrate that this treatment is being offered to or requested by patients in a UK fertility clinic with the claim that this treatment increases live birth rate or improves other treatment outcome. This could be contained in patient information leaflets, website content or anonymised conversations between patients and fertility clinic staff. (max. 300 words)

Patient facing information regarding platelet rich plasma (PRP) therapies are beginning to appear on the websites of HFEA licenced centres and their affiliated satellites. In addition, a limited number of clinics have been identified as offering the treatment on the UK market at additional cost to the patient.

Whilst it is acknowledged that the patient-facing information recognises that the treatment is still experimental, there is a concern that the evidence selected in support of the treatment is limited and non-specific to the patient groups being targeted. In addition, the HFEA have begun receiving enquiries from patients searching for PRP treatment at UK licenced clinics and queries as to whether we will be adding this treatment to our **rated list**. This suggests that patients could benefit from clear and impartial information on these treatments.

Intrauterine and intraovarian PRP therapies are also becoming increasingly available on the European fertility market, being offered at centres in Greece, Poland and Spain. As it is known that UK patients travel to these countries for treatment, it may be appropriate for the HFEA to provide information on their evidence base.

Please provide any recommendations made by professional bodies, e.g. NICE, ESHRE, RCOG, BFS or ASRM, for or against the use of this treatment in fertility patients. (max. 500 words)

At the time of writing, no public guidance or commentary on the use of PRP in fertility treatment has been issued by the National Institute for Health and Care Excellence (NICE), the Royal College of Obstetricians and Gynaecologists (RCOG), the British Fertility Society (BFS), or Association of Reproductive and Clinical Scientists (ARCS). However, the **Donor Selection Guidelines** (issued by the Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Professional Advisory Committee, JPAC) make reference to PRP therapy in the context of **body piercing** and **osteoarthritis** and advise a deferral period of at least four months before whole blood and blood components can be donated.

Current evidence base

Effectiveness

To be included in the HFEA add-on review list, a treatment needs to lack published evidence about its effectiveness. Please provide peer-reviewed published evidence that this treatment add-on **is or is not effective at increasing live birth rate or improving other treatment outcomes**, i.e. the extent to which this treatment is or is not able to deliver the promised benefits. Please include references to any relevant published data as appendices to this form. For example, you may wish to include references to data from animal studies, large data studies, research on human embryos, or clinical trial data. Study outcomes should include live birth rate as a primary or secondary outcome. (max. 500 words)

Research is predominantly limited to patient groups with conditions affecting implantation/ability to sustain pregnancy or ovarian reserve, as described above.

Intrauterine infusion/injection

There are 25 systematic reviews with meta-analyses on intrauterine PRP (5,18–41). Outcomes considered by authors include live birth rate, clinical pregnancy rate, implantation rate, miscarriage rate, and endometrial thickness, amongst other secondary outcomes.

The systematic reviews on intrauterine infusion of PRP for patients with recurrent implantation failure (RIF) (20,23–26,28,34,35) find improved pregnancy outcomes in patients treated with PRP compared to controls across the following parameters: clinical pregnancy rate (20,23,24,26,28,35), chemical pregnancy rate (20,28,35), implantation rate (20,23,26,35), live birth rate (20,23,24), and endometrial thickness (25,35). However, as highlighted by Kaur et al. (2024), low quality evidence may restrict confidence in results (34).

Relative efficacy of intrauterine PRP to treat RIF was most recently considered in the network meta-analysis published by Jiang et al. (2025). When compared to infusion with granulocyte colony-stimulating factor (G-CSF), human chorionic gonadotrophin (HCG), or peripheral blood mononuclear cells (PBMCs) against placebo or no-treatment, PRP emerged as the most effective treatment for increasing both clinical pregnancy and live birth rates in this subpopulation (29). This reiterated findings of the earlier analyses by Jin et al. (2022) and Kong et al. (2023) (22,39) but was contrary to the analysis conducted by Busnelli et al. (2021) (19).

The two meta-analyses which compared intrauterine PRP for thin endometrium, found that PRP infusion significantly improved endometrial thickness, implantation rate, and live birth rate (31,32).

Intraovarian injection

Six systematic reviews with meta-analyses on intraovarian injection of PRP consider its clinical potential for patients with poor ovarian reserve markers (including diminished ovarian reserve, poor ovarian response, and premature ovarian insufficiency) (11–16). Underlying research included both randomised and non-randomised studies, dominated by observational research (42–62).

Meta-analyses predominantly considered ovarian reserve parameters, such as serum hormone levels (including anti-Mullerian hormone, AMH, and follicle stimulating hormone, FSH) and antral follicle count, as primary outcomes measures. Where available, IVF parameters (including oocyte and embryo count, fertilisation rate, cycle stimulation parameters, chemical/clinical pregnancy rates, and live birth rates) were considered as secondary outcomes.

Regarding ovarian reserve parameters, analysis by Vahabi Dastjerdi et al. (2024), Éliás et al. (2024), Panda et al. (2020), and Li et al. (2023) provided positive evidence that AMH levels increased following PRP treatment (11–13,15). FSH was reported to decrease after PRP treatment by Vahabi Dastjerdi et al. (2024), Éliás et al. (2024) and Panda et al. (2020) – indicating that treatment has a positive effect (11–13). However, in the analysis by Maged et al. (2024) neither increase reached statistical significance (14).

For IVF parameters, the number of retrieved oocytes and embryos created was found to be improved after PRP treatment in analysis by Vahabi Dastjerdi et al. (2024), Éliás et al. (2024), Panda et al. (2020), Maged et al. (2024), and Li et al. (2023). Two meta-analyses noted that the assessment of clinical, chemical and live birth rates was not possible due to limited data (14,15).

Summary

Despite promising findings, many authors advised caution, highlighting that research comparisons were limited by risk of bias (due to small sample size or single-centre design) and/or heterogeneity in patient inclusion criteria, PRP preparation, and treatment protocols (2). As reiterated by Katsika et al. (2025) and conclusions of numerous systematic reviews, the current evidence is insufficient to inform clinical practice. This highlights the need for further well-designed, high-powered studies that identify patient populations who may benefit from PRP (8).

In addition, Vaidakis et al. (2024) analysed both the use of intrauterine and intraovarian administration of autologous PRP for patients undergoing assisted reproduction in a **Cochrane review** (3). Evaluating 12 RCTs published prior to January 2023 across three different comparisons, authors concluded that there is currently insufficient evidence to support its use in routine clinical practice.

Good practice recommendations for the use of platelet rich plasma (PRP) were given by the ESHRE add-ons working group in 2023[ii]. Neither intrauterine administration for thin endometrium/RIF, nor intraovarian injection for poor ovarian response (POR)/premature ovarian insufficiency (POI) is recommended for use in clinical practice (17). The ESHRE recommendations cite low-quality evidence, a lack of a proper multicentre RCT, and absence of safety data as the justification for these recommendations. It is however acknowledged that the available data regarding intrauterine administration of PRP shows promise, but that studies considered involved small sample sizes, heterogeneous patient populations, and were subject to overrepresentation by a single research group. As such, this application of PRP needs to be further investigated through well designed studies (17).

Safety

If there is evidence that this treatment is **not** safe or there is risk of harm, for either the patients or the children born after the use of this treatment or may reduce treatment effectiveness, please outline it here. Please include references to any relevant published data as appendices to this form. For example, you may wish to include references to data from animal studies, large data studies, research on human embryos, or clinical trials data. (max. 500 words)

At present, there is limited evidence addressing the safety of PRP therapies in the context of fertility treatment (2,9,17). Studies referencing potential harms report an absence of side effects or notable complications following the procedures (2,63). However, there is some low-certainty evidence that intrauterine infusion may be associated with an increased risk of preterm delivery (OR 8.02, 95% CI 1.72 to 37.33; 120 women) (3,64). Patient information on intrauterine PRP, issued by **No1 Fertility (Australia)**, notes that uncommon complications associated with the procedure include bruising, bleeding, spotting, subtle swelling, pain, and rarely, fever and infection.

Further investigation to understand the impact of exposing the ovaries, endometrium and embryos in the endometrial cavity to PRP is needed before conclusions on the safety of this treatment can be drawn. These should assess both the short- and long-term safety of the treatment to both the patients and resulting child (2,9,17).

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[ii] Note: ESHRE recommendations considered research published prior to 10th August 2022.

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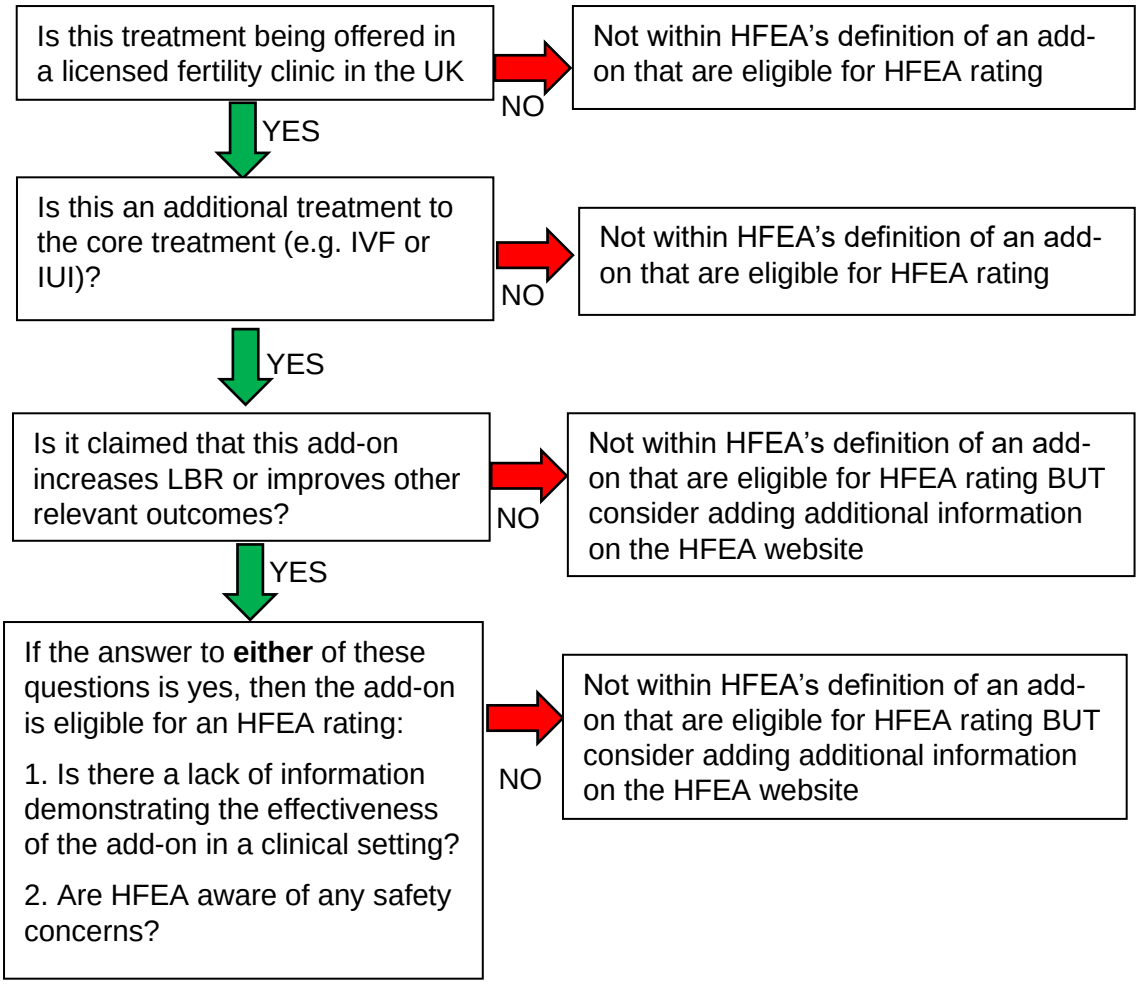
Annex B: SCAAC treatment add-on application decision tree



Impact of the microbiome on fertility and fertility treatment outcomes

Details about this paper

Area(s) of strategy this paper relates to:	Supporting scientific and medical innovation
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	6
Paper number:	HFEA (09/06/2025) 006
Meeting date:	09 June 2025
Author:	Molly Davies, Policy Manager (HFEA)
Annexes	None

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to: • Advise the executive if they are aware of any other recent developments; • Consider the research findings and the quality of the evidence, drawing conclusions on what influence the microbiome may have on fertility treatment outcomes; and • Review whether any outputs from the HFEA are required to address the implications of microbiome testing during fertility treatment.
Resource implications:	To be determined
Implementation date:	To be determined
Communication(s):	To be determined
Organisational risk:	Low

1. Introduction

1.1. The microbiome refers to the community of microorganisms – primarily bacteria, but also fungi and viruses – that inhabit various parts of the human body, including the gut, skin, and reproductive tract. Research in this area investigates how microbial populations may influence reproductive health and outcomes, for example through imbalances in microbiome composition. If the composition of the microbiome is shown to be related to fertility or fertility treatment outcomes, understanding this relationship will have implications for managing infertility and there may be potential for development of interventions to improve outcomes for patients.

1.2. The 'Impact of the microbiome on fertility and fertility treatment outcomes' topic was introduced to the SCAAC's horizon scanning prioritised list in **February 2018** and discussed at the **February 2019** and **October 2023** SCAAC meetings.

1.3. In 2023, the Committee acknowledged that there had been a substantial increase in published literature exploring the role of vaginal and endometrial microbiota in fertility, with evidence suggesting that such microbial environments may influence pregnancy outcomes for patients using assisted reproductive technologies (ART). In particular, the presence of Lactobacillus in the female reproductive tract had been heavily researched for its positive associations with fertility, with an altered microbiome or dysbiosis thought to be associated with poorer treatment outcomes. The SCAAC have not yet commented on research relating to the male microbiome.

1.4. It was additionally noted that the research was moving from being testing-focused to the development of treatments, with members observing an increase in patient engagement with the research and a rise in commercialisation of microbiota testing and marketing of biotic supplements. Although commercial diagnostic tests that assess the endometrial microbiome were available on the market, the committee concluded that current testing methods and interventions lacked clinical uptake and could not justify inclusion on the **treatment add-ons rated list**.

1.5. The **European Society of Human Reproduction and Embryology** (ESHRE) have since published their **good practice recommendations on add-ons in reproductive medicine** and do not consider microbiome testing or treatment as an add-on (Lundin et al., 2023). However, the European Association of Urology Guidelines on Male Sexual and Reproductive Health has been updated to address the use of probiotic treatment (Minhas et al., 2025).

1.6. It was agreed that the SCAAC would continue to monitor developments in this topic, including considering the potential importance of the wider microbiome in relation to infertility. In the most recent horizon scanning review, conducted in **February 2025**, this topic was determined to be a medium priority area, noting that there is growing public interest in the microbiome and increasing commercialisation of testing available within the private fertility sector.

1.7. This paper presents literature published between September 2023 and May 2025 investigating the possible relationship between the microbiome and fertility or fertility treatment outcomes. The Executive notes that this paper is not an assessment of study validity.

2. Research developments

The Female Microbiome and Reproduction

Female reproductive tract microbiome

General reproductive tract microbiome and fertility outcomes

2.1. Peng et al. (2025) analysed the vaginal and uterine microbiota of 120 women undergoing frozen embryo transfer, finding that non-pregnant women had higher microbial diversity and lower Lactobacillus abundance in both the vagina (91.66 % vs 74.50 %) and uterus (37.27 % vs 33.45 %), as well as elevated Gardnerella in the vagina (3.92 % vs 12.12 %). Uterine microbiome diversity did not differ significantly, but reduced Pseudomonas levels were observed in non-pregnant women. A Lactobacillus-dominated microbiome was associated with higher pregnancy rates.

2.2. Väinämö et al. (2023) investigated the role of vaginal microbiota in IVF outcomes, finding that a Lactobacillus crispatus-dominant profile was positively associated with clinical pregnancy and live birth. The study also reported intra-individual microbiota shifts between non-pregnancy and pregnancy states, highlighting the dynamic nature of the vaginal microbiome during early human reproduction. These results support microbiota screening or modulation to optimise embryo transfer timing.

2.3. This prospective cohort study conducted by Bielfeld et al. (2024) examined whether an adverse vaginal microbiome in subfertile women can spontaneously improve over time. Among 76 patients sampled before fertility treatment, those with a favourable microbiome (high/medium profile) proceeded with treatment, while those with an unfavourable profile (low) postponed and were resampled over successive cycles. Of the 23 patients with an initial low profile, 75% shifted to a more favourable profile within 3 months. Presence of Lactobacillus crispatus was linked to a higher likelihood of spontaneous improvement. These findings suggest the vaginal microbiome fluctuates naturally, and an initially adverse profile can improve without intervention.

2.4. The large cross-sectional study by Wang et al. (2024d) examined the vaginal microbiome profiles of 1,411 women (1,255 undergoing embryo transfer) and their association with infertility and IVF outcomes. Using 16S rRNA sequencing and metagenomic analysis, the authors found that both very high and very low levels of Lactobacillus are associated with reduced pregnancy rates, whereas moderate Lactobacillus abundance (~80%), particularly of L. crispatus and L. iners, is associated with pregnancy rates (54.35–57.73%). The presence of Gardnerella vaginalis (CST[1] IV-B) was found to yield comparable pregnancy rates to high-Lactobacillus CSTs (I-A and III-A). Additionally, nonpregnant women were found to exhibited higher levels of antibiotic resistance genes, mainly hosted by Proteobacteria and Firmicutes.

2.5. The observational study by Kadogami et al. (2023) assessed the species-specific effects of uterine Lactobacillus dominance on IVF outcomes. While Lactobacillus spp. (primarily L. crispatus, L. iners, L. gasseri, and L. jensenii) were generally associated with improved outcomes,

[1] Community state types (CSTs) describe vaginal microbiota profiles which are grouped based on dominant bacterial species.

the implantation rate was lowest in women dominated by L. iners. This study indicates that dominance of different Lactobacillus species may not confer the same reproductive benefit.

2.6. By introducing specific Lactobacillus (L. rhamnosus, L. reuteri, L. crispatus) or Gardnerella vaginalis strains into hormone-manipulated mice models, Rahman et al. (2023) investigated the role of oestradiol and progesterone on probiotic, eubiotic and dysbiotic vaginal microbiota states. Authors found that that oestrogen, but not progesterone, was essential for sustained colonisation by both Lactobacilli species and G. vaginalis. Oestrogen increased vaginal glycogen levels, supporting bacterial persistence.

2.7. Hasan et al. (2024) compared vaginal microbiomes of five infertile and five fertile women using 16S and shotgun metagenomics. Infertile women showed lower microbial diversity and higher abundance of Lactobacillus iners, L. gasseri, and Gardnerella vaginalis, while fertile women had more diverse communities and fungal presence (Penicillium citrinum, 62.5%). Community State Types (CSTs) varied across individuals, with L. johnsonii found exclusively in infertile samples. Despite its small sample size, the study suggests an association between reduced microbial diversity and specific taxa with female infertility.

2.8. The prospective multicentre study by Chopra et al. (2024) examined vaginal microbiota differences between fertile and idiopathically infertile women using 16S rRNA sequencing. Infertile women displayed altered alpha and beta diversity compared to fertile controls, with increased abundance of Gardnerella, Prevotella, Atopobium, and Enterococcus. In contrast, Lactobacillus iners predominated in fertile participants. The findings suggest that dysbiosis of the vaginal microbiota may play a role in idiopathic infertility.

2.9. Souza et al. (2023) conducted a systematic review of 18 studies involving 2,011 patients to assess the role of Lactobacillus spp. in female fertility. Fertile patients exhibited a Lactobacillus-dominated vaginal microbiota associated with positive reproductive outcomes, while infertile patients showed dysbiotic profiles. The findings suggest that bacterial composition could serve as a basis for personalised diagnostic and therapeutic approaches in fertility care.

2.10. Nishio et al. (2024) analysed the cervicovaginal metabolome and microbiome of IVF patients to identify factors influencing pregnancy outcomes. Although metabolism decreased in both groups at embryo transfer, pregnant patients (n = 10) exhibited a notable reduction in pyruvate. Non-pregnant patients (n = 13) showed inverse correlations between amino acid metabolites and anaerobic microbiota. These findings suggest that cervicovaginal metabolic profiling, particularly pyruvate metabolism, may serve as a potential marker for predicting IVF success.

2.11. Cortés-Ortíz et al. (2025) characterised the vaginal microbiota of 136 Mexican women with primary or secondary infertility. Using qPCR on cervical swabs, they found significant microbial differences between the two groups. Lactobacillus crispatus and L. gasseri dominated in primary infertility, alongside high levels of Gardnerella vaginalis and Fannyhessea vaginae. HPV and sexually transmitted bacteria correlated with G. vaginalis.

2.12. Balla et al. (2024) reviewed findings from the Human Microbiome Project and subsequent research, highlighting that a Lactobacillus-dominated microbiome in the female genital tract is associated with improved fertility and ART outcomes. In contrast, dysbiosis, marked by reduced Lactobacilli and increased diversity of pathogens like Gardnerella and Prevotella, is linked to

infertility, implantation failure, and pregnancy complications. Despite growing evidence, the absence of standardised protocols and microbial biomarkers limits clinical application.

2.13. The case report by Sola-Leyva et al. (2025) provides interesting insights into microbiome variation observed between uteri in a case of uterus didelphys. Regarding the microbiome, significant differences were found between the uteri; notably in the right uterus, a clear non-dominance of lactobacilli and the presence of genera such as Staphylococcus, Streptococcus, and Acinetobacter was reported. The right uterus presented a less 'favourable' microenvironment, a characteristic that was also reflected in the right cervix.

2.14. Current evidence on the role of the female reproductive tract microbiome on reproductive outcomes has been further investigated by Li et al. (2024c), Xiao et al. (2024) and Karadbhajne et al. (2025). Reiterating findings from above studies, authors suggest that dominant Lactobacillus presence enhances conception rates, while dysbiosis contributes reproductive complications. The reviews highlight microbial markers as emerging tools and advocate for further mechanistic studies to guide targeted microbial interventions in infertility treatment.

Recurrent implantation failure and recurrent pregnancy loss

2.15. Zhang et al. (2024d) investigated the endometrial and vaginal microbiota in 32 women with repeated implantation failure (RIF) and 18 controls undergoing fertility treatment. Using 16S rRNA sequencing and qPCR, authors reported that the RIF group exhibited higher endometrial microbiota diversity and reduced Lactobacillus, with increased Gardnerella and Acinetobacter abundance. These microbial changes correlated with lower expression of key endometrial receptivity markers, including homeobox A11, integrin αvβ3, and VEGF. The study links endometrial dysbiosis to impaired receptivity in unexplained RIF.

2.16. The cross-sectional metagenomic study by Su et al. (2024) investigates reproductive tract dysbiosis in infertile women undergoing IVF, aiming to identify microbial biomarkers of implantation failure. Authors revealed a strong positive association between Lactobacillus abundance and successful embryo implantation, while patients with multiple implantation failures exhibited significantly altered microbial compositions and reduced Lactobacillus levels. Metagenomic analysis also identified enrichment of the L-lysine biosynthesis pathway and elevated vaginal pH in implantation failure patients. A diagnostic model based on microbial signatures demonstrated good discriminatory power (AUC = 0.913 for IS vs. multiple implantation failure; AUC = 0.784 in validation), suggesting the clinical potential of microbiota profiling.

2.17. Liu et al. (2024a) used metagenomic sequencing to compare the vaginal microbiota of 37 women with RIF to 43 women who achieved pregnancy after their first frozen embryo transfer. The RIF group showed significantly higher alpha diversity ($P < 0.05$) and increased abundance of the bacterial families Actinomycetaceae ($P = 0.013$), Ruminococcaceae ($P = 0.013$), the genera Actinomyces ($P = 0.028$), Subdoligranulum ($P = 0.013$), and species Prevotella timonensis ($P = 0.028$), and Lactobacillus jensenii ($P = 0.049$). Amongst these, Subdoligranulum was the most strongly associated genus with RIF. These results suggest distinct microbial profiles may contribute to implantation failure in IVF patients.

2.18. The prospective study by Wei et al. (2024) investigated associations between vaginal microecology, endometrial microbiota, and pregnancy outcomes during frozen embryo transfer. Normal vaginal microecology, marked by lower pH and leukocyte esterase negativity, was linked

to higher clinical pregnancy rates and found to influence endometrial microbial composition. Although endometrial microbiota did not differ significantly across pregnancy outcome groups, transvaginal Lactobacillus supplementation improved clinical pregnancy rates in patients with prior failed cycles.

2.19. Blazheva et al. (2024) investigated endometrial microbiota and immune cell profiles in patients with RIF (n = 107) and recurrent pregnancy loss (RPL) (n = 93). Using real-time PCR and flow cytometry, authors identified disrupted microbial communities in most patients with RIF and RPL, characterised by the absence of Lactobacillus spp. and the presence of more than 10% dysbiotic bacteria. Endometrial dysbiosis was frequently associated with alterations in endometrial immune cells, including lymphocytes, T cells, and uterine natural killer cells. ART outcomes were found to be improved following treatment with antibiotic, probiotic and antifungal agents.

2.20. Bai et al. (2024) analysed uterine cavity microbiota in early pregnancy using 16S rRNA sequencing in patients with unexplained recurrent pregnancy loss (URPL) versus controls. Microbial presence was detected in 100% of URPL cases but in none of the patients undergoing induced miscarriages during early pregnancy, with Lactobacillus and Curvibacter identified as dominant taxa. Notably, Curvibacter abundance negatively correlated with peripheral NK cell counts ($r = -0.759$, $p = 0.018$), suggesting that alterations in the dominant microbiota may lead to adverse pregnancy outcomes.

2.21. Garmendia et al. (2024) reviewed evidence linking dysbiosis in the vaginal, endometrial, and gut microbiota to RPL, highlighting that reduced Lactobacillus crispatus is associated with increased local inflammation and immune dysregulation. Gram-negative bacteria, viral infections, and microbial metabolites may trigger immune activation, leading to a pro-inflammatory Th1/Th17 profile and diminished Treg and tolerogenic NK cells. The authors suggest that microbiota modulation, especially vaginal transplantation of L. crispatus, may promote immune tolerance, and further work is needed to investigate probiotic efficacy and the effect of hormone stimulation on microbiota.

2.22. Scarfò et al. (2024) investigated the relationship between endometrial dysbiosis, oestrogen metabolism, and reproductive failure in patients with infertility and RIF (n = 40). Dysbiotic endometrial samples showed elevated inflammatory markers (IL-1β, HIF-1α), reduced IGF-1, increased β-glucuronidase activity, and heightened ERβ expression. Lactobacilli abundance was inversely correlated with both β-glucuronidase activity and ERβ expression, suggesting a possible mechanistic link between dysbiosis, estrobolome disruption, and impaired endometrial receptivity.

2.23. A number of narrative reviews have additionally considered the potential role of the vaginal and endometrial microbiome in RIF. These reviews reiterate the suggestion that a loss of Lactobacillus dominance and increased microbial diversity may compromise endometrial receptivity by promoting local inflammation and immunological disruption, though the mechanisms remain ill defined. Overall, these narrative reviews reinforce the need for systematic, high-quality research to establish causal relationships and inform clinical practice in assisted reproduction (Hiraoka et al., 2023; Gao et al., 2024a; Han, 2024; Lafioniatis et al., 2024; Rokhsartalab Azar et al., 2024; Kumar et al., 2025).

Endometriosis and adenomyosis

2.24. The cross-sectional study by Li et al. (2025a) employed metabolomic, microbiomic (5R 16S rRNA), and transcriptomic analyses to compare endometrial profiles across women with endometriosis (n = 91), adenomyosis (n = 56), and healthy controls (n = 97). Distinct microbial and metabolic signatures were identified for patients with endometriosis and adenomyosis, with shared alterations in lipid metabolism and unique taxa linked to Pseudomonadota (formerly Proteobacteria). Integrative multi-omic and machine learning approaches enabled accurate classification and revealed condition-specific immune and signalling pathway disruptions. The study underscores divergent yet overlapping pathophysiology in endometriosis and adenomyosis, highlighting microbiota-metabolite-immune interactions.

2.25. Zhu et al. (2024) analysed uterine and peritoneal fluid microbiota in 26 endometriosis patients and 31 controls with tubal obstruction-related infertility. Advanced-stage endometriosis (stage III–IV) was associated with reduced Lactobacillus and increased Pseudomonas, Enterococcus, Dubosiella, and Klebsiella in peritoneal fluid. Distinct microbial shifts in uterine fluid also emerged by endometriosis stage, indicating that as endometriosis progresses the microbiota of the peritoneal and uterine fluid continue to change. Pathway analysis suggested that these microbial changes may impair endometrial receptivity, contributing to endometriosis-associated infertility.

2.26. Ono et al. (2024) conducted a prospective study of 43 women with RIF to examine the uterine endometrial microbiome and its relationship with endometriosis. Women with endometriosis had significantly higher bacterial loads and diversity, with Dialister (41.7% vs. 3.3%) and Streptococcus (58.3% vs. 16.1%) species more frequently detected than in those without endometriosis. Dialister presence was independently associated with endometriosis (OR = 10.97, p = 0.036). These findings suggest that specific microbial signatures in the uterine endometrium microbiome may contribute to RIF in endometriosis patients.

2.27. Wang et al. (2025) present a narrative review summarising current evidence linking microbial dysbiosis to the development and progression of endometriosis, highlighting the role of microbiota in inflammation, immune modulation, oestrogen regulation, metabolism, and the gut-brain axis. The review emphasises the diagnostic delay in endometriosis and explores the potential for microbiota and their metabolites as early, non-invasive diagnostic markers and therapeutic targets.

Endometritis

2.28. Han et al. (2024c) conducted a cross-sectional study of patients undergoing infertility assessment to evaluate vaginal microbiota differences in those with (n = 49) and without (n = 49) chronic endometritis (CE). Using 16S rRNA sequencing, the study identified significant differences in microbial composition and function, with Enterobacter, Prevotella, Faecalibacterium, and Phascolarctobacterium emerging as microbial markers for CE. A predictive classifier based on these markers achieved an AUC of 83.26%, supporting the diagnostic potential of vaginal microbiome profiling for CE.

2.29. The prospective study by Zhang et al. (2024a) characterised the endometrial microbiota in RIF patients with and without CE using 16S rRNA gene sequencing. Patients with CE exhibited greater microbial diversity and distinct taxonomic profiles, with authors identifying Proteobacteria, Aminicenantales and Chloroflexaceae as characteristic of CE. Lactobacillus, Acinetobacter,

Herbaspirillum, Ralstonia, Shewanela, and Micrococcaceae were more prevalent in non-CE individuals. These microbiota differences correlated with adverse reproductive outcomes, and functional profiling revealed enrichment of immune-related and metabolic pathways in the CE (chronic endometritis) group.

2.30. Břečka et al. (2024) provide a narrative review on the pathophysiology, diagnosis, and treatment of CE in infertile women. They highlight that CE is often asymptomatic but may negatively affect IVF success rates, potentially due to alterations in the endometrial microbiome. The authors emphasise diagnostic challenges due to the absence of standardised criteria and underscore the importance of hysteroscopy and histological evaluation, while noting the limitations of these methods. Antibiotic therapy appears to improve reproductive outcomes, but more robust studies are required to clarify CE's role in recurrent implantation failure.

Polycystic ovary syndrome and the microbiome

2.31. The cross-sectional study by Zhao et al. (2025) examined the vaginal microbiota of infertile women with polycystic ovary syndrome (PCOS) and tubal factor infertility (TFI) undergoing IVF (n = 85), compared to controls (n = 37), using vaginal swab samples collected during the implantation window. Authors found a higher abundance of Lactobacillus iners and Pseudomonas spp. in the non-pregnant group, suggesting these taxa may be negatively associated with IVF success.

2.32. Chudzicka-Strugała et al. (2024) retrospectively analysed 594 PCOS patients and found that 64% exhibited vaginal pH ≥ 4.4 with suspected bacterial vaginosis (BV). Elevated Gardnerella vaginalis was found in more than half (56.8%) of PCOS women (n = 380) with suspected BV. Authors concluded that BV appears to be more common in patients with PCOS, potentially due to chronic inflammation and abnormalities in the vaginal microbiome. BV may therefore be an unrecognised cause of infertility for PCOS patients.

2.33. The review by Cocomazzi et al. (2024) discusses emerging associations between vaginal microbiota dysbiosis and gynaecological conditions, including endometriosis, PCOS, PID (pelvic inflammatory disease), and cancers. It highlights that reduced Lactobacillus abundance in the vagina and related reproductive sites is linked to increased risk and poorer prognosis in gynaecological malignancies. While mechanistic links remain unclear, the review positions microbiota profiling as a potential diagnostic and therapeutic avenue in oncogynaecology.

Genital tract infections (including bacterial vaginosis)

2.34. van den Tweel et al. (2024a) conducted a prospective cohort study of 53 women undergoing IUI, IVF, or ICSI to examine how hormonal fertility treatments affect the vaginal microbiome. A reduction in Lactobacilli abundance and increased incidence of bacterial vaginosis (BV) were observed during treatment, with 24% testing BV-positive and 17% converting from BV-negative to BV-positive. The authors propose that timing and treatment strategies to mitigate microbiome deterioration may improve ART outcomes.

2.35. Maksimovic Celicanin et al. (2024) conducted a systematic review and meta-analysis of 25 studies involving 6,835 IVF patients to assess the impact of vaginal dysbiosis, including BV and aerobic vaginitis (AV), on reproductive outcomes. Dysbiosis was associated with increased early pregnancy loss (relative risk = 1.49) and reduced clinical pregnancy rate (relative risk = 0.82), though no significant effect was observed on live birth or biochemical pregnancy rates. The

findings highlight a partial but clinically relevant link between VD and IVF outcomes, despite mechanistic uncertainties.

2.36. The narrative review by Pérez-Ibave et al. (2025) discussed the pathophysiological mechanisms of BV. The authors proposed ten hallmarks of BV and noted high recurrence rates despite antibiotic therapy, underscoring the need for improved, microbiota-targeted treatments.

2.37. George et al. (2024) reviewed the role of Prevotella species in female genital tract infections, highlighting that P. bivia, P. amnii, and P. timonensis possess distinct virulence factors and antibiotic resistance, contributing to upper tract infections via ascension from untreated BV. Loss of protective Lactobacillus and microbial imbalance were identified as key drivers of immune disruption in female genital tract infections.

2.38. Klasner et al. (2024) reviewed the interplay between host immunity, vaginal microbiota, and Chlamydia trachomatis (CT) in spontaneous infection clearance. While untreated CT can cause infertility and pelvic inflammatory disease, emerging evidence suggests the vaginal microbiota may influence infection outcomes. Knowledge gaps remain regarding mechanisms that could inform future protective interventions.

2.39. Córdova et al. (2024) conducted a systematic review of eight studies evaluating the association between vulvovaginal candidiasis infection and infertility in women of reproductive age. Across 909 infertile and 2,363 fertile patients, meta-analysis found no significant association between candidiasis and infertility (OR = 1.44; 95% CI: 0.86–2.41; p = 0.17). Sensitivity analysis did not alter the outcome, suggesting that candidiasis is not a contributing factor to female infertility.

Follicular fluid microbiota

2.40. Ou et al. (2023) conducted a systematic review of studies investigating microbial presence in follicular fluid (FF) and its association with IVF outcomes. Among 289 patients, FF-positive cases showed lower pregnancy rates (19.7% vs. 32.2%) though the difference was not statistically significant (OR: 0.57, P = 0.11). Fertilisation rates were similar between groups. Lactobacillus spp. appeared beneficial, whereas non-Lactobacillus microbes may have adverse effects. Evidence quality was low.

Uterine fibroids

2.41. Bensouda et al. (2024) conducted 16S rRNA sequencing to compare uterine microbiota in patients with endometrial polyps or fibroids versus controls. Polyps were associated with higher alpha diversity and distinct microbial communities, particularly in catheter-tip samples. Sampling method significantly influenced microbiome profiles, underscoring the need for methodological standardisation.

2.42. Don et al. (2023) provided a narrative review proposing seven mechanistic hypotheses by which uterine fibroids may impair fertility, focusing on both mechanical and molecular pathways. These include interference with sexual function, gamete transport, uterine peristalsis, microbiome alteration, inflammatory signalling, molecular endometrial changes, and disrupted angiogenesis. The review argues that better understanding of these mechanisms is critical for shifting from generic surgical approaches to pathophysiology-targeted therapies that preserve fertility.

Caesarean scar

2.43. van den Tweel et al. (2024b) conducted a systematic review to assess the impact of caesarean scar defects (niches) on live birth rates following assisted reproduction, and to explore whether niches influence the vaginal microbiome. Six retrospective cohort studies (n = 5070) were included, showing a pooled adjusted odds ratio of 0.58 (95% CI 0.48–0.69), indicating reduced live birth rates in women with a niche. Only three studies assessed microbial composition in relation to CS or niche, providing insufficient evidence to confirm a link between niche-associated dysbiosis and ART outcomes.

2.44. The study by Chen et al. (2023b) investigates the role of fungal dysbiosis in caesarean section scar diverticulum (CSD), a condition linked to infertility due to persistent inflammation. Using metagenomic and mass spectrometry, the authors demonstrate that abnormal fungal species in CSD alter bacterial abundance by modulating key metabolites, contributing to bacterial dysbiosis. Notably, fungi such as Lachnellula suecica, Clavispora lusitaniae, and Ophiocordyceps australis were found to disrupt Lactobacillus populations by reducing metabolites essential for their maintenance. These findings challenge the sufficiency of antibiotic-only treatments and point to fungi as critical targets for future therapeutic strategies.

Viral agents

2.45. Da Costa et al. (2023) performed metagenomic analysis of vaginal secretions from 46 patients and found Torque teno virus (TTV) in 47.1% of infertile women versus 0% of fertile controls (p = .0035). TTV was more prevalent in women with male factor infertility (55.6%) and ovulation disorders (42.9%). TTV detection negatively correlated with Lactobacillus crispatus dominance (p = .0184), suggesting immune or microbial dysregulation. No differences in bacteriophage composition (including Lactobacillus phages) were observed between fertile and infertile groups.

Gut microbiota and female reproductive health

General infertility

2.46. Both (Cheng et al., 2025) and Fu et al. (2025) analysed the National Health and Nutrition Examination Survey data (collected by the Centre for Disease Control and Prevention) to investigate the link between the Dietary Index for Gut Microbiota (DI-GM) and female infertility. Both studies found that higher DI-GM scores were significantly associated with reduced infertility prevalence (adjusted OR = 0.89). Specifically, individuals with DI-GM ≥6 had markedly lower infertility risk (40%) compared to those with lower scores.

2.47. Using the same dataset, Xiao et al. (2025) examined the association between DI-GM, flavonoid intake, and female infertility. As above, higher DI-GM scores were significantly associated with reduced infertility risk (aOR = 0.30, p = 0.006), particularly in women under 35. Among women ≥35 years, moderate flavonoid intake also showed a protective effect (aOR = 0.19, p = 0.009). The study highlights age-specific dietary strategies that may support gut microbiota and reduce infertility risk.

Polycystic ovary syndrome (PCOS)

2.48. Chen et al. (2023a) compared gut mycobiota in 17 PCOS patients and 17 controls, finding reduced fungal diversity and distinct microbial compositions in PCOS. PCOS patients showed increased Saccharomycetaceae and Saccharomyces, and decreased Trichosporonaceae and

Aspergillus. Functional pathway differences suggest fungal dysbiosis may contribute to PCOS pathogenesis.

2.49. In a further study by the same group, Chen et al. (2024b) analysed the bacterial diversity and community structure of PCOS patients (n = 17) compared to healthy controls (n = 17). In this study, PCOS patients exhibited reduced microbial diversity, lower levels of Firmicutes and Bacteroidota, and enrichment of Bifidobacterium, with depletion of 11 genera including Bacteroides, Ruminococcus, and Roseburia. Functional profiling (PICRUSt2) revealed distinct microbial metabolic pathways in PCOS. These findings suggest gut dysbiosis may contribute to PCOS pathophysiology and could be a target for intervention.

2.50. Zhu and Zhang (2024) conducted a systematic review on gut microbiome alterations in adults with PCOS, focusing on microbial function. While overall microbial diversity was not significantly reduced, PCOS was associated with increased abundance of Proteobacteria, Bacteroides, Enterococcus, and Escherichia-Shigella, alongside species-level enrichment of Ruminococcus gnavus, Parabacteroides distasonis, and Bacteroides fragilis. Functional analysis revealed altered pathways linked to glucose, lipid, bile acid, and protein metabolism, suggesting a microbiota-mediated metabolic contribution to PCOS pathophysiology.

2.51. The review by Salehi et al. (2024) explores gut microbiota dysbiosis in women with PCOS, noting elevated levels of Porphyromonas spp., Bacteroides coprophilus, and Faecalibacterium prausnitzii, alongside reduced short-chain fatty acid-producing bacteria. This microbial imbalance is linked to insulin resistance and reproductive dysfunction. Probiotic supplementation was shown to improve microbial composition and SCFA production, thereby alleviating PCOS symptoms and potentially improving fertility outcomes.

2.52. Chen et al. (2024a) used mouse models to assess the impact of hyperandrogenism and oestrogen deficiency on insulin resistance and gut microbiota composition, simulating features of PCOS. While dihydrotesosterone or ovariectomy alone did not induce insulin resistance, their combination led to both metabolic dysfunction and distinct gut microbial shifts, including alterations in Rikenellaceae and Mucispirillum schaedleri. These findings suggest that hormonal interactions contribute to dysbiosis and metabolic traits in PCOS.

2.53. The role of gut microbiota dysbiosis in the pathogenesis of PCOS has been further discussed in the review articles by Dilliyappan et al. (2024), Khobragade et al. (2024), Zhou et al. (2024) and Senthilkumar and Arumugam (2025). Collectively these articles highlight mechanisms by which the microbiome influences endocrine and metabolic dysfunction, and discuss emerging therapeutic strategies to target the microbiome for PCOS prevention and treatment. These include dietary interventions, probiotics and metabolic modulation, as presented below.

Infertility and obesity

2.54. Bellver et al. (2024) conducted a multicentre observational study comparing oral, gut, vaginal, and endometrial microbiota in infertile women stratified by BMI. While no significant differences were found in the gut Bacteroidetes/Firmicutes ratio, patients with obesity showed increased Parasutterella and Roseburia, and a higher prevalence of Streptococcus-dominated endometrial microbiota (>50%). Vaginal and endometrial profiles were similar, supporting ascension as a colonisation pathway. The findings suggest altered endometrial microbial profiles in obesity may contribute to poorer reproductive outcomes.

2.55. The experimental study by Wen et al. (2024) explores how a high-fat diet impairs ovarian function in female mice depending on the age of exposure. The most severe reproductive disruption occurred when a high-fat diet was initiated at post-puberty (6 weeks old). Through faecal microbiota transplantation, they demonstrated that gut dysbiosis plays a causal role in impairing fertility – particularly by elevating L-saccharopine, a metabolite shown to disrupt mitochondrial homeostasis and estradiol synthesis via inhibition of the AMPKα/MFF-mediated fission pathway. Intervention with the compound AICAR, an AMPKα activator, was found to ameliorate these effects and restore oocyte quality.

Endometriosis and genealogical conditions

2.56. Wang et al. (2024e) used Mendelian randomisation to assess causal links between gut microbiota and endometriosis-associated infertility (EAI). Their analysis identified Actinomyces bacteria increased the risk of EAI (OR = 1.657), however Holdemania and Ruminococcaceae NK4A214 were found to have protective effects. Sensitivity analyses supported the robustness of the findings, and no horizontal pleiotropy was detected.

2.57. Marcos et al. (2024) used 16S rRNA sequencing to investigate microbial profiles across the female reproductive and gastrointestinal tract in 21 patients with endometriosis and other infertility-related conditions. The study identified distinct, site-specific microbiota patterns, with Lactobacillus being commonly shared between endometrium and vagina, while Haemophilus was uniquely associated with endometriosis cases across gastrointestinal sites. These findings support the concept of a "female holobiont," where systemic microbial homeostasis may influence reproductive health.

2.58. Using 16S sequencing and metabolomics, the study by Talwar et al. (2024) identified that patients with endometriosis had a reduced presence of the beneficial gut microbial metabolite, 4-hydroxyindole. Functional assays demonstrated that 4-hydroxyindole suppresses endometriosis-associated inflammation and hyperalgesia in both mouse and human xenograft models. The altered metabolomic profile also shows strong similarities to that found in inflammatory bowel disease, suggesting a possible association between the conditions.

2.59. Hamamah et al. (2024) proposed a mechanistic link between intestinal and endometrial dysbiosis and endometriosis through the modulation of key cytokines IL-17 and IL-33. The paper suggests that elevated oestrogen levels promoted by gut dysbiosis activate these cytokines, contributing to endometriosis. This supports the existence of a gut–endometrial axis influencing reproductive health.

2.60. Tang et al. (2024b) employed a two-sample Mendelian randomisation approach to investigate causal associations between gut microbiota and site-specific endometriosis, as well as endometriosis-induced infertility. The study identified multiple genera linked to endometriosis across different anatomical locations, including the ovary, fallopian tube, pelvic peritoneum, and rectovaginal septum, and three genera associated with infertility related to endometriosis. These findings highlight the potential role of gut microbiota in the pathogenesis of endometriosis and its reproductive consequences.

2.61. Lu et al. (2024) used Mendelian randomisation to assess causal relationships between metabolites and reproductive endocrine disorders, including PCOS, endometriosis, and female infertility. The study identified metabolites such as 1-palmitoylglycerophosphocholine (positively

associated with PCOS) and phenylacetate (negatively associated with infertility), highlighting metabolite-specific risk and protective factors. These findings support a mechanistic link between metabolic alterations and reproductive pathogenesis.

2.62. A number of recent narrative reviews have additionally examined the emerging role of the gut microbiome in endometriosis, alongside other infertility-associated conditions (including PCOS and gynaecological cancers). These reviews discuss evidence considering the microbiota-gut-brain axis, emphasising the need for rigorous, standardised research to clarify associations and inform microbiome-targeted diagnostics and personalised interventions (Favaron et al., 2024; Guo and Zhang, 2024; Hearn-Yeates et al., 2024; Pérez-Prieto et al., 2024; Tang et al., 2024a; Wang et al., 2024b).

Other infertility pathologies

2.63. The review by Zhang et al. (2024b) explores the role of the gut microbiota in modulating iron metabolism and ferroptosis pathways in female infertility, highlighting an association between iron overload and ferroptosis to hypogonadism, ovarian dysfunction, impaired embryonic development, and reduced endometrial receptivity. The authors propose that targeting these mechanisms may offer novel therapeutic strategies for infertility, particularly in women with haematological disorders.

Oral microbiome

2.64. Ye et al. (2024) investigated how periodontitis-related oral dysbiosis affects the female reproductive tract and whether the probiotic bacteriocin nisin can mitigate these effects. In a polymicrobial mouse model of periodontal disease, DNA from oral pathogens was detected in reproductive organs, with infection reducing uterine microbiome diversity and increasing inflammatory cytokines (IL-6, TNF-α). Nisin treatment reduced pathogen load, modulated the reproductive tract microbiome, and suppressed inflammation, suggesting circulatory transmission of oral pathogens and a therapeutic potential for nisin.

2.65. Marcickiewicz et al. (2025) reviewed evidence linking oral cavity dysbiosis to systemic inflammation and its associations with female infertility-related conditions, including PCOS and endometriosis. The review also explored the impact of oral microbiota on gestational outcomes such as preterm birth and miscarriage, underscoring the potential systemic effects of oral dysbiosis on reproductive health through inflammatory and hormonal pathways.

Interventions and therapeutic strategies for the female microbiome

Restoration of the local reproductive tract microbiome

2.66. Naghi Jafarabadi et al. (2024) conducted a randomised clinical trial to assess whether intravaginal probiotic use before frozen embryo transfer (FET) improves pregnancy outcomes in women with RIF (recurrent implantation failure). Although both chemical and clinical pregnancy rates were slightly higher in the probiotic group, the differences were not statistically significant. The study concluded that intravaginal probiotics did not significantly improve outcomes and called for further investigation into optimal use parameters.

2.67. Yang et al. (2025) conducted a systematic review and meta-analysis of 10 RCTs and two in vivo/in vitro studies on the impact of probiotics on sexual function, hormones, and fertility outcomes. Probiotics improved sexual function, notably in women on antidepressants, and

enhanced sexual function index scores. Combined treatments (e.g., Lactofem with Letrozole/SSRIs) resulted in 10% pregnancy rates versus 0% in controls. Probiotics reduced menopausal symptoms by 66%, improved sperm parameters, lowered the LH/FSH ratio (3.0 to 2.5), and increased testosterone. Overall, probiotics were linked to improved pregnancy rates and reproductive health outcomes.

2.68. Kamrani et al. (2025) conducted a double-blind randomised control trial investigating probiotics in patients with RIF (recurrent implantation failure). Probiotics were found to modulate the Th1/Th2 cytokine ratio, reducing pro-inflammatory cytokines (TNF-α, IFN-ɣ) and increasing anti-inflammatory cytokines (IL-10, IL-4), alongside a decrease in Th1 cells and an increase in Th2 cells. This immunomodulation led to improved implantation outcomes, with a significantly higher clinical pregnancy rate in the probiotic group ($p = 0.037$).

2.69. Hu et al. (2024) conducted a retrospective study of 254 patients with CE undergoing FET to compare outcomes between antibiotic-only treatment and a combined regimen of doxycycline plus vaginal Lactobacillus. While the combination group had a non-significant increase in biochemical pregnancy rate (70.4% vs. 64.7%), it showed a significantly lower rate of premature rupture of membranes (33.3% vs. 50.0%, $p = 0.037$). Overall, findings suggest potential benefits of adjunctive probiotic use, though evidence remains inconclusive.

2.70. The randomised blinded trial by Tanha et al. (2023) investigated the effect of the vaginal probiotic Lactovag on vaginal microbiota normalisation and pregnancy outcomes in FET cycles. A total of 103 women undergoing ART were assigned either to receive Lactovag suppositories or no microbiome intervention. While clinical pregnancy (23.4%) and fetal heart detection (21.3%) were more frequent in the Lactovag group, differences between groups were not statistically significant ($P > 0.05$). However, the presence of grade A embryos significantly increased pregnancy odds (OR: 1.53, $P = 0.001$), and this was further enhanced with Lactovag use (OR: 1.68, $P = 0.008$). Authors concluded that vaginal microbiota may influence reproductive outcomes and that probiotics like Lactovag might offer a supportive role, although findings require cautious interpretation given limited statistical significance.

2.71. He et al. (2024b) investigated the use of Lactobacillus crispatus chen 01 (isolated from healthy women) for treating CE. In vitro and mouse models showed that L. crispatus chen 01 inhibited pathogens, reduced inflammation (downregulating TLR, MyD88, and p65/p-p65), improved endometrial histology, and enhanced implantation processes via the Wnt/β-catenin pathway. Pregnancy rates increased dramatically in mice (100% vs 0% in controls). In a clinical trial, L. crispatus chen 01 supplementation improved progesterone levels, pregnancy rates (87.18% vs 76.19% with antibiotics alone), and endometrial pathology in CE patients, highlighting its potential as a probiotic intervention to improve fertility outcomes.

2.72. Sakamoto et al. (2024) compared two treatment regimens for improving uterine microflora in patients with RIF and non-Lactobacillus dominant profiles. Treatment B (oral and vaginal metronidazole followed by combined oral and vaginal probiotics) was from to significantly improve Lactobacillus occupancy (90.0% vs. 41.2%, $p = 0.0127$), though its impact on IVF outcomes remains undetermined.

2.73. Di Pierro et al. (2023) retrospectively evaluated the effect of oral Lactobacillus crispatus M247 in 160 women undergoing ART. Treated women had higher clinical pregnancy rates (OR = 1.56), with the effect most pronounced in the blastocyst subgroup under age 43 and BMI >18.6, where

clinical pregnancy rose from 28.4% to 44.5% (OR = 2.08, $p < 0.05$). Live birth rates were also higher in the probiotic group (12.5% vs. 7.5%) and increased by 200% in the blastocyst subgroup (OR = 3.64, $p = 0.05$).

2.74. Maleki-Hajiagha et al. (2025) conducted a systematic review of six prospective studies (n = 850) assessing vaginal probiotics before embryo transfer. A non-significant increase in clinical pregnancy rates was observed overall (RR 1.19; P = 0.07). Subgroup analysis showed no differential effect in women with or without recurrent implantation failure. No significant differences were found for biochemical, ongoing pregnancy, or miscarriage rates. Findings suggest possible benefit, but evidence remains inconclusive.

2.75. Bakun et al. (2023) studied 30 infertile women, comparing 20 with endometriosis undergoing ART who received the probiotic Femina Probiz (containing Lactobacullus and Bifidobacterium strains) to 10 tubal infertility controls. Probiotic supplementation occurred over one month prior to ART, and NLRP3 inflammasome levels were measured pre- and post-treatment. Authors concluded that incorporating probiotics into ART treatment led to a substantial reduction in mRNA expression of the NLRP3 inflammasome.

2.76. Liu et al. (2024c) investigated whether a recombinant Lactobacillus crispatus strain expressing granulocyte colony-stimulating factor (G-CSF) could better treat thin endometrium than G-CSF alone in a mouse model. The engineered strain significantly improved endometrial thickness, angiogenesis, gland number, and embryo number, while reducing fibrosis and pathogenic taxa. Mechanistically, it appeared to act via the PI3K/AKT pathway and upregulated key angiogenic and hypoxia-related markers.

2.77. The preclinical study by Fan et al., (2025) describes the development of a novel injectable hydrogel loaded with Lactobacillus to support endometrial regeneration. Designed for improved retention and bioactivity, the hydrogel transitions from fluid to a stable nanonetwork at body temperature, maintaining bacterial viability and modulating the uterine microenvironment. In a rat model of endometrial injury, it persisted for over 21 days and promoted structural and functional repair, suggesting potential for future therapeutic use in uterine infertility.

2.78. Raimundo et al. (2025) assessed the effect of Ligilactobacillus salivarius PS11610 supplementation on IVF outcomes. Treatment (1×10^9 CFU every 12 hours for one month pre-IVF) demonstrated antimicrobial activity against pathogens linked to reproductive tract dysbiosis. While embryo quality was lower in some categories for women receiving the probiotic, the FET group showed significantly improved live birth rates (26.4% vs 17.9%) and biochemical pregnancy rates (42.6% vs 34%). The study suggests that despite mixed results on embryo quality, L. salivarius PS11610 may enhance ART outcomes.

2.79. Santana and Póvoa (2024) reviewed six studies evaluating the impact of probiotics on reproductive outcomes in women undergoing ART. While five studies reported improved pregnancy rates, only one reached statistical significance; another found a significant reduction in miscarriage rates despite no increase in pregnancy. The review highlights uncertainty around whether probiotics meaningfully modulate the female genital tract microbiome or sustain such changes over time.

2.80. Patki et al. (2025) presented expert consensus from a multidisciplinary meeting in India on the role of Lactobacillus-based probiotics in managing infertility and RIF (recurrent implantation

failure). Experts agreed that microbial dysbiosis contributes to RIF and endorsed oral probiotic use pre-embryo transfer to enhance implantation and reduce miscarriage risk, though recommendations are not based on new empirical data.

2.81. Li et al. (2025b) conducted a narrative review of endometrial receptivity assessment methods in infertile women, highlighting a shift from morphological evaluation to molecular and microbiota-based markers. Advances such as the endometrial receptivity array and uterine microbiome profiling are emphasised for their role in personalising ART treatment and improving implantation outcomes.

Antibiotics

2.82. Alemu et al. (2024) performed a systematic review and meta-analysis (n = 1,206,583) on preconception antibiotic exposure and reproductive outcomes. Macrolides and sulfonamides use was associated with increased infertility risk (FR: 0.65 and OR: 2.35, respectively), while beta-lactams (non-penicillin G) and quinolones were linked to reduced infertility odds. Antibiotic exposure also raised miscarriage (RR: 1.34) and congenital anomaly risks (OR: 1.85 for trimethoprim). This study highlights risks associated with preconception antibiotic exposure.

2.83. The Cochrane review by Ameratunga et al. (2023) evaluated whether administering antibiotics before or during embryo transfer in ART cycles improves reproductive outcomes. Two randomised controlled trials considering 377 patients were included in the analysis. Due to low certainty of evidence, it was unclear whether antibiotics given prior to or at the time of embryo transfer improved live birth rates (odds ratio (OR) 0.48, 95% CI 0.10 to 2.23) or clinical pregnancy rates (OR 1.01, 95% CI 0.67 to 1.55; $I^2 = 0\%$); however, administration may reduce genital tract colonisation slightly (OR 0.59, 95% CI 0.37 to 0.95). Overall, the findings suggest insufficient evidence to recommend routine antibiotic use during embryo transfer, highlighting the need for larger, high-quality studies to clarify potential benefits or harms.

Modulating the gut microbiome

2.84. Ramzan et al. (2024) reviewed the role of probiotics in managing PCOS highlighting how dysbiosis in the gut microbiota contributes to PCOS pathogenesis through mechanisms involving insulin resistance, hyperandrogenism, inflammation, and hormonal dysregulation. The authors suggest that probiotic strains from Bifidobacterium, Lactobacillus, Clostridium, and Enterococcus may improve PCOS symptoms by enhancing intestinal barrier integrity, reducing inflammation, and modulating insulin and sex hormone signalling via short-chain fatty acids. However, they acknowledge that more robust clinical studies are required to validate efficacy.

2.85. Hariri et al. (2024) conducted a triple-blind RCT in 56 women with PCOS, assessing 12-week synbiotic supplementation on health-related quality of life. Synbiotics significantly improved PCOSQ-26 domains including infertility (P = 0.027), weight, body hair, and emotional wellbeing, as well as physical health scores (SF-12), though perceived stress remained unchanged.

2.86. The study by Kukaev et al. (2024) investigated gut microbiota dysbiosis and short-chain fatty acids in patients with PCOS, finding a reduction in beneficial bacteria (Clostridium leptum group, Prevotella spp.) and an overgrowth of opportunistic species (Clostridium perfringens, C. difficile, Staphylococcus spp., Streptococcus spp.). PCOS patients also exhibited elevated faecal levels of acetic acid which was inversely correlated with BMI, insulin resistance, and systemic inflammation. Treatment with metformin was found to restore acetic acid but not serum short

chain fatty acids. The study proposes a predictive model based on serum butyric and valeric acid from metformin response, supporting microbiota-targeted strategies in fertility care.

2.87. Li et al. (2024b) reviewed emerging evidence linking gut microbiota to PCOS through activation of brown adipose tissue. Brown adipose tissue activity, shown to be reduced in PCOS, can be modulated by microbial metabolites (such as short-chain fatty acids and bile acids) produced by the gut microbiome. Therapies such as cold exposure, metformin, or GLP-1 receptor agonists may enhance this pathway, highlighting the gut microbiome as a possible therapeutic target in PCOS.

2.88. Xiong et al. (2024) investigated the effects of GLP-1 receptor agonists (liraglutide and semaglutide) in DHEA-induced PCOS mice. Both drugs modulated the alpha and beta diversity of gut microbiota and improved metabolic parameters. Liraglutide showed the ability to reverse the altered microbial composition and the disrupted microbiota functions caused by PCOS, while semaglutide elevated Helicobacter, negatively correlated with body weight. Microbiota shifts may underlie GLP-1RA efficacy in PCOS treatment.

2.89. Megli et al. (2024) used a mouse model to investigate how diet influences vaginal microbiota composition and Streptococcus agalactiae colonisation. High-fat, low-fibre diets promoted persistent colonisation, independent of obesity or glucose intolerance. Findings highlight diet as a modifiable factor affecting vaginal microbiota and potentially reproductive outcomes.

2.90. The randomised controlled trial by Talebi et al., (2024) investigated the effects of early time-restricted feeding (eTRF), alone or combined with probiotic supplementation, versus a standard calorie-restricted diet on metabolic, hormonal, inflammatory, and oxidative stress markers in overweight and obese women with PCOS. The 8-week trial assigned participants to one of three arms (eTRF + probiotics, eTRF + placebo, or calorie restriction + placebo), assessing outcomes including insulin resistance, sex hormones, oxidative stress, and anthropometric measures. The addition of probiotics to eTRF conferred no added benefit. Authors concluded that eTRF, with or without probiotics, offered no advantage over traditional calorie restriction in managing PCOS-related outcomes.

2.91. The narrative review by Türkoğlu et al. (2024) explores the role of dietary patterns in the management of endometriosis, highlighting evidence that diets such as Mediterranean, anti-inflammatory, vegetarian, and low-FODMAP may alleviate symptoms. The review identifies key dietary elements that modulate inflammation and potentially affect disease progression, including fibre, omega-3 fatty acids, plant-based proteins, and antioxidants. It also evaluates emerging evidence on gut microbiota's role in endometriosis pathophysiology, suggesting that microbiome-targeted interventions may serve as adjunct therapies. Finally, the review considers how artificial intelligence may advance personalised nutrition strategies in endometriosis care.

2.92. The preclinical study by Feng et al., (2024) investigated the role of the gut microbiome in chemotherapy-induced premature ovarian insufficiency (CIPOI) using a cisplatin-induced mouse model. Faecal microbiota transplantation from CIPOI mice impaired ovarian health, while multi-omics analysis revealed reduced levels of Limosilactobacillus reuteri and its metabolite β-resorcylic acid (β-RA). Supplementation with either restored hormonal balance, preserved follicular reserve, and prevented granulosa cell apoptosis via modulation of the SRY-box 7 (SOX7)/BAX pathway. Critically, β-RA pretreatment protected oocyte quality, embryonic development, and foetal viability, suggesting a protective mechanism along the gut-ovary axis.

Traditional medicine

2.93. Zheng et al. (2024) investigated the herbal compound Sparganium stoloniferum-Curcuma phaeocaulis (SL-EZ) in a rat model of endometriosis. SL-EZ inhibited ectopic lesion growth and restored gut microbial balance, notably increasing Lactobacillus gasseri and Lactobacillus johnsonii. Metabolomic and sequencing analyses revealed SL-EZ modulates multiple gut microbial metabolic pathways, supporting its therapeutic efficacy in treating endometriosis.

2.94. Yin et al. (2025) investigated the effects of the traditional Chinese medicine Yi-Qi-Qing-Shi-Hua-Yu (YQQSHY) on pelvic inflammatory disease in a rat model. YQQSHY treatment was found to reduce uterine tissue damage and fibrosis, lowered pro-inflammatory cytokines, and increased anti-inflammatory factors. Additionally, YQQSHY significantly altered the composition and diversity of intestinal microbiota, suggesting a link between gut health and treatment.

The Male Microbiome and Reproduction

Male reproductive tract microbiome and microbial transmission

Characterisation of male reproductive tract microbiota and association with seminal quality

2.95. Li et al. (2024a) characterised the upper reproductive tract microbiota in male rats using 16S rRNA sequencing and identified tissue-specific bacterial genera, including Methyloperoxococcus in testes and Jeotgalicoccus in the epididymis. Correlations were found between microbial abundance and sperm parameters, suggesting a potential link between microbiota composition and male fertility.

2.96. Faniev et al. (2025) conducted a comparative analysis of the taxonomic structure of the testicular and urethral microbiota of patients with non-obstructive azoospermia (n = 62) in ART protocols with positive (n = 16) and negative (n = 46) outcomes. Whilst the frequency of occurrence of the Streptococcaceae family was more common in patients with negative ART outcomes, Enterococcaceae and Brevibacteriaceae families were more common in patients with positive ART outcomes.

2.97. Han et al. (2024a) conducted a multi-omics study on asthenozoospermia (AZS), identifying significant differences in seminal microbiota and metabolites across AZS severity levels. Reduced levels of Pseudomonas and Serratia, and increased Uruburuella and Vibrio, were observed in more severe AZS cases. The metabolite hexadecanamide, positively associated with beneficial genera, was shown to enhance sperm motility and upregulate target genes PAOX and CA2 in sperm cells. These findings link specific microbial-metabolite interactions to impaired motility and suggest hexadecanamide as a potential therapeutic agent for AZS.

2.98. The observational study by Osadchiy et al. (2024) investigated associations between the semen microbiome and abnormal semen analysis parameters in men undergoing fertility evaluation. Using 16S rRNA sequencing and multivariate analyses, the study found that men with abnormal sperm motility had a significantly higher abundance of Lactobacillus iners, while men with abnormal sperm concentration showed increased levels of Pseudomonas stutzeri and Pseudomonas fluorescens, but lower levels of Pseudomonas putida. These findings suggest that specific microbial taxa, including L. iners, may influence male fertility, warranting further investigation into the role of the semen microbiome in idiopathic infertility.

2.99. Kukoyi et al. (2025) conducted a meta-analysis on Ureaplasma urealyticum and semen quality, revealing significant reductions in ejaculate volume, sperm concentration, motility, and morphology in infected males compared to controls. Increased seminal leukocyte count and elevated levels of IL-1β, IL-6, IL-8, TNF-α, CD4+, and CD8+ T cells were observed, alongside heightened sperm DNA fragmentation and apoptosis. These findings suggest U. urealyticum impairs sperm quality via inflammatory responses and oxidative stress.

2.100. He et al. (2024) combined 16S rRNA sequencing and untargeted metabolomics to investigate the role of the seminal microbiome in high sperm DNA fragmentation index, a marker of poor fertility outcomes. They found specific microbial profiles, particularly Lactobacillus iners, were enriched in patients with high fragmentation indexes, along with increased activity in butanoate and purine degradation pathways, suggesting microbiota-driven metabolic damage to sperm DNA. The study proposes the microbiome as a novel therapeutic target for male infertility associated with high DNA fragmentation.

2.101. Gomes et al., (2023) conducted a systematic review of seven studies to evaluate the relationship between microbiota composition and seminal quality. The review found that Lactobacillus was more prevalent in men with normal semen parameters, whereas Prevotella was more common in men with seminal abnormalities. The findings suggest that dysbiosis, particularly reduced Lactobacillus and increased pathogenic species, is associated with male infertility.

2.102. Liang et al. (2025) employed Mendelian randomisation to assess the causal relationship between immune cell subtypes and male infertility using FinnGen and GWAS data. Mendelian randomisation identified 23 immune phenotypes associated with male infertility, with six protective and 17 posing risks. No reverse causality was found, and sensitivity analyses confirmed the robustness of the findings. This study suggests a genetic-level causal link between immune cell types and male infertility.

2.103. Asgari et al. (2025) compared seminal bacteria in 80 infertile and 80 fertile men, identifying a higher prevalence of bacteriospermia in the infertile group ($p<0.05$). Ureaplasma urealyticum was the most abundant species (7.5%, $p<0.05$), followed by Enterococcus faecalis (6.25%, $p>0.05$), with no detection of Streptococcus agalactiae in abnormal samples. Ureaplasma urealyticum was associated with reduced sperm motility and morphology, while Enterococcus faecalis and Streptococcus agalactiae showed no impact. The study suggests bacteriospermia, particularly U. urealyticum, compromises seminal quality and fertilisation potential.

2.104. Bragina et al. (2024) investigated microcolonial forms of Mycoplasma hominis and their impact on spermatogenesis using samples from 125 fertile men and 93 infertile patients. Microcolonies, identified through molecular and electron microscopy methods, were found 2.5 times more frequently in patients with poor sperm quality than classical M. hominis colonies. These microcolonial forms attached to spermatozoa and displayed a unique ultrastructure with concentric layers (12–14 nm periodicity). The findings suggest that microcolonial M. hominis may contribute to male infertility.

2.105. The preprint study by Bhagwat et al. (2025) investigated the effects of bacterial vaginosis-associated toxins lipopolysaccharide (LPS) and vaginolysin (VLY) on sperm function. Mouse and human sperm exposed to these toxins exhibited increased intracellular calcium, impaired capacitation, reduced motility, and decreased acrosomal exocytosis ($p<0.05$), leading to diminished fertilisation capacity in vitro. The LPS-induced effects were mediated via Toll-like

receptor 4 and could be blocked by polymyxin-B, highlighting a mechanism independent of CatSper. These findings suggest that BV toxins disrupt sperm function and contribute to infertility.

2.106. Mowla et al. (2025) examined the seminal microbiota and sperm quality in 223 men with reproductive disorders (including RPL, male infertility, and unexplained infertility) and controls. Semen clustered into three genera-dominant groups: Streptococcus, Prevotella, and Lactobacillus/Gardnerella, with Prevotella-dominant samples showing higher microbial richness ($p<0.001$), diversity ($p<0.001$), and bacterial load ($p<0.0001$). No global association was found between bacterial composition/load and sperm parameters.

2.107. Baud et al. (2024) integrated microbiota profiling and metabolomics to investigate seminal bacteria's influence on semen metabolite composition in infertile couples. While overall metabolite profiles were not significantly altered by microbiota composition, levels of certain metabolites, such as urocanate (adjusted $P < 0.001$), were elevated in abnormal semen and enriched in samples dominated by Prevotella spp. ($P < 0.05$). Urocanate's immunomodulatory properties suggest a potential link between seminal dysbiosis and semen quality. This study highlights a possible mechanistic pathway by which the seminal microbiome may contribute to male infertility.

2.108. Shrestha et al., (2024) examined the prevalence of bacteriospermia in 217 semen samples from infertile men in Nepal, finding bacteria present in 25.3% of samples, with Staphylococcus aureus as the predominant isolate. Semen volume was significantly reduced with bacteriospermia ($p = 0.001$), while sperm concentration, motility, morphology, and vitality were negatively affected but not significantly. The study suggests bacterial infection impairs semen parameters and may contribute to male infertility. These findings provide a baseline for future research on bacteriospermia's role in reproductive health.

2.109. Jin et al. (2024a) investigated the role of the antimicrobial peptide DEFB119 in male infertility, focusing on its impact on the seminal microbiome. In patients with abnormal spermiograms ($n = 57$), modularity of microbial metacommunities was markedly reduced compared to controls ($n = 30$), and elevated seminal DEFB119 was linked to decreased bacterial diversity and community structure. Recombinant DEFB119 reduced sperm progressive motility and showed species-specific antimicrobial activity, suggesting its dual role in host-microbiome interactions and male fertility. This study implicates DEFB119-mediated dysbiosis in sperm dysfunction.

2.110. Jendraszak et al. (2024) conducted a narrative review of 51 studies, focusing on the male genitourinary microbiome and its potential role in male fertility. They highlighted anatomical variation in microbial communities across regions such as the skin, urethra, and seminal glands. Evidence suggests associations between bacterial diversity in testicular tissue and sperm quality, with emerging interest in microbial exchange between sexual partners. The review underscores the need for standardised methodologies and longitudinal studies to clarify causal links and inform clinical translation.

2.111. Neto et al. (2024) conducted a narrative review of 37 studies (9,310 participants) on the seminal microbiome and its relationship with male infertility. Next generation sequencing based studies confirmed bacterial presence in semen, with high interindividual variability and microbial dominance patterns. Couples often shared microbial taxa between the seminal and vaginal microbiomes, including Staphylococcus and Streptococcus, particularly in cases of known

infertility. While bacteria in IVF culture media did not impact pregnancy rates, the seminal microbiome was linked to broader reproductive outcomes.

2.112. The review paper by Grande et al. (2024) summarised the current evidence on alterations in semen microbiota associated with infertility, male tract infections, and HPV. Key findings include increased Prevotella and Pseudomonas linked to poor semen parameters, reduced Lactobacillus crispatus as a marker of low semen quality, and altered microbiota profiles in prostatitis and HPV. The authors advocate for future research into the "couple genital microbiota" due to its potential relevance to fertility.

2.113. Corral-Vázquez et al., (2024) reviewed current knowledge of the seminal microbiome, highlighting its complexity, dynamic composition, and emerging associations with male fertility. Certain bacterial clusters have been linked to healthy semen profiles, while others correlate with infertility markers. The review also discusses external influences such as lifestyle, gut microbiota, and probiotic use, and addresses methodological challenges affecting data interpretation.

2.114. Davies et al., (2024) reviewed evidence linking the seminal microbiome to declining sperm quality and male infertility, highlighting mechanisms such as bacterial-induced reactive oxygen species and DNA damage. Next-generation sequencing has revealed conserved bacterial clusters in semen, but conflicting results across studies due to methodological inconsistencies and small sample sizes limit clinical applicability. Associations between specific microbial profiles and abnormal semen parameters are emerging, but robust evidence is lacking.

2.115. Potiris et al. (2025) reviewed the impact of oxidative stress (OS) and inflammation on male infertility, highlighting the role of ROS-induced DNA, protein, and lipid damage. Pro-inflammatory cytokines like TNF-α and IL-6 were shown to disrupt spermatogenesis and promote oxidative damage, while infections from E. coli and C. trachomatis were linked to sperm dysfunction. The review emphasises the need for early detection and targeted therapies, including microbiota modulation, antimicrobials, anti-inflammatories, and antioxidants. These strategies aim to mitigate oxidative damage and improve fertility outcomes.

2.116. Sciorio et al. (2025) conducted a review on immune-mediated male infertility, highlighting the roles of infections, inflammation, and autoimmunity in impairing sperm quality. Epididymitis, testicular trauma, vasectomy, and infections contribute to antisperm antibody formation and oxidative stress, reducing motility and migration. Sperm-immobilising antibodies inhibit function, while assisted reproductive treatments like ICSI, IVF, and IUI can help overcome immune barriers. The study underscores the need for improved diagnostic tools, understanding immune-pathology, and addressing legal concerns in male infertility.

2.117. Chatzokou et al., (2025) critically reviewed evidence linking the semen microbiome to male fertility and reproductive outcomes, emphasising the role of next-generation sequencing in uncovering microbial diversity. The review highlights the opposing effects of genera such as Lactobacillus (beneficial) and Prevotella (detrimental) on sperm quality and DNA integrity, with implications for both natural conception and ART outcomes. Despite emerging associations, there is currently no standardised diagnostic or therapeutic approach. The authors call for development of clinical algorithms and further research into microbiome-targeted fertility strategies.

2.118. Henkel (2024) reviewed current evidence on leukocytospermia and bacteriospermia, conditions affecting up to 30% of infertile men, and their roles in male infertility. Bacterial infections trigger leukocyte infiltration, leading to oxidative stress through cytokine and reactive oxygen species (ROS) production, damaging sperm function. While antibiotics, antioxidants, and anti-inflammatories are used, no standardised management protocol exists, and the impact on assisted reproduction outcomes remains unresolved.

Partner Microbiota Dynamics

2.119. Molina et al. (2025) introduced the concept of the "seminovaginal microbiome," encompassing the interactive microbiota of both sexually active partners. This review synthesised next-generation sequencing studies to explore how microbial exchange during intercourse influences reproductive health, ART outcomes, and susceptibility to infections. It also highlights the understudied microbial dynamics in same-sex and transgender individuals, advocating for a more inclusive and integrative approach to reproductive microbiome research.

2.120. Berard et al. (2025) reviewed the influence of the urogenital microbiomes and genital tract inflammation in both sexes on infertility and IVF outcomes. Associations between altered female reproductive tract microbiota, inflammation, and reduced IVF success were highlighted, along with evidence linking male urogenital microbiota to impaired semen quality. The review also addressed microbial transmission between partners and its potential impact on reproductive outcomes.

2.121. Alqawasmeh et al. (2024) investigated microbial transmission from semen and follicular fluid into spent embryo culture media in 61 ART-treated couples. Microbial presence in culture media was detected in 82.5% of cases, with semen as the primary source in conventional IVF. Staphylococcus spp. and Streptococcus anginosus in semen were negatively associated with sperm motility and count ($p < 0.001$), and specific follicular fluid genera correlated with types of female infertility. However, no associations were found between microbiota and ART success outcomes (including fertilization rate, embryo development, number of available embryos, and clinical pregnancy rate).

Gut microbiota and male fertility

2.122. Xi et al. (2023) used Mendelian randomisation to investigate causal links between gut microbiota and infertility. Specific taxa, such as Eubacterium oxidoreducens (OR = 2.05) and Lactococcus (OR = 1.45), were associated with increased risk of male infertility, while others like Eubacterium rectale (OR = 0.31) and Ruminococcaceae NK4A214 (OR = 0.54) were protective. Several genera were also causally associated with female infertility. Sensitivity analyses confirmed the robustness of findings. The study supports microbiota-based monitoring and personalised interventions in reproductive medicine.

2.123. Zhang et al. (2024c) conducted a bidirectional Mendelian randomisation study to assess causal links between gut microbiota and reproductive system diseases, including male and female infertility. The analysis identified 61 causal associations; for instance, Eubacterium hallii was protective against premature ovarian failure but pathogenic in endometriosis, while Erysipelatoclostridium was associated with PCOS, endometriosis, epididymitis, and orchitis. Intestinibacter was linked to male infertility and sexual dysfunction, and reverse analysis indicated that female infertility-related diseases more strongly altered gut microbiota composition than male

ones. The study supports a bidirectional causal relationship between gut microbiota and infertility-related conditions.

2.124. The review by Ashonibare et al. (2024) explores the emerging gut microbiota-gonadal axis and its influence on reproductive physiology in both sexes. Drawing predominantly from animal studies, the authors describe how gut microbiota modulate steroid hormone levels, insulin sensitivity, immune function, oxidative stress (via ROS generation), and potentially even local gonadal microbiota composition. These interactions are proposed to influence gametogenesis, gonadal function, and systemic reproductive capacity. While the findings highlight mechanisms, such as microbial control of oestrogen metabolism and immune-mediated disruption of gonadal environments, the review highlight the lack of human data and calls for translational studies to validate these effects in clinical settings.

Mechanistic insights

2.125. Wang et al. (2024a) demonstrated in a mouse model that cholestasis impairs spermatogenesis by disrupting liver tryptophan metabolism, reducing AHR ligand levels, and downregulating androgen synthesis genes. Supplementation with AHR ligand ITE improved testosterone synthesis. Gut microbiota alterations were linked to this dysfunction, identifying a cholestasis-gut-testis axis in male infertility.

2.126. Qu et al. (2025) investigated the reproductive toxicity of berberine (BBR) in male mice and found that BBR reduced serum testosterone, sperm concentration, mating rate, and fecundity. These effects were mechanistically linked to a BBR-induced decrease in the gut bacterial family Muribaculaceae. Restoration of Muribaculaceae levels through fecal microbiota transplantation or direct supplementation reversed the reproductive impairments, with ornithine metabolism and LDLR-mediated testosterone synthesis identified as key pathways.

2.127. Jin et al. (2024) investigated the effects of dapagliflozin on diabetes-induced male infertility in db/db mice. Using integrated multi-omics analyses, they showed that dapagliflozin improved sperm concentration and motility, reshaped gut microbiota (notably increasing beneficial Lachnospiraceae species), and altered key metabolites across the gut–plasma–testis axis. Proteomic and mechanistic analyses suggested reduced testicular apoptosis and oxidative stress, partly through downregulation of 2'-deoxyinosine.

2.128. Panghal and Jena (2023) reviewed evidence linking type 1 diabetes mellitus to male infertility via gut-testis axis dysfunction. Gut dysbiosis, inflammation, and oxidative stress were identified as key mediators of gonadal damage, suggesting potential therapeutic targets to mitigate diabetes-related male reproductive impairment.

2.129. Pan et al. (2024) conducted a cross-sectional study comparing the gut microbiota of 60 men with asthenozoospermia (AS) and 48 healthy controls using 16S rRNA sequencing. AS patients exhibited significantly lower alpha diversity and distinct beta diversity profiles. Genera such as Escherichia_Shigella and Prevotellaceae_UCG_001 were enriched in AS and negatively correlated with sperm motility. Functional predictions highlighted disruptions in pathways including steroid biosynthesis and meiosis.

2.130. Han et al. (2024b) used Mendelian randomisation to evaluate causal relationships between gut microbiota and male reproductive disorders, including infertility and abnormal spermatozoa. Eight microbial groups were identified as causally linked, with Eubacterium oxidoreducens and

Streptococcaceae increasing risk, while Bacteroidaceae and Porphyromonadaceae were protective. No evidence of pleiotropy was found, and functional enrichment supported biologically relevant pathways. The study provides strong evidence for a gut–testis axis in male infertility.

2.131. Chen et al. (2024c) present a narrative review on the role of the testis-gut microbiota axis in male infertility, synthesising evidence on how gut dysbiosis may impair spermatogenesis and motility through mechanisms including immune modulation, oxidative stress, endotoxin-mediated inflammation, and hormonal signalling. The authors discuss the potential of interventions such as probiotics, prebiotics, synbiotics, faecal microbiota transplantation, and herbal extracts to restore microbial balance and improve sperm quality. The review calls for deeper mechanistic studies to substantiate therapeutic potential.

2.132. Guo et al. (2024) investigated the role of gut microbiota in orchitis-induced male infertility using antibiotic-induced dysbiosis and LPS-induced orchitis mouse models. Dysbiosis exacerbated inflammation, disrupted oxidative stress enzyme activity, reduced testosterone levels, and increased blood-testosterone barrier permeability, which were partially restored by faecal microbiota transplantation. Antibiotics and LPS co-treatment led to more severe inflammation and BTB disruption than LPS alone, but faecal microbiota transplantation mitigated these effects. This study highlights gut microbiota's involvement in orchitis pathogenesis, suggesting potential for microbiome-targeted therapies.

2.133. Hao et al. (2024) conducted a systematic review and meta-analysis of 935 studies (1947–2023) examining internal ammonia (NH_3) and hydrogen sulfide (H_2S) production, male infertility, and gut microbiota. Results showed NH_3 and H_2S negatively correlated with Lactobacillus levels (linked to improved fertility) and positively with Bacteroides (linked to reduced fertility), with meta-analysis confirming statistical significance ($p<0.001$). The study proposes a gut microbiota-inner gases male fertility axis, highlighting the potential impact of microbial gas production on reproductive health.

Mendelian randomisation studies

2.134. The study by Fu et al. (2023) used a two-sample Mendelian randomisation approach to investigate the causal relationship between gut microbiota composition and male infertility risk. Using GWAS data from the MiBioGen and FinnGen consortia, several microbial genera were identified as either risk-enhancing (e.g., Allisonella, Anaerotruncus, Barnesiella, Intestinibacter, Lactococcus) or protective (Bacteroides, Romboutsia, and Ruminococcaceae groups). The findings suggest a causal role for specific microbiota in male reproductive dysfunction.

2.135. The Mendelian randomisation study by Zou et al. (2024) explores the causal relationship between gut microbiota, inflammatory cytokines, and male infertility using genome-wide association study (GWAS) data from over 120,000 participants. The authors identify two gut microbes, Anaerotruncus (which increases infertility risk) and Bacteroides (which decreases infertility risk), as having significant causal associations with male infertility. Additionally, hepatocyte growth factor (HGF) is found to reduce risk, while monocyte chemotactic protein 3 (MCP-3) increases it. Mediated Mendelian randomisation analysis suggests that HGF partially mediates the protective effect of Bacteroides on MI (38.9% mediated effect), indicating a plausible microbiota-cytokine-fertility axis.

2.136. Deng et al. (2024) used Mendelian randomisation to assess causal relationships between gut microbiota and male infertility, identifying one family and four genera with significant associations. Notably, Anaerotruncus was linked to a nearly twofold increased risk of infertility (OR = 1.96, p = 0.016). Multiple randomisation methods confirmed the robustness of findings, with minimal heterogeneity or pleiotropy.

2.137. Zhang et al. (2023).used a two-sample Mendelian randomisation approach to explore causal relationships between gut microbiota and infertility, employing data from 18,340 individuals. Protective effects were identified for the Ruminococcaceae NK4A214 group in both male (OR = 0.61) and female (OR = 0.85) fertility, while Anaerotruncus (OR = 1.81) was linked to male infertility and several taxa (e.g., Betaproteobacteria, Burkholderiales) had adverse effects on female fertility. Results were consistent across multiple MR methods, with no evidence of pleiotropy or heterogeneity. These findings support a causal role of specific gut microbiota in reproductive health.

2.138. Wang and Kang (2025) used Mendelian randomisation to evaluate the causal role of gut microbiota in male fertility, including infertility, abnormal spermatozoa, and erectile dysfunction. Decreased Ruminiclostridium was associated with increased male infertility risk (OR = 0.54, p = 0.045), while reduced Prevotella was linked to abnormal sperm (OR = 0.67, p = 0.046). Lachnospiraceae NC2004 group was positively associated with erectile dysfunction. No heterogeneity or pleiotropy was detected. These findings provide novel evidence of a gut microbiota-male fertility axis.

2.139. Wu et al. (2025) used two-sample Mendelian randomisation to examine causal links between gut microbiota and male reproductive health issues. Protective genera included Erysipelatoclostridium, Parasutterella, Ruminococcaceae UCG-009, and Slackia against prostatitis, while genera such as Faecalibacterium and Sutterella were detrimental. Causal relationships were also identified between certain bacteria and orchitis, epididymitis, male infertility, and abnormal spermatozoa, with links to sperm proteins like SPACA3, SPACA7, SPAG11A, and others. These findings suggest a microbiome-mediated pathway for reproductive inflammation and dysfunction, offering potential for diagnostic and therapeutic strategies.

Reviews

2.140. The review by Cannarella et al. (2024) critically examine the impact of obesity on male infertility, integrating meta-analytic evidence showing a negative effect of excessive body weight on both conventional and biofunctional sperm parameters. The authors explore mechanisms including gut microbiome alterations, chronic inflammation, and hormonal imbalances, proposing these as potential molecular targets for diagnostics and treatment, though several pathways remain insufficiently understood.

2.141. Muñoz et al. (2024) reviewed multifactorial causes of declining male fertility, highlighting roles of oxidative and nitrosative stress mediated by gut microbiota, environmental exposures, and infections. Emerging interventions include prebiotics and psychological support, though treatment efficacy and safety, such as potential DNA damage from antioxidant overuse, remain uncertain.

2.142. Lv et al. (2024) reviewed evidence on the role of gut microbiota and their metabolites in male reproductive health, focusing on sperm quality, testicular structure, sex hormone regulation, sexual behaviour, and probiotic supplementation. Gut dysbiosis may impair the blood-testis

barrier, alter sex hormone synthesis and circulation, and affect the hypothalamic-pituitary-testis axis. Metabolites can also influence sexual arousal via central nervous system pathways. While probiotic supplementation shows promise in improving male fertility, mechanisms remain incompletely understood.

2.143. Magoutas et al. (2025) reviewed male infertility, highlighting its contribution to 40-50% of cases and the often unknown underlying causes. The review discusses how testicular microenvironment and diet may affect fertility, emphasising the limited research on their combined effects. It highlights emerging evidence linking diet and the seminal microbiome to semen quality, suggesting that dietary interventions could improve male fertility. This approach could reduce reliance on assisted reproduction and shift the fertility conversation to include male factors.

2.144. Hsu et al. (2024) reviewed the role of epigenetics, particularly DNA methylation, in spermatogenesis and male infertility, highlighting altered methylation in genes such as MEST, H19, and MTHFR. The study discussed how microbial infections can induce immune responses that disrupt host epigenetic regulation, potentially contributing to infertility. It also noted associations between dysregulated lncRNA levels and sperm motility and count. The authors suggest that better understanding sperm methylation could inform diagnostics for male infertility.

2.145. Ughade et al. (2024). reviewed the role of genital tract dysbiosis in fertility, highlighting how microbial imbalances in both female and male reproductive tracts impair sperm function, mucosal immunity, and reproductive outcomes. Mechanisms include altered vaginal pH, inflammation, and decreased sperm viability and motility. The review also discusses diagnostic strategies and therapeutic options such as probiotics, prebiotics, and antimicrobial agents aimed at restoring microbial balance and improving fertility.

2.146. The review by Wang et al. (2024c) examines the role of microbiota across male and female reproductive systems in both humans and animals, highlighting how dysbiosis may impair gamete quality, implantation, and embryonic development. The paper outlines mechanisms including metabolic and epigenetic disruption, and proposes microbiota modulation as a promising strategy for diagnosing and treating reproductive disorders. Findings support the potential of microbiota-based interventions to improve reproductive outcomes and reduce pregnancy complications.

Interventions and therapeutic strategies for the male microbiome

2.147. The systematic review by Oliveira et al. (2024) evaluates four RCTs on probiotic supplementation in men with idiopathic infertility, including oligozoospermia, asthenozoospermia, and teratozoospermia. Probiotics, particularly Lactobacillus and Bifidobacterium, showed significant improvements in sperm parameters, including motility-through antioxidant effects and DNA protection from ROS. The findings support probiotics as a potential therapeutic intervention in male infertility.

2.148. Asadi et al. (2023) investigated the impact of probiotic supplementation (FamiLact®) on semen parameters post-varicocelectomy in infertile men. Over three months, the probiotic group showed significant improvements in sperm concentration (33.7 ± 22.5 vs. 21.1 ± 16.1 × 10^6/mL, P = 0.046) and sperm morphology (15.0 ± 8.9% vs. 12.0 ± 11.5%, P = 0.016) compared to placebo, while changes in semen volume and motility were not significant. The study suggests probiotics can

enhance semen quality following varicocelectomy, offering a cost-effective and well-tolerated treatment adjunct.

2.149. Dong et al. (2024) conducted an integrated RNA-seq, metabolomics, and 16S rDNA analysis to assess the effects of the prebiotic chitosan oligosaccharide (COS) on male reproduction. COS promoted germ cell proliferation, testicular development, and expression of meiotic proteins (DDX4, DAZL, SYCP1), while enhancing antioxidant capacity and upregulating testicular steroid proteins STAR and CYP11A1. COS also activated the PI3K-Akt pathway and altered gut microbiota, influencing serum metabolites to support spermatogenesis. These findings suggest COS may enhance male fertility through combined antioxidant, metabolic, and microbiome-mediated mechanisms.

2.150. Jalilvand et al. (2024) examined the protective effects of the antioxidant olibanum on oxidative stress and apoptosis in testes and sperm dysfunction induced by lipopolysaccharide, a particle of gram-negative bacteria, in male rats. Lipopolysaccharide (1 mg/kg) significantly increased germ cell apoptosis (TUNEL assay) and reduced sperm count and morphology. Olibanum at both 100 mg/kg and 200 mg/kg doses significantly reduced apoptotic germ cell counts and improved sperm count and morphology. These findings highlight a potential therapeutic value for olibanum when in ameliorating lipopolysaccharide-induced oxidative damage and spermatogenic dysfunction.

2.151. Calamai et al. (2024) applied a novel flow cytometry method to measure sperm ROS in 131 subfertile men and 31 healthy donors. Subfertile patients showed significantly higher ROS production (14.22% vs. 9.75%, $p<0.01$), with no correlation to age, semen parameters, or sperm DNA fragmentation (sDF). Bacteriospermia sharply increased ROS (31.61% vs. 14.20%, $p<0.01$), and 29% of subfertile patients exceeded the established ROS threshold. The findings indicate that sperm oxidative stress, largely independent of conventional semen analysis, may provide additional diagnostic insights into male infertility.

2.152. Juhász et al. (2024) identified Lactiplantibacillus plantarum SNI3 as a novel probiotic with pro-reproductive effects in male mice. Four weeks of administration increased testis size, testosterone, sperm count, and fertility. Faecal microbiota transplantation from treated mice replicated these effects in microbiome-depleted recipients. Metabolomic analysis identified γ-glutamyl-glutamate (γ-GluGlu) as a key testicular metabolite induced by L. plantarum; exogenous γ-GluGlu increased sperm count independently of testosterone.

2.153. Kotarska (2024) examined the effects of Lactobacillus rhamnosus culture supernatant on mouse sperm, finding no significant impact on viability, motility, or genome integrity, though high concentrations were toxic. The study highlights the protective potential of lactobacilli metabolites against hydrogen peroxide-induced DNA damage. These findings suggest that appropriately dosed probiotic or postbiotic preparations taken by women are unlikely to harm their partners' sperm. However, the therapeutic use of lactobacilli for male fertility remains inconclusive.

2.154. Hajian et al. (2025) investigated the protective effects of heat-killed Lactobacillus reuteri on bile duct ligation-induced male reproductive toxicity in rats. The probiotic improved sperm viability and luteinising hormone levels, while reducing oxidative stress markers and inflammatory gene expression (TNF-α and IL-6) compared to controls ($p \leq 0.05$). Histological analysis showed enhanced testicular structure and function in the treated group. These results suggest that heat-

killed L. reuteri mitigates cholestasis-related male infertility via antioxidant and anti-inflammatory mechanisms.

2.155. Lee et al. (2024) examined the effects of three probiotics (L. rhamnosus, L. fermentum, L. paracasei) on mouse sperm motility and vitality. Probiotic treatment increased sperm motility by 30–40% compared to untreated samples and enhanced vitality through upregulation of mitochondrial activity, involving AMPK and SIRT1. All probiotics tested improved mitochondrial function-related protein levels in sperm. These findings suggest probiotics may enhance sperm motility and represent a potential therapeutic option for male infertility.

2.156. Lakhe et al. (2024) presented a case report of a 33-year-old male with bacteriospermia caused by Escherichia coli and Klebsiella pneumoniae, resulting in compromised sperm motility, count, morphology, and high DNA fragmentation index. Antibiotic and antioxidant treatments improved sperm DNA integrity and resolved microbial colonisation. This led to successful IVF and embryo transfer, resulting in a positive pregnancy.

2.157. Raad et al. (2023) assessed the effects of Lactobacillus plantarum secretions on sperm quality during cryopreservation in infertile men. After thawing, samples with 10^8 CFU/mL secretions showed no significant decrease in progressive motility compared to fresh semen, unlike controls. Lactobacillus plantarum secretions also better preserved sperm DNA integrity, particularly in non-normozoospermic samples. These findings suggest that incorporating these secretions into freezing media may protect sperm motility and DNA from cryodamage.

2.158. Liu et al. (2024a) investigated the effects of Lactiplantibacillus plantarum 1008 (LP1008) on testicular function in male mice with high-fat-diet-induced obesity. LP1008 treatment improved blood cholesterol, insulin resistance, and testicular testosterone levels, while enhancing antioxidative enzyme activity (SOD, CAT, GPX) and reducing lipid peroxidation. It also mitigated testicular apoptosis, inflammation, and autophagy, and altered gut microbiota by reducing Ruminococcaceae and increasing Bifidobacteriaceae diversity. These findings suggest LP1008 ameliorates obesity-induced male infertility by enhancing antioxidant capacity and gut microbiota regulation.

2.159. Hao et al. (2025) investigated how acupuncture affects the gut-testis axis in asthenozoospermic mice, using a cyclophosphamide-induced model. Acupuncture was found to improve sperm motility, testicular pathology, hormone levels, and gut-testis barrier function, with specific gut microbiota shifts (e.g., increased Bacteroidota and Faecalibaculum) and metabolite changes linked to sperm motility. Faecal microbiota transplantation validated that gut microbiota modulation contributed to acupuncture's effects. This study suggests acupuncture enhances reproductive function via gut-testis axis restoration.

2.160. Monteiro et al. (2024) conducted a narrative review on precision nutrition in subfertile couples. Evidence supports Mediterranean-style diets and personalised dietary interventions – using nutrigenetics, microbiota testing, and metabolic profiling – to improve fertility outcomes by modulating inflammation, oxidative stress, and metabolic health (Monteiro et al., 2025).

2.161. Kaltsas et al. (2023) explored the relationship between genital tract microbiota dysbiosis and male infertility, emphasising mechanisms such as inflammation, oxidative stress, and sperm damage. The authors highlight microbial signatures common in infertile men, including reduced microbial diversity and dominance of pathogenic species. The paper also discusses emerging therapeutic

approaches, including probiotics, prebiotics, targeted antimicrobials, and faecal microbiota transplantation, and advocates for a precision medicine model tailored to individual microbial and pathophysiological profiles.

Mechanistic insights into the microbiome and reproductive health

Host interactions

2.162. Potter et al. (2024) used dual-species RNA-sequencing to examine interactions between Neisseria gonorrhoeae and human neutrophils. Gonococcal survival was linked to suppression of iron acquisition genes and adaptation to oxidative stress. Host polymorphonuclear leukocytes upregulated pro-inflammatory and adhesion-related genes. Findings elucidate mechanisms of immune evasion contributing to chronic infection and infertility risk.

2.163. Rodriguez et al. (2025) review the immune regulation mechanisms within the male reproductive tract, focusing on the role of the blood-testis barrier and blood-epididymal barrier in protecting spermatogenic cells from immune-mediated damage. They explain how the immune-privileged status of the testis can be exploited by pathogens (bacteria and viruses), leading to inflammation, germ cell loss, and potentially infertility. The review also addresses the fine balance between antimicrobial defence and the risk of autoimmunity, highlighting the testis as both a site of immune tolerance and vulnerability.

2.164. Elovitz et al. (2023) (preprint) used RNA- and ATAC-sequencing to study cervicovaginal epithelial responses to vaginal bacteria. G. vaginalis triggered NLRP3 inflammasome activation and pro-inflammatory gene expression, while L. crispatus had minimal inflammatory impact and induced epigenomic silencing in epithelial cells. Findings highlight distinct host-microbe interactions with potential relevance for reproductive health.

2.165. Zhang et al. (2025) investigated the impact of dyslipidaemia on ART outcomes, endometrial transcriptome, and microbiome in women with RIF. RIF patients with dyslipidaemia exhibited significantly lower implantation, pregnancy, and live birth rates, and showed increased prevalence of non-receptive endometrial transcriptomic profiles and pathogenic bacteria. Transcriptomic analysis revealed dysregulation in cholesterol biosynthesis and immune pathways, with elevated CD56^dim^ NK cells and an altered macrophage M1/M2 ratio. These findings suggest that dyslipidaemia contributes to implantation failure through microbiome-associated immune dysregulation.

2.166. Joseph et al. (2024) examined bacterial extracellular vesicles (bEVs) from Gardnerella vaginalis, Mobiluncus mulieris, and Lactobacillus crispatus, identifying distinct proteomic profiles and immune responses. bEVs from G. vaginalis and M. mulieris were internalised by cervical and vaginal epithelial cells and triggered TLR2-mediated proinflammatory cytokine responses, unlike L. crispatus. This study highlights a mechanistic pathway by which pathobiont-derived bEVs may contribute to adverse reproductive outcomes through host immune activation.

2.167. Lingasamy et al., (2024) reviewed current research on the immunome-microbiome interface in the female reproductive tract, focusing on its influence on immune regulation and reproductive disorders. The review discusses the roles of IgA and IgM, immune tolerance, and the potential of technologies such as PhIP-Seq and mFLOW-Seq for microbiome analysis. While mechanisms remain poorly defined, understanding these bidirectional interactions may advance diagnostics and therapeutic strategies in infertility and pregnancy management.

2.168. Qin et al. (2024) present a narrative review exploring how lipopolysaccharide (LPS), a component of Gram-negative bacteria, affects female fertility through immune activation via TLR signalling. Authors summarised the current understanding of how LPS-induced inflammation impairs ovarian function and reproductive outcomes, and outline the complex, multifactorial mechanisms implicated. The review also discusses potential therapeutic strategies to mitigate LPS-associated fertility decline.

2.169. Shen et al. (2023) investigated how LPS, impairs reproductive function through the tryptophan-kynurenine pathway. LPS activated p38, NF-κB, and JNK signalling, increasing Ido1 expression and kynurenine levels, which reduced oestradiol and FSH, impaired ovulation, and decreased pregnancy and offspring survival rates. In vitro, kynurenine reduced oestradiol production and altered granulosa cell function. The findings suggest bacterial endotoxins contribute to infertility by disrupting ovarian hormone signalling.

2.170. Gutzeit et al. (2025) used integrated human Cervix and Vagina Chips to demonstrate that cervical mucus protects against epithelial damage and inflammation induced by BV-associated dysbiosis. Proteomic analysis identified differentially abundant proteins, suggesting potential biomarkers and therapeutic targets for managing BV and preserving vaginal health.

2.171. Yu et al. (2024) developed fallopian tube (FT) organoids from patient tissue to study inflammatory responses to vaginal bacterial species relevant to pelvic inflammatory disease and tubal factor infertility. When exposed to Lactobacillus crispatus or Fannyhessea vaginae, FT organoids exhibited distinct inflammatory gene expression patterns, notably CXCL-family gene upregulation in response to F. vaginae. These responses originated from epithelial cells, not immune cells, indicating the organoids' utility for dissecting host-pathogen interactions in the upper reproductive tract.

2.172. Chenafi-Adham et al. (2024) reviewed the role of human papillomaviruses (HPVs) in infertility, highlighting their higher prevalence in the seminal fluid of men with idiopathic infertility. HPVs may impair male fertility by reducing sperm quality and inducing anti-sperm antibodies, while in women they are associated with increased miscarriage risk and impaired trophoblast implantation. Co-infections, particularly with Gardnerella vaginalis, and dysbiosis may exacerbate HPV-related fertility issues. The review also discusses prevention and treatment strategies, including vaccination.

2.173. The scoping review by Lindsay et al. (2023) synthesises findings from 76 studies on endometrial innate immune responses to bacterial and viral infections in human and animal models. It highlights cytokine and chemokine production in response to pathogens like E. coli and C. trachomatis, and identifies key knowledge gaps, including limited data on viral interactions and downstream effects on implantation and pregnancy. Emerging model systems (e.g. organoids, immune co-cultures) are proposed to advance mechanistic understanding.

2.174. Rivera et al. (2025) investigated the effects of bacterial toxin-induced Rho GTPase activation on human sperm structure and function. In vitro exposure to an E. coli-derived activator increased RhoA GTPase activity and intracellular calcium under non-capacitating conditions, while decreasing progressive motility and increasing non-progressive motility and acrosome reaction. Structural analysis showed more abnormal sperm morphology, including vacuoles in the sperm head. These findings suggest that bacterial infections disrupt key sperm functions via Rho

GTPase pathways, highlighting its potential as a biomarker for sperm quality in infection-associated infertility.

2.175. The preprint publication by Dohadwala et al. (2025) demonstrated that BV-associated sialidase enzymes remodel human sperm glycocalyx, increasing susceptibility to complement lysis, agglutination, and impaired cervical mucus transit. This damage, driven by Gardnerella vaginalis and Prevotella timonensis, suggests that BV-associated sialidases compromise sperm survival and function. The findings propose a mechanism by which BV may exacerbate adverse reproductive outcomes such as preterm birth and infertility.

2.176. Da Silva et al. (2025) examined the role of epididymal proton-secreting clear cells (CCs) in immune responses during LPS-induced epididymitis in mice, mimicking microbial invasion by Gram-negative bacteria. CCs shifted to a proinflammatory phenotype, upregulating cytokines and chemokines, downregulating sperm maturation genes, and undergoing morphological changes. These responses disrupted immune-epithelial interactions, reduced sperm motility, and caused epithelial damage, with decreased mononuclear phagocyte projections and increased dendritic cell and neutrophil activity. The study highlights how microbial signals like LPS can trigger epididymal immune activation via CCs, linking microbiome disturbances to male infertility and suggesting new diagnostic and therapeutic targets.

2.177. Wei et al. (2025) investigated the antioxidant icariin (ICA) as a potential treatment for obesity-induced male infertility in a high-fat diet-induced obese mouse model and TM3 cell studies. ICA significantly reduced body weight changes, pyroptosis (via NLRP3 inflammasome suppression), insulin resistance, and testicular spermatogenic dysfunction. In TM3 cells, ICA also mitigated inflammation and pyroptosis induced by LPS + Nig and insulin resistance, improving metabolic and reproductive markers. The study suggests ICA may improve male fertility by addressing inflammation and insulin resistance and calls for further investigation of ICA in relation to the gut microbiome.

2.178. Anton et al. (2025) used RNA sequencing to study how cervicovaginal epithelial cells respond to bacterial culture supernatants. Gardnerella vaginalis induced pro-inflammatory and pro-apoptotic gene expression, while Lactobacillus crispatus triggered transcriptional and epigenomic changes suggesting protective effects, including reduced chromatin accessibility. The study highlights how modulating the vaginal microbiome could improve reproductive health by influencing host–microbe interactions.

Methodological and translational considerations

Preclinical modelling platforms

2.179. Gulati et al. (2024) highlight the limitations of existing preclinical models for studying female reproductive tract diseases and introduce the human vagina chip as a promising organ-on-a-Chip platform. This model allows co-culture of primary human vaginal epithelial and stromal cells with microbial consortia under dynamic fluid flow, replicating key physiological and microbial interactions. The study provides a detailed protocol for its development and demonstrates its utility in modelling healthy versus dysbiotic vaginal environments.

2.180. Zhang et al., (2025a) evaluated three in vitro models for studying E. coli-induced endometritis in organoid systems: air-liquid interface (ALI), microinjection, and direct infection. They found that direct infection of endometrial organoids in suspension culture most closely mimicked ascending

bacterial infection, particularly in terms of epithelial adhesion, invasion, and barrier disruption. Compared to ALI and microinjection models, direct infection was more practical, cost-effective, and conducive to live observation, despite needing further refinement.

2.181. The review by Kaya et al. (2024) outlines the importance of accurate models of the female reproductive tract to study complex host-microbiota interactions relevant to disorders such as infertility, endometriosis, and cervical cancer. The paper evaluates the use of organoid and microfluidic technologies that replicate key features of the female reproductive tract, including epithelial differentiation, immune responses, hormonal cycling, and microbial dynamics. It also discusses the integration of patient-derived organoids with microfluidic platforms as an emerging strategy for personalised medicine and mechanistic discovery.

Sampling collection and handling

2.182. Turner et al. (2023) compared two self-collection methods for vaginal microbiome analysis in healthy volunteers, including tampons and lower vaginal swabs. The study found no significant differences in microbiome profiles between the two collection methods or between fresh and frozen samples. Additionally, tampons were highly acceptable to participants, with 100% willing to use them for sample provision and over half reporting routine use. The prevalence of dysbiosis was 42.9%.

2.183. Culturomics-based methods to analyse endometrial microbiota and correlate the results with ongoing pregnancy was investigated in a prospective cohort study by Cariati et al. (2023). Following embryo transfer, detection of bacteria by culturomics from catheter tips were considered as a method to detect pathogen growth. Using this approach, 68 (73.92%) patients tested positive for one or more microbes and 25 patients (26.08%) had no microbial growth. The testing methods was concluded to be reliable and offer a means to improve diagnosis and treatment strategies.

2.184. The prospective study by Koedooder et al. (2024) investigated the similarity between self-collected midstream urine and vaginal microbiota samples in subfertile women undergoing IVF/ICSI. Using intergenic spacer profiling, they found a high correlation ($R^2 = 0.78$) between sample types, though urine samples had reduced species richness. Vaginal sampling was deemed more representative, and the study supported the use of vaginal microbiota profiling over urine for predicting fertility treatment outcomes.

2.185. Gao et al. (2024b) conducted a prospective pilot study comparing patient- and physician-collected vaginal microbiome samples in women undergoing IVF/ICSI. Using the IS-Pro technique on 444 samples from 222 patients, the study found high similarity between sampling methods (mean cosine similarity ≥ 0.93) and no significant differences in microbial composition or species abundance. These findings validate self-sampling as a reliable, patient-friendly alternative for vaginal microbiome assessment in fertility care.

2.186. Tuddenham et al. (2024) examined whether vaginal microbiota composition measured by 16S rRNA sequencing is altered by room temperature shipping versus immediate freezing. Using self-collected samples preserved in two different stabilising solutions, they found complete concordance in CST classification and no significant differences in bacterial abundance or taxa-level composition between shipped and immediately frozen samples.

Diagnostic assay evaluation

2.187. Maldonado-Barrueco et al., (2024) assessed the diagnostic utility of the Allplex™ Bacterial Vaginosis Plus (ABVP) NAAT in 74 women with infertility, endometriosis, or recurrent pregnancy loss. While Lactobacillus and Gardnerella vaginalis were detected with moderate sensitivity and high specificity, no significant BV-associated bacterial differences were found. The ABVP assay showed limited diagnostic value for these conditions.

2.188. Tian et al. (2024) retrospectively evaluated the Vaginal Microecology Evaluation System (VMES) to monitor vaginal microbiome changes during IVF in tubal factor infertility patients. They found that controlled ovarian stimulation (COS) led to increased rates of bacterial vaginosis and dysbiosis, and that microbiome profiles before and after COS were independently associated with live birth and early miscarriage rates. VMES was predictive of IVF outcomes, supporting its potential clinical utility.

2.189. Polifke et al. (2024) compared vaginal and endometrial microbiomes in 71 IVF patients with implantation failure or recurrent pregnancy loss, using different 16S rRNA sequencing schemes. When comparing V1-V2 and V2-V3 rRNA sequencing, differences were observed for a small number of species, including Bifidobacterium sp., Propionibacterium sp. and Staphylococcus sp. Despite this rRNA sequencing schemes were otherwise consistent, confirming that endometrial microbiomes differ substantially from their vaginal counterparts – being significantly more diverse and enriched in species such as Corynebacterium, Staphylococcus, Propionibacterium and Prevotella.

2.190. Davidson et al. (2024) reviewed methodological variability in next-generation sequencing (NGS) studies of the female genital tract microbiome in ART contexts. Key inconsistencies were found across sample handling, DNA extraction, sequencing approaches, and bioinformatics, undermining reproducibility. Long-read sequencing offers promise, but standardisation is urgently needed to enhance clinical utility.

2.191. Lüll and Org (2024) reviewed the methodological challenges in studying the endometrial microbiome, noting that Lactobacillus dominance may reflect vaginal contamination rather than true uterine colonisation. A consensus on sampling techniques is lacking, complicating efforts to define a healthy endometrial microbial profile. The authors argue that standardising protocols is essential to reliably integrate endometrial microbiome insights into diagnostics and reproductive health management.

2.192. The review by Vanstokstraeten et al. (2024) discusses methodological challenges in studying the low-biomass microbiota of the upper female reproductive tract, particularly the endometrium. It critiques current sequencing methods due to contamination risks and highlights culturomics as a promising, though currently impractical, alternative for clinical use.

2.193. Yuan et al. (2024) reviewed the role of the endometrial microbiota in maintaining uterine health and its susceptibility to hormonal and physiological changes. Microbial imbalance is associated with uterine disorders such as endometritis and cancer, and future interventions may include antibiotics, probiotics, prebiotics, or microbiota transplantation. However, methodological limitations, such as contamination risks and lack of standardised evaluation protocols, impede current research progress.

3. Conclusions

3.1. Since this topic was last discussed in October 2023, publication of further research suggests that the relationship between the female reproductive tract microbiome and reproductive success is more nuanced that initially thought.

3.2. The evidence finds that a Lactobacillus-dominated microbiome, particularly enriched in L. crispatus, is consistently associated with favourable ART and natural fertility outcomes. However, emerging evidence suggests that reproductive success is not solely dependent on overall diversity or Lactobacillus presence, but also on the dominance of specific species and their functional roles. Furthermore, distinct microbial signatures linked to infertility-related pathologies, such as endometriosis and PCOS, are beginning to emerge.

3.3. Microbiome research has additionally expanded to consider the role of male reproductive tract, revealing that seminal and genital microbial communities may influence sperm quality, motility and fertility outcomes. This research indicates that both patients' microbiomes may play a role in infertility diagnostics and treatment outcomes.

3.4. Accumulating evidence supporting the role of gut microbiota and male/female reproductive health is beginning to elucidate the mechanistic pathways through which this occurs. This includes how systemic immune regulation, hormone balance and microbial metabolites influence reproductive outcomes, highlighting further potential avenues for diagnostic, predictive and therapeutic interventions.

3.5. Despite growing interest in the role of the reproductive tract microbiome in infertility, the evidence base remains experimental. With limited consensus on the microbial profiles that warrant clinical concern, the evidence supporting diagnostic testing and targeted treatments remains inconclusive. Some studies have reported positive effects of interventions (from biotic treatment to microbial transfer and dietary modulation); however, others have highlighted concerns regarding potential risks, including the disruption of beneficial microbiota and inconsistent efficacy.

3.6. To reliably integrate microbiome-targeted diagnostics and therapies into clinical fertility care, authors have called for further robust mechanistic research including independently validated trials. Despite this, commercial diagnostic tests that assess the endometrial and seminal microbiome (such as the EMMA and ALICE tests) are already being offered to patients in the UK by some clinics.

4. Recommendations

4.1. Members are asked to:

- Advise the executive if they are aware of any other recent developments;
- Consider the research findings and the quality of the evidence, drawing conclusions on what influence the microbiome may have on fertility treatment outcomes; and
- Review whether any outputs from the HFEA are required to address the implications of microbiome testing during fertility treatment.

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Health outcomes for ART patients (including gestational surrogates and egg donors)

Details about this paper

Area(s) of strategy this paper relates to:	Regulating a changing environment
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	7
Paper number:	HFEA (09/07/2025) 007
Meeting date:	09 June 2025
Author:	Dharmi Deugi, Scientific Policy Officer (HFEA)
Annexes	Annex A – A brief review and opinion of the evidence base on risks to gestational surrogates by Professor Stuart Campbell

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to: • Advise the executive if they are aware of any other recent developments. • Consider the research findings and the quality of the evidence and draw conclusions on any identified risks for patients undergoing ART, including for gestational surrogates and egg donors. • Review whether any outputs from the HFEA are required addressing health outcomes for ART patients (including gestational surrogates and egg donors).
Resource implications:	To be determined

Implementation date:	To be determined
Communication(s):	To be determined
Organisational risk:	Low

1. Introduction

1.1. Assisted reproductive technology (ART) includes techniques such as in vitro fertilisation (IVF), intra cytoplasmic sperm injection (ICSI) and intrauterine insemination (IUI). Fertility treatments are generally very safe – most women are no more likely to experience problems with their health or pregnancy than women who have conceived naturally. However, there are some risks, which range from mild discomfort to more serious conditions, such as Ovarian Hyperstimulation Syndrome (OHSS), or developing longer-term health issues. Research on this area investigates the risk of obstetric outcomes following ART, such as pre-eclampsia and hypertensive disorders of pregnancy, the relationship between fertility drugs and cancer incidence later in life, as well as psychological issues.

1.2. The **HFEA's Code of Practice** (Guidance Note 4.8 and 4.9) requires licensed clinics to provide certain information to patients undergoing fertility treatment about the treatment and associated risks. It specifically states that before treatment is offered, the centre should give a woman seeking treatment and her partner, if applicable, information about possible outcomes and risks of treatment. This includes the likely outcomes of the proposed treatment, potential immediate and longer-term risks of treatment and any treatment add-ons used. The patient(s) should also be informed of the nature and potential risks of any alternative treatment options available, as well as the risks of using emerging or unproven treatments. In addition, the centre should also provide information about the possibility of developing OHSS. The HFEA website provides information about the **risks of fertility treatment** for patients.

1.3. In 2001, the HFEA reviewed evidence of the association between the use donated gametes and embryos and the incidence of hypertensive problems and pre-eclampsia in pregnancy[1].

1.4. The topic of health outcomes for ART patients was added to the SCAAC's horizon scanning prioritisation as a high priority topic in **February 2025** following recent research on this topic, for example the Velez et al (2024) study that looks at morbidity in gestational carriers as well as wider media interest and parliamentary questions asking about the long term health impact of egg donation and surrogacy in recent years.

1.5. There are two ongoing research projects looking at the long-term effects of ART on patients using the **HFEA Register**, including; **Prolonged effects of ART on health of women and their children: a record linkage study for England (PEARL)** and **Associations between ART and women's mental health: an investigation using clinical data linkage**. One project investigating cancer risk in women after IVF has been completed (Williams et al., 2018).

1.6. As this is a newly introduced topic, the research highlighted in this paper has been published between January 2015 and May 2025. Where there was limited research published in the last 10 years, earlier studies have also been referenced. This paper provides a summary of the findings described in published literature and is not an assessment of study validity. A brief review and

[1] **HFEA Chairs Letter (2001)**

opinion of the evidence base on risks to gestational surrogates by Professor Stuart Campbell is also presented in Annex A.

2. Research findings

Risk factors associated with ART

2.1. A committee opinion published (2016) by the American College of Obstetricians and Gynaecologists (ACOG) addresses perinatal and obstetric risks associated with ART. It highlights that although these risks may be higher in multifetal gestations, even singletons achieved with ART and ovulation induction (OI) may have a higher risk than those naturally conceived. However, it remains unclear to what extent these associations might be related to the underlying cause(s) of infertility. The committee also emphasizes that underlying maternal health conditions and past obstetric complications may influence perinatal outcomes in ART pregnancies. Recommendations include completing a medical evaluation before ART or OI procedures to ensure patients are in good health, counselling women about the risks associated with treatment (especially risks associated with multifetal pregnancy and the option in such cases for multifetal reduction), adhering to guidelines recommending the transfer of a limited number of embryos, and addressing any maternal health problems or inherited conditions.

2.2. A commentary by (Smith et al., 2021) highlights that infertility treatments can elevate the risk of complicated pregnancies. Although such research has pointed towards higher risks in individuals with cardiovascular risk factors, it is less clear whether these risks are compounded in individuals with preexisting cardiovascular disease (CVD). The authors stress the importance of clinicians being informed about the cardiovascular implications of infertility treatments to support patient-centred reproductive decision-making. They also emphasize the need for further research in this area due to existing gaps in knowledge regarding the short and long-term cardiovascular implications of ART among individuals with and without CVD.

2.3. A review by (Guan et al., 2023) notes that women undergoing ART often have pre-existing cardiovascular risk factors such as obesity, hypertension, dyslipidaemia, and diabetes. Additionally, ART procedures, including ovarian stimulation and embryo transfer (ET) can increase cardiometabolic demands, especially in cases of multifetal pregnancies. The review highlights that ART may be associated with an increased incidence of adverse pregnancy outcomes like pre-eclampsia (PE), which are linked to both immediate and long-term cardiovascular complications. Notably, the risk of PE appears to be greater with frozen embryo transfers (FETs) compared to fresh ETs. Whilst current research suggests a correlation between ART and elevated CVD risk, the authors emphasize that causality has not been definitively established. They advocate for prospective and mechanistic studies to better understand this, and recommend comprehensive cardiovascular risk screening before and during pregnancy for women considering or undergoing ART. This approach provides an opportunity to implement preventive strategies and connect patients to long-term care aimed at managing cardiometabolic health.

Risk associated with IVF using autologous oocytes

2.4. A retrospective cohort study by (Farland et al., 2022) utilised data between 2004 and 2017 from Massachusetts state vital records linked to the Society for Assisted Reproductive Technology

Clinic Outcome Reporting System and hospital observational/inpatient stays. The study investigated the causes of hospitalisations up to 8 years after live birth among women who used ART, medically assisted reproduction (MAR), and unassisted sub fertile (USF) delivery and compared that to those with fertile delivery. Among 492,515 deliveries, 5.6% used ART, 1.6% used MAR, and 1.8% were USF. Compared with fertile deliveries, deliveries that used ART or MAR or were USF were more likely to have hospital utilization (inpatient or observational stay) for any reason for up to 8 years of follow-up. ART deliveries had an increased risk of hospitalization for conditions of the cardiovascular system, overweight/obesity, diabetes, reproductive tract, digestive tract, thyroid, respiratory system, and cancer. Furthermore, deliveries with MAR and subfertility had similar patterns of hospitalization as ART deliveries.

Obstetric/pregnancy related complications

2.5. A retrospective cohort study by (Lei et al., 2019) compared pregnancy complications between 2,256 conceptions by ART and 6,768 spontaneous conceptions (SC) over a 3-year period (2013-2015) at the Beijing Obstetrics and Gynaecology Hospital. After adjustment for maternal age, gravidity, parity, maternal education, smoking, alcohol consumption, and body mass index (BMI), pregnancies conceived by ART were associated with a significantly increased incidence of gestational diabetes, gestational hypertension, and intrahepatic cholestasis of pregnancy (ICP) compared with SC. These associations were similar for the singleton group. In the twin group, only the incidence of ICP was significantly higher than in controls. The study found that pregnancies conceived by ART were associated with perinatal complications, including placental abruption, premature rupture of membranes (PROM), postpartum haemorrhage (PPH) and polyhydramnios. The singleton group had a similar result with PA, but not with foetal membranes ruptures before labour and polyhydramnios. There were no significant differences in the incidence of these perinatal complications in the twin group. Compared with the control group, significantly increased incidences of gestational hypertension, GDM, ICP, PROM, PPH, were observed in both FET subgroups. Fresh subgroups showed a higher incidence of gestational hypertension, GDM, ICP, and PPH than the control group. The authors argue that patients who underwent ART were at increased risk of several adverse pregnancy outcomes compared with SC.

2.6. A meta-analysis by (Qin et al., 2016) looked at 50 cohort studies to determine whether there are any increases in pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies after ART (n = 161,370) compared with natural conception (NC, n = 2,280,241). The ART singleton pregnancies had a significantly increased risk of pregnancy-induced hypertension (PIH), gestational diabetes mellitus (GDM), placenta previa, antepartum haemorrhage and PPH. Sensitivity analysis yielded consistent results concluding that the ART singleton pregnancies are associated with higher risks of adverse obstetric outcomes.

2.7. A retrospective cohort study by (Singh et al., 2022) included 1,125 IVF conceived singletons (AP group) and 7,193 SC singletons (SP group), from a single tertiary infertility centre (2011-2020). Maternal outcomes like gestational hypertension, PE, GDM, oligohydramnios, chorioamnionitis, operative and instrumental delivery were significantly different in the two groups ($p<0.05$). The AP group had a significantly increased risk of GDM and PIH as compared to the SP group. IVF significantly increases the risk of abruption by 2 times ($p=0.028$) and independently increases the risk of caesarean section by 3.1-fold ($p<0.001$).

2.8. A retrospective multicentre cohort study from 2019 to 2021 by (Salmanov et al., 2023) compared the risk of maternal complications in pregnancies conceived by ART (n = 1,361) with those conceived naturally (19,801) across 14 Women's Hospitals from 8 regions of Ukraine. Compared to natural pregnancies, statistically significant increases were noted in GDM, PE, thyroid-related diseases, placenta previa and PPH in ART pregnancy. The occurring rates of anaemia and liver-related diseases were also elevated in ART pregnancy compared to natural conception, but with no statistically significant difference. The authors concluded that women who conceived by ART were at increased risks of several adverse pregnancy outcomes compared with women who conceived naturally.

2.9. A systematic review and meta-analysis by (Chih et al, 2021) included 85 studies to evaluate the association between ART and hypertensive disorders of pregnancy (HDP) or PE relative to spontaneous conception (SC). Numbers needed to harm (NNH) were calculated based on absolute risk differences between exposure and control groups. Compared to SC, IVF/ICSI singleton pregnancies and multiple pregnancies were both associated with higher odds of HDP. Singleton pregnancies with oocyte donation (OD) had the highest odds of HDP out of all groups analysed. FET resulted in higher odds of HDP than fresh embryo transfer. The associations between IVF/ICSI pregnancies and SC were similar for PE. Most interventions had an NNH of 40 to 100, while singleton and multiple oocyte donation pregnancies had particularly low NNH for HDP (16 and 10, respectively). The authors argue that IVF/ICSI pregnancies are at higher odds of HDP and PE than SC, irrespective of the plurality. The odds were especially high in FET and OD pregnancies.

Risk of cancer

Risk of reproductive cancers

2.10. A review of meta-analyses by (Saso et al., 2025) included 11 meta-analytical reviews consisting of 188 studies to assess the validity of the association between the development of female-specific malignancies including ovarian, endometrial, breast, and cervical cancer after fertility treatment (FT: controlled ovarian stimulation [COS] and/or in IVF or ICSI). A statistically significant increase in incidence of ovarian cancer and borderline ovarian tumours was observed. The incidence of ovarian cancer was higher with FT and IVF specifically. For borderline ovarian tumours, the incidence was higher, not only with FT overall and IVF, but also according to the fertility drug regimen applied: clomiphene citrate (CC) only, human menopausal gonadotropin only, and CC and human menopausal gonadotropin combined. When using the threshold for statistical significance, the meta-analyses relevant to ovarian cancers remained statistically significant. However, none of the examined associations could claim either strong or highly suggestive evidence.

2.11. A registry-based cohort study by (Sandvei et al., 2023) compared ovarian cancer risk for women who gave birth after ART vs natural conception (NC). Through linkage of nationwide registry data, the study followed 3,303,880 initially nulliparous women in Denmark (1994-2014), Finland (1990-2014), Norway (1984-2015) and Sweden (1985-2015) from first pregnancy ≥22 weeks to ovarian cancer, emigration, death or end of follow-up (2014/2015). After adjusting for age, parity, maternal birth year and country, and for body mass index and smoking in subsamples, 2,683 participants developed ovarian cancer (135 after ART and 2,548 after NC (incidence rates 11.6 and 5.5 per 100,000 person-years, respectively)) during a mean follow-up of 14.4 years. The risk was higher

for women who gave birth after ART compared to NC. Associations were stronger for conventional IVF than for ICSI.

2.12. A systematic review and meta-analysis by (Gennari et al., 2015) of 20 population-based cohort studies assessed the potential association between hormonal infertility treatments (HITs) and breast cancer (BC) risk, by analysing BC incidence in women undergone HITs. Subgroup analyses were performed by type of intervention (IVF vs. NO IVF), follow-up duration (<10 vs. >10 years), and type of control (population vs. infertile). In the seven studies with the IVF procedure, no increase in BC risk was observed, in the three NO IVF studies, an increased BC risk was identified. A borderline interaction between type of intervention (IVF vs. NO IVF) and BC risk was observed. An increased risk with longer follow-up (≥10 vs. <10 years) was detected. Authors argue that although HITs are not associated with an increased BC risk, this cannot be ruled out with older treatment protocols based on long-term administration of clomiphene.

2.13. A large, population based, data linkage cohort study by (Williams et al., 2018) used HFEA data on women who had ART in Great Britain from 1991 – 2010 to investigate the risks of ovarian, breast, and corpus uteri cancer. HFEA fertility records were linked to the NHS Central Registers of England, Wales, and Scotland and SIRs were calculated by use of age, sex, and period specific national incidence rates. 255,786 women contributed. No significant increased risk of corpus uteri cancer was found during an average of 8.8 years' follow-up. This study found no significantly increased risks of BC overall. An increased risk of in situ BC was detected, associated with an increasing number of treatment cycles. There was an increased risk of ovarian cancer, both invasive and borderline. Increased risks of ovarian tumours were limited to women with endometriosis, low parity, or both. It was concluded that no increased risk of corpus uteri or invasive BC was detected in women who had ART, but increased risks of in situ BC, and invasive and borderline ovarian tumours (possibly linked to patient characteristics) were found in this study.

Kidney dysfunction

2.14. A case series study by (Nagy et al., 2025) gathered all consecutive patients (from 2021 to 2024) followed by Mansoura University Hospital's Obstetric Nephrology Service or admitted to its Nephrology and Gynaecology Department during pregnancy with a diagnosis of acute or chronic kidney function (CKD) impairment after conceiving with ART. Of approximately 150 pregnancies referred to Obstetric Nephrology, 6 were referred for acute or acute-on-chronic kidney function impairment, or nephrotic syndrome after conceiving via ART. In one patient, CKD was overlooked and later progressed to kidney failure; one had probable CKD, but discontinued follow-up before confirmation; and one had a kidney malformation, diagnosed during pregnancy. All presented with early or very early severe hypertension and proteinuria, before 20 weeks, while pre-eclampsia and the hypertensive disorders of pregnancy are conventionally defined as developing after 20 weeks of gestation. The study concluded that severe early-pregnancy kidney impairment after ART is probably more frequent than previously reported.

Fresh versus frozen embryo transfer

2.15. A retrospective cohort study by (Zhang et al., 2021) assessed perinatal and maternal outcomes after autologous FET (n = 1663) in comparison to fresh ET (n = 3964) in women of advanced maternal age (AMA) with ≥35 years in reproductive medical centres from 2009 to 2014. Women

who underwent FET had an increased risk of hypertensive disorders of pregnancy (HDP) [1.1 % vs. 0.4 %, adjusted OR (95 % CI): 2.76 (1.39-5.51); p = 0.004].

2.16. A retrospective study by (Zhang et al., 2024) compared maternal outcomes such as, pregnancy induced hypertension (PIH), PE, GDM, placental abruption (PA), placenta accreta spectrum (PAS) and post-partum haemorrhage (PPH) between fresh ET, FET, and SC groups. FET was associated with higher risks for PIH, PE, PAS and PPH. Fresh ET was associated with higher risks for PA, and fresh ET is an independent risk factor for PA. Embryo status (fresh or frozen) is a key factor affecting the maternal outcomes in ART treatments.

2.17. A systematic review and meta-analysis by (Roque et al., 2018) including 6 studies examined the obstetric outcomes in IVF/ICSI singleton pregnancies after FET and fresh ET. When comparing pregnancies that arose from FET or fresh ET, there was an increase in the risk of obstetric complications in pregnancies resulting from FET when compared to those emerging from fresh ET, including in PIH, PE, and placenta accreta. There were no significant differences in the risk between the FET and fresh ET groups when evaluating placenta previa.

Embryo developmental stage and cryopreservation method

2.18. A retrospective study by (Onogi et al., 2022) analysed the impact of developmental stage and cryopreservation method of transferred embryos on maternal and obstetric outcomes in a large single-centre cohort of women (n = 36,827) who underwent oocyte retrieval followed by their first ET between 2008 and 2017. Patients underwent a single fresh cleavage-stage ET (SFCT), single vitrified-warmed cleavage-stage ET (SVCT) or single vitrified-warmed blastocyst transfer (SVBT). Pregnancy complications were analysed in 7,502 singleton births (SFCT, 3,395 cycles; SVCT, 586 cycles; and SVBT, 3,521 cycles). Multivariate logistic regression analysis revealed that the adjusted odds ratio (aOR) for hypertensive disorders in pregnancy was significantly lower in the SVBT group than in the SFCT group. The aOR for low-lying placenta was lower in the SVBT group than in the SFCT group. The aOR for placenta previa was lower in the SVCT and SVBT groups than in the SFCT group. This study demonstrates reassuring outcomes for SVBT (in terms of a lower incidence of pregnancy complications) compared to SFCT.

Endometrial preparation method for FET

2.19. A retrospective by (Li et al., 2023) analysed obstetric outcomes in different endometrial preparation methods for frozen-thawed ET. Endometrial preparation during FET was performed in the natural cycle (NC) with timing based on monitoring of the naturally occurring luteinizing hormone (LH) peak or in human chorionic gonadotropin (hCG)-triggered modified natural cycles (MNC), artificial cycle (AC) with hormone replacement therapy cycle and cycle with ovulation induction (OI). After adjusting for the effect of gravidity, parity, pre-pregnancy BMI and number of miscarriages, the AC group had higher but not significantly different rates of gestational hypertension, PE and intrahepatic cholestasis of pregnancy (ICP) than women in OI and MNC groups. Significant differences were observed in the rates of placental adherence and post-partum haemorrhage (PPH) (24.33% in AC vs. 13.07% in OI, p = 0.003, 24.33% in AC vs. 16.24% in MNC, p = 0.002) among the three groups. In singletons, significant differences were observed in the rates of placental adherence (14.09% in AC vs. 8.57% in MNC, p = 0.002), AC and MNC groups had higher risk of PPH compared with the OI group (18.36% in AC vs. 12.38% in MNC, p = 0.042 and 7.69% in OI vs. 18.36% in AC, p = 0.013).

Multiple pregnancies

2.20. A meta-analysis by (Qin et al., 2015) looked at 39 cohort studies involving 146,008 multiple births to study pregnancy-related complications and outcomes in multiple pregnancies resulting from ART vs. natural conception (NC). Multiple pregnancies from ART were associated with a higher risk of premature rupture of membranes, with sensitivity analysis yielding similar results. Authors conclude that multiple pregnancies generated via ART, may be associated with higher risks of pregnancy-related complications and adverse pregnancy outcomes.

2.21. A retrospective population-cohort analysis by (Arian et al., 2021) used natality data from the US National Center for Health Statistics (2015-2017) to compare maternal outcomes among twin pregnancies conceived as result of different types of fertility treatments (OI, IUI, ICSI, IVF; n = 24,411) with those of spontaneous conception (SC, n = 152,951) twin pregnancies. Maternal risks of gestational hypertension and GDM were significantly higher in the OI/IUI and ART groups compared with the SC group. The overall prevalence of early adverse maternal outcomes, medical complications and obstetric complications including the risk of unplanned hysterectomy, intensive care unit admission, maternal blood transfusion, and perineal laceration were also significantly higher in the OI/IUI and ART groups compared with the SC group, even after adjusting for several potential confounders.

2.22. A retrospective analysis by (Gulersen et al., 2022) using the US Centers for Disease Control and Prevention (CDC) Natality Live Birth database (2016-2019) compared pregnancy outcomes in twin pregnancies conceived by IVF (n = 39,356) to SC (n=376,204). Compared to spontaneously conceived twin pregnancies, those conceived by IVF were associated with an increased risk of GDM, hypertensive disorders of pregnancy (HDP), maternal intensive care unit admission, maternal blood transfusion, and unplanned hysterectomy. The study concludes that IVF twin pregnancies represent a subgroup of twins with an increased risk of several adverse pregnancy outcomes, compared to those conceived spontaneously.

Cross border reproductive care

2.23. A retrospective chart review including 4,457 deliveries at a tertiary public hospital from February 2023 to January 2024 by (Rodriguez et al., 2025) compared maternal outcomes from IVF pregnancies conceived domestically in the US and through cross-border reproductive care (CBRC) mainly in Central and South America (including Panama, Dominican Republic, Brazil and Colombia). Among all deliveries, 95 were conceived via IVF, out of which 24.2% were conceived through CBRC. The incidence of HDP were significantly higher in CBRC pregnancies compared to domestic IVF pregnancies ($p < 0.05$). Trends toward increased rates of PPH and multiple gestations were also observed in the CBRC group, although were not statistically significant. The study highlights a significantly higher percentage of hypertensive disorders among CBRC pregnancies, while other maternal and outcomes were comparable to domestic IVF pregnancies.

2.24. A study by (Jaspal et al., 2019) determined whether women (n = 65) from a single large tertiary centre seeking NHS care for IVF multiple pregnancies were more likely to have sought IVF treatment overseas and whether this was associated with different maternal and neonatal outcomes. 38 women who were older and had more pre-existing medical conditions had IVF overseas. 11 pregnancies used donor embryos, of which 10 were from overseas treatment. 75% of women treated overseas conceived a triplet or higher order pregnancy compared to fewer than 10% of women who conceived in the UK. Almost half of all women treated overseas had more

than two embryos transferred. Overseas IVF pregnancies had poorer obstetric and neonatal outcomes: 24% of live born babies died in the neonatal period compared to 0% in the UK group. The average neonatal costs per baby born from overseas IVF were £20,600 which is 2.5 times higher than for those whose mothers conceived in the UK. Higher order multiple pregnancies are greatly over-represented by those undergoing IVF in overseas clinics. These are associated with poorer obstetric and neonatal outcomes. Improving NHS provision of fertility services might improve outcomes for the mother and babies while reducing the long-term burden to both fertility patients and the NHS.

Advanced maternal age

2.25. A retrospective analysis by (Yuan et al., 2025) utilised propensity score matching (PSM) on 128 cases in the IVF group and 196 cases in the natural conception (NC) group, selected from the Second Nanning People's Hospital between January 2020 and December 2023 to compare pregnancy outcomes in singleton pregnancies among nulliparous women of advanced maternal age (AMA). Before matching, early pregnancy haemoglobin (HB) levels, and GDM incidence was higher in the IVF group compared to the NC group, with statistically significant differences ($P < 0.05$). However, there were no statistically significant differences in late pregnancy HB, HDP, placenta previa, and placental abruption (PA) between the two groups. Following matching, there were no statistically significant differences in early and late pregnancy HB, GDM, HDP, placenta previa, and PA between the two groups ($P > 0.05$).

Research on IVF using donated material

IVF using donor eggs

Obstetric/pregnancy-related complications

2.26. A study protocol for a prospective multicentre cohort study (Bentem at al., 2019) aims to study the association between a high number of human leucocyte antigen (HLA) mismatches between foetus and mother and the development of HDP, including pregnancy induced hypertension (PIH) and PE. High number of HLA mismatches is defined as ≥5 foetal–maternal HLA mismatches based on discrepancy on the HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ antigens. Secondary objective is the association of high number of HLA mismatches and the severity of PE, time to the development of PE and development of other pregnancy complications, including spontaneous miscarriage, haemolysis elevated liver enzymes and low platelet count (HELLP), GDM and (severe) preterm birth. The design includes women (n = 200) with pregnancies conceived via oocyte or embryo donation or surrogacy and, women with either naturally conceived (n = 146) and non-donor IVF pregnancies (n = 146), matched for age and ethnicity. Expected results include a higher degree of pregnancy complications in OD pregnancies compared with IVF and NC, a higher number of HLA mismatches between mother and foetus in OD pregnancies compared with IVF and NC pregnancies. Also expected is an association between the development of hypertensive pregnancy complications and a higher number of HLA mismatches, and a higher number of HLA mismatches between mother and foetus in women who conceived through OD with severe hypertensive complication, as well as the development of severe PE at an earlier gestational age.

2.27. A retrospective study conducted by (Tarlatzi et al., 2017) at the Vrije Universiteit Brussel (VUB) Erasme Fertility Clinic evaluated whether pregnancies resulting from oocyte donation (OD) have a higher risk of obstetric complications compared with autologous oocyte IVF pregnancies. Propensity score matching on maternal age and parity was carried out to compare 144 OD singleton pregnancies resulting in delivery beyond 22 gestational weeks, with 144 pregnancies achieved through IVF and ICSI with autologous oocytes. All pregnancies were achieved after fresh ET. Singleton pregnancies after OD were associated with a significantly higher risk for PE, PIH and caesarean delivery ($p < 0.05$), compared with pregnancies using autologous oocytes.

2.28. A meta-analysis by (Blázquez et al., 2016) determined whether there is a higher incidence of PE in pregnancies achieved by OD compared with IVF autologous oocyte pregnancies. The systematic review included 11 retrospective and prospective cohort studies of women reporting results on the association between OD vs. IVF and PE. The results showed that OD is a risk factor for the development of PE compared to IVF cycles, with a weighted OR of 3.12 under a fixed effects method (FEM: no heterogeneity between the studies). The meta-regression analysis showed that neither multiple pregnancies nor patient age significantly explained the variability of the effect of oocyte donation on PE. The study concludes that OD pregnancies confer a threefold increase in the likelihood of developing PE than those achieved by own oocyte IVF.

2.29. A retrospective cohort study by (Modest et al., 2019) compared the risk of ischemic placental disease (IPD), PE, PA, small for gestational age (SGA), or intrauterine foetal demise due to placental insufficiency between donor (n = 262) and autologous IVF (n = 3,501) pregnancies with non-IVF pregnancies (n = 35,321), as well as donor IVF pregnancies with autologous IVF pregnancies (2000-2015). SGA was defined as birthweight < 10th percentiles for gestational age and sex. Compared with non-IVF pregnancies, IPD was more common among donor IVF pregnancies and autologous IVF pregnancies, adjusted for age and parity. IVF pregnancies were more likely to be complicated by preeclampsia, placental abruption and SGA.

Risk of cancer and other longer-term diseases

2.30. A retrospective cohort study by (Klement et al., 2024) evaluated the association between women conceiving through OD and future cancer-related morbidity, as compared with women (matched for age and number of children) conceiving through IVF with autologous oocytes (AO), SC, and nulliparas (664 OD to 664 AO, 700 OD to 700 SC, and 700 OD to 700 nulliparas). Cancer-related morbidity rates were comparable between OD and other groups, but compared with nulliparas, a trend was noted. Survival analysis curves were not significantly different, although a trend was shown in the curve comparing to nulliparity ($p = 0.07$). In a Cox regression model corrected for BMI, smoking and HRT exposure, cancer in the OD group did not differ compared to other groups. It was concluded that women conceiving through OD do not have increased risk for cancer-related morbidity in the decade following delivery.

2.31. A retrospective observational study by (Fassio et al., 2019) assessed the importance of kidney function and maternal comorbidity in egg donation (ED, n = 296 singleton pregnancies who delivered between 2008-2019) pregnancies in comparison to 1,407 low-risk singleton pregnancies delivered between 2009-2016. Logistic multiple regression analysis tested: PE; pregnancy induced hypertension (PIH); preterm delivery; small for gestational age; explicatory variables: age; BMI; parity; comorbidity (kidney diseases; immunologic diseases; thyroid diseases; other). In keeping with ED indications, maternal age was high (44 years). Comorbidity

was common: at least one potential comorbid factor was found in about 40% of cases (kidney disease: 3.7%, immunologic 6.4%, thyroid disease 18.9%, other-including hypertension, previous neoplasia and all other relevant diseases-10.8%). No difference in age, parity and BMI was observed in ED women with and without comorbidity. Patients with baseline renal disease or "other" comorbidity had a higher risk of developing PE or preterm delivery after ED. PE was recorded in 23% vs. 9%, OR: 2.513 (CI 1.066-5.923; p = 0.039); preterm delivery: 30.2% vs. 14%, OR 2.565 (CI: 1.198-5.488; p = 0.044). Limiting the analysis to 124 cases (41.9%) with available serum creatinine measurement, higher serum creatinine was correlated with risk of PE and preterm delivery. This study suggests that the risk of PE after ED is modulated by existing comorbidities. While the cause effect relationship is difficult to ascertain, the relationship between serum creatinine and outcomes suggests that more attention is needed to baseline kidney function and comorbidity.

Multiple pregnancies

2.32. A secondary analysis of the observational, prospective, population-based cohort study of twin pregnancies (JUmeaux Mode d'Accouchement) by (Korb et al., 2020) compared the risk of serious maternal complications (regrouped within severe acute maternal morbidity (SAMM): non-severe pre-eclampsia (PE), placenta praevia and planned mode of delivery) during pregnancy and the postpartum in twin pregnancies according to mode of conception (NC, non-IVF fertility treatment, including IUI and ovarian stimulation, IVF, ICSI or OD). Data was collected from 176 French maternity units (n = 8,748) among which 67.3% conceived naturally, 9.8% had non-IVF fertility treatment, 14.9% had IVF with AO, 4.2% had ICSI with AO and 3.8% used OD. Overall, 538 (6.1%) developed SAMM. Women with twin pregnancy after any type of MAR had a higher risk of SAMM than those with a natural twin pregnancy, after adjustment for confounders. This association varied according to MAR procedure. The risk of SAMM was higher among women with IVF using either AO or OD compared with the reference group and higher after OD compared with AO. Conversely, the risk of SAMM for women with non-IVF fertility treatment and with ICSI using AO did not differ from the reference group. The study showed an increased risk of SAMM in women with twin pregnancies after MAR, notably after IVF using autologous oocytes and particularly after oocyte donation.

IVF using donor sperm

2.33. A systematic review and meta-analyses by (Pohjonen et al., 2022) investigated whether use of donor sperm in IUI, in IVF or in ICSI treatments affect maternal risks compared with SC or use of partner sperm in IUI, IVF or ICSI. The results demonstrated an increased risk for pre-eclampsia (PE) and hypertensive disorders of pregnancy (HDP) in pregnancies resulting from IUI with donor sperm compared with IUI with partner sperm. For HDP and PE, no difference was found between IVF with donor sperm vs. partner sperm.

2.34. A retrospective cohort study by (Kennedy et al., 2019) compared the risk of HDP among IVF pregnancies conceived with autologous gametes (own eggs and partner sperm) and those conceived with donor sperm, donor egg (and partner sperm) and donor embryo within Australia between 2009 and 2017. The final cohort contained 1,435,578 and 239 pregnancies conceived by donor sperm, donor egg, and donor embryo, respectively, and 13,191 controls. Compared to control pregnancies, there was no increase in the risk of HDP among pregnancies conceived via

donor sperm. Subgroup analysis was performed for a cohort where parity was known (n = 4551), and of these, 305 multigravida pregnancies were conceived via donor sperm. Among this cohort, no increased risk of PE or pregnancy induced hypertension (PIH) was found. A significantly increased risk for HDP was associated with donor egg use. However, the association was no greater among pregnancies conceived with donor embryos than among the donor oocyte group. These findings suggest that exposure to new sperm may not be implicated in the pathogenesis of preeclampsia.

Research on egg donors

2.35. A retrospective cross-sectional survey by (Söderström-Anttila et al., 2016) included women who had donated oocytes between 1990 and 2012 at three fertility clinics. The study used a self-administered questionnaire with 75.2% response rate with a mean follow up time of 11.2 years and a mean respondent age of 42 years. Immediate complications occurred in 7.2% (42/582) of the donation cycles and the most common complication was OHSS in 5.0% of the donations. There were no reports of ovarian or uterine cancer and only one case of breast cancer. After the donation, 11.5% of the donors experienced unsuccessful attempts to become pregnant.

2.36. A retrospective survey by (Tober et al., 2023) investigated the experiences of US oocyte donors (n = 420) with OHSS and possible correlations between OHSS severity and number of oocytes retrieved, trigger type, and prior OHSS history (between February 2019 and September 2020). On cycles where donors reported receiving gonadotropin-releasing hormone (GnRH) agonist triggers (n = 337), they reported milder OHSS compared to cycles with human chorionic gonadotropin (hCG) or dual triggers. Among donors undergoing multiple retrieval cycles, the severity of OHSS in second cycles was strongly associated with OHSS severity in first cycles. It was concluded that, self-reported OHSS in oocyte donors is lower in GnRH antagonist stimulation protocols combined with GnRHa trigger and in cycles where donors reported fewer than 30 oocytes retrieved. Donors who reported severe OHSS on a prior cycle were significantly more likely to experience severe OHSS on a subsequent cycle.

2.37. A retrospective study by (Kramer et al., 2009) investigated medical and psychological issues of US based oocyte donors (n = 155) through an online questionnaire completed on average 9.4 years after first donation. Questions asked about medical complications, contact with the clinic used, and information exchange or contact with people conceived from their eggs. Reported medical complications were OHSS (30.3%) and infertility (9.6%). Following egg donation, 2.6% of women reported contact from the IVF clinic for medical updates. On the questionnaire, 34.2% of women reported medical changes they thought would interest donor children; half said that they had attempted to report these changes to the clinic with variable results. Donors had frequently not sought information about pregnancy outcomes because of confusion around 'anonymity' or 'confidentiality'.

2.38. A cross-sectional mixed methods survey by (Adlam et al., 2025) evaluated physical outcomes and psychosocial experiences of oocyte donors (n=363) after donation across 3 age cohorts (ages: 22-71 years, M = 38.8). Most donors (89.5%) reported a positive overall experience. Self-reported physical outcomes, including changes to menstrual cycles, ovulation, or fertility, were reported by 21% of participants after donation. 41.4% reported procedural pain, and 10.5% reported OHSS. Anxiety (25.8%) and depression (23.2%) were the most common self-reported

diagnoses. Participants screened clinically significant rates of alcohol/drug misuse, with 50% of those reporting depressive symptoms. Anonymity was the most common qualitative response for reported emotional distress (17%) and regret (20%). The authors argue that most participants felt their oocyte donation experience was positive despite reported pain, menstrual cycle changes, and emotional distress. Depression and anxiety were the most common self-reported diagnoses. Alcohol/drug misuse was associated with depression, indicating the importance of screening oocyte donors for mental health and drug/alcohol misuse. Concerns included lack of communication after procedure and lack of information provided on long-term health outcomes indicating that clinicians can incorporate this when counselling this population.

2.39. A cross-sectional study by (Gonzalo et al., 2019) evaluated the health of oocyte donors and their experience through a telephone interview guided by a semi-structured questionnaire covering several aspects of reproductive health and personal experience. Of the 38 women achieving a pregnancy after donation, five reported six pregnancy complications. Two out of 121 (2%) women reported being in menopause (aged 41 and 45). Twenty-three women (19%) reported gynaecological issues and eight (7%) reported fertility problems, although only four consulted a specialist. Most of the women highlighted positive feelings about their donation (93%) and 155 (97%) would recommend donating. Less than half (44%) mentioned some negative aspects, mainly related to physical discomfort: injections (17%), pain (14%), and side effects of ovarian stimulation (8%). The impact of donation on women's life was mostly favourable, with the majority of participants reporting positive aspects and recommending donation, although some negative feelings as physical discomfort also arose. Therefore, more comfortable stimulation protocols could be developed.

2.40. A committee opinion published (2020) by the Practice Committees of the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology, Birmingham, Alabama highlights the various possible risks oocyte donors are exposed to including OHSS, acute procedural risks, such as pelvic infection and ovarian torsion, ovarian and breast cancer, as well as future ovarian reserve and psychological issues. Although there are no clearly documented long-term risks associated with oocyte donation, and as such, no definitive data upon which to base absolute recommendations, given the possible cumulative risks to and future needs of an individual donor, the committees recommend that it may be reasonable to limit the number of stimulated cycles for a given oocyte donor to no more than six.

Risk of breast cancer

2.41. A commentary by (Schneider et al., 2017) emphasises the need to create egg donor registries to facilitate long-term studies on egg donors. Until this information is available, the authors call for more realistic explanations to egg donors about the lack of knowledge of long-term risks as well as more transparent informed consent documents.

2.42. A commentary by (Fauser and Valesco 2017) addresses concerns about a potential link between oocyte donors (OD) and BC risk, with authors stating that current research presents conflicting evidence and is limited by for example inclusion bias. Furthermore, large-scale studies on women undergoing IVF have not demonstrated a significant increase in BC risk. In conclusion, the authors note with some degree of confidence that the data currently available and the overall risk calculations do not support increased BC risk in OD, and that sufficient and high-quality registry data of oocyte donor follow-up are required to generate more robust information.

Psychological outcomes

2.43. A systematic review by (Moghaddam et al., 2021) studied the psychosocial consequences of oocyte donation in donors across 14 studies. Psychosocial challenges of donors were obtained in three dimensions including short and long-term psychological reactions to treatment complications, emotional reactions to their function as an oocyte donor, and emotional reactions to the resulting offspring resulting and related social challenges. According to the existing studies, oocyte donation is a challenging process with short and long-term psychosocial consequences for donors, concluding that in order to prevent the feasible psychosocial hazards caused by the donation process, it is necessary to provide oocyte donors with psychosocial support, proper counselling, and awareness of the facts and possible issues ahead.

2.44. An anonymous survey study by (Blakemore et al., 2019) assessed the experiences and psychological outcomes of 36 oocyte donors from one fertility centre who underwent oocyte donation (anonymous or directed/known) between 2008 and 2019 at the NYU Langone Fertility Center. Majority of the respondents were between 2 and 10 years since donation and reported a high prevalence of psychiatric symptoms or diagnoses post-donation. The majority of donors reported positive thoughts and feelings toward their donation process as well as to the knowledge of children born from their donation. Negative comments about donation were in the minority but focused on unexpected aspects about the process or outcome. The authors concluded that despite a high reported prevalence of psychiatric symptoms, the majority of respondents felt positively about the donation experience.

2.45. A prospective study by (Kazemi et al., 2016) examined psychiatric symptoms in 63 oocyte-donating women compared to 63 women providing their own oocytes for IVF (in couples with male infertility). They were evaluated pre- and post-ovulation-induction in regard to hypochondriasis, anxiety, social impairment, and depression. The mean hypochondriasis score for oocyte-donators was significantly lower than for women providing their own oocytes, prior to ovulation-induction (5.03 vs. 6.59). However, after ovulation-induction and oocyte retrieval this score rose to 6.66 among oocyte-donors, whereas it remained essentially unchanged among women providing their own oocytes. The mean anxiety score for oocyte-donating women also rose following this procedure, from 5.87 to 7.65. Depression scores for both groups remained similar, before and after the procedure. Results showed that at the beginning of the Assisted Reproduction Program (ARP) donating women have the same conditions as own oocyte women regarding depression and anxiety but after egg collection, they suffered more damages regarding hypochondriasis and anxiety aspects.

Research on gestational surrogates

2.46. A narrative review by (McCoy et al., 2024) applies evolutionary theory to explain how and why pregnancy is riskier with an unrelated embryo, including risk of pre-eclampsia (PE) and other diseases due to a special immune challenge. This is because the unrelated embryo and gestational carrier have fewer matching genes—therefore exacerbating symptoms of evolutionary maternal-foetal conflict. Hypertensive disorders and various placental pathologies are more likely in young and healthy carriers of unrelated embryos. The authors also discuss micro chimerism in egg donation pregnancies, whereby wholly foreign cells pass from mother to

embryo and vice-versa, concluding that surrogates and egg donors should be told of the increased risks they face compared to other similarly fertile, young women.

2.47. A systematic review by (Phillips et al., 2019) included 36 studies examining the maternal and perinatal outcomes of surrogate pregnancies. It was found that maternal complications associated with surrogate pregnancies include hypertensive disorders of pregnancy, postpartum haemorrhage, and gestational diabetes, highlighting that surrogacy as a route to parenting is not without risk to the surrogate.

Obstetric/pregnancy related complications

2.48. A retrospective cohort study by (Woo et al., 2017) compared maternal outcomes between singleton live births achieved with commissioned versus spontaneously conceived embryos carried by the same gestational surrogate (n = 124). In comparison to spontaneous births (n = 249), surrogate births (n = 103) had significantly higher obstetrical complications, including GDM, hypertension, antibiotic requirement during labour, and PPH.

2.49. A retrospective cohort study by (Peters et al., 2018) used data from the VU Medical Centre Amsterdam over a 10-year period to analyse reproductive and obstetric outcomes in 63 gestational carriers. These women underwent a total of 184 single embryo transfers using analogous oocytes (AO) from 60 intended mothers resulting in 35 ongoing singleton pregnancies. 20.6% of pregnancies were complicated by HDP, 52.9% had induced labour, caesarean delivery (CD) rate was 8.8% and PPH occurred in 23.5%. Authors conclude an increased risk for adverse obstetric outcomes in surrogate mothers for hypertensive disorders and post-partum haemorrhage compared with non-surrogacy pregnancies.

2.50. A population-based cohort study by (Velez et al., 2024) determined the risk for severe maternal morbidity (SMM) in a group of singleton births of which 97.6% were by unassisted conception, 1.8% by IVF, and 0.1% by gestational carriage. Secondary outcomes included HDP, CD, preterm birth, and PPH. Respective risks for SMM were 2.3%, 4.3%, and 7.8%. The weighted relative risks (wRRs) were 3.30 (95% CI, 2.59 to 4.20) comparing gestational carriage with unassisted conception and 1.86 (CI, 1.36 to 2.55) comparing gestational carriage with IVF. HDP, PPH and preterm birth before 37 weeks were also significantly higher in gestational carriers than either comparison group. The authors note that among singleton births after 20 weeks, a higher risk for SMM and adverse pregnancy outcomes was seen among gestational carriers compared with women who conceived with and without assistance.

2.51. A retrospective cohort analysis by (Pavlovic et al., 2020) compared perinatal outcomes between 78 commissioned cycles (CC) and 71 spontaneous cycles (SC) by the same gestational carriers (GC). Commissioned pregnancies were significantly associated with an increased composite incidence of perinatal complications (such as, pre-eclampsia, gestational mellitus diabetes, postpartum haemorrhage and placental abruption) compared with spontaneous pregnancies before and after adjusting for age ($p < 0.05$). There was a higher frequency of preterm delivery and hypertensive disorders in the commissioned group compared to the spontaneous group. Out of the 7 patients that had complications in the spontaneous pregnancy cohort, only 2 of those patients subsequently had a complication in their commissioned cycle. Commissioned cycles confer a greater incidence of composite perinatal complications and were independently associated with a lower average gestational age when compared with spontaneous pregnancies

carried by the same GC despite a confirmed healthy uterine environment, sperm samples, and donor oocytes.

2.52. A systematic review and meta-analysis (by Matsuzaki et al., 2024) included six studies from 2011 to 2023 to assess obstetric outcomes in 28,300 gestational carrier (GC) pregnancies and 1,270,662 non-GC pregnancies. Comparator studies revealed lower odds of caesarean delivery and comparable rates of hypertensive disorders, preterm birth, and low birth weight in GC pregnancies vs non-GC ART pregnancies. Comparatively, GC pregnancies had higher odds of hypertensive disorders vs general (non-GC ART and non-ART) pregnancies with comparable caesarean delivery risk. Although severe maternal morbidity and mortality was rare among GCs, the authors argue that overall GC pregnancies posed higher risks than non-GC pregnancies and contributing factors may include ART procedures and increased rates of multiple gestations which influence adverse perinatal outcomes in GC pregnancies.

Multiple pregnancies

2.53. A retrospective cohort study by (Osmundsen et at., 2023) determined surrogacy incidence in an ART conceived twin population, and the association with an increased rate of complications in twin surrogacy pregnancies. Over the 10-year period, 36 of 249 pregnancies were identified as gestational surrogates. The incidence of GDM was higher among surrogates than other non-surrogate twin pregnancies ($p < 0.05$), while other complications such as, PE and hypertension were lower.

Research on ovulation stimulating drugs

Risk of cancer

2.54. A meta-analysis by (Li et al., 2025) evaluated 40 studies assessing the effect of ovarian stimulating drugs on ovarian tumours. The secondary objective was to assess this effect in different sub-groups. Compared with unexposed infertile women and unexposed general population, ovarian-stimulating drugs increased the risk of invasive ovarian cancer ($p < 0.05$) and borderline cancer ($p < 0.05$) in women receiving any fertility drugs. The sub-group analysis showed a higher risk of both invasive and borderline ovarian cancer in infertile women using ovarian-stimulating drugs compared to infertile women with no exposure and the general unexposed population. An increased risk of ovarian cancer was observed in nulliparous women, but not in parous women. In addition, a cumulative clomiphene dose of <900 mg and a number of gonadotropin cycles ≥6 were factors that increased the risk of invasive ovarian cancer. Combined treatment with clomiphene and gonadotropin was associated with an increased risk of borderline cancer.

2.55. An updated review by (Rizzuto et al., 2019) looked at 13 case-control and 24 cohort studies (adding nine new cohort and two case-control studies compared to the 2013 review) with a total of 4,684,724 women to examine the risk of ovarian cancer in women using infertility drugs when compared to the general population or untreated infertile women. Two cohort studies reported an increased incidence of invasive ovarian cancer in exposed sub fertile women compared with unexposed women. One reported a standardised incidence ratio (SIR) of 1.19 (95% CI, 0.54 to 2.25) based on 17 cancer cases. The other cohort study reported a hazards ratio (HR) of 1.93 (95% CI 1.18 to 3.18), and this risk was increased in women remaining nulligravid after using

clomiphene citrate (CC) (HR 2.49, 95% CI 1.30 to 4.78) versus multiparous women (HR 1.52, 95% CI 0.67 to 3.42) (very low-certainty evidence). The slight increase in ovarian cancer risk among women having between one and three cycles of IVF was reported but was not clinically significant. There was no increase in risk of invasive ovarian cancer after use of infertility drugs in women with the BRCA mutation according to one cohort and one case-control study. The certainty of evidence assessed using Grading of Recommendations Assessment, Development and Evaluation (GRADE) was very low. For borderline ovarian tumours, one cohort study reported increased risk in exposed women with an SIR of 3.61 (95% CI 1.45 to 7.44), and this risk was greater after treatment with CC (SIR 7.47, 95% CI 1.54 to 21.83) based on 12 cases. In another cohort study, the risk of a borderline ovarian tumour was increased, with an HR of 4.23 (95% CI 1.25 to 14.33), for sub fertile women treated with IVF compared with a non-IVF-treated group with more than one year of follow-up. A large cohort reported increased risk of borderline ovarian tumours, with HR of 2.46 (95% CI 1.20 to 5.04), although based on 17 cases. A significant increase in serous borderline ovarian tumours was reported in one cohort study after the use of progesterone for more than four cycles (RR 2.63, 95% CI 1.04 to 6.64). A case-control study reported increased risk after CC was taken, with an SIR of 2.5 (95% CI 1.3 to 4.5) based on 11 cases, and another reported an increase especially after human menopausal gonadotrophin was taken (OR) 9.38, 95% CI 1.66 to 52.08). Another study estimated an increased risk of borderline ovarian tumour, but this estimation was based on four cases with no control reporting use of fertility drugs. The certainty of evidence assessed using GRADE was very low. However, although some studies suggested a slight increase in risks of ovarian cancer and borderline ovarian tumour, none provided moderate- or high-certainty evidence.

2.56. A retrospective cohort study (Bjørnholt et al., 2015) assessed the risk of borderline ovarian tumours following exposure to fertility drugs in a cohort of 96,545 Danish women referred to all Danish fertility clinics from 1963-2006 with a median length of 11.3 years for follow-up. All women were followed for first occurrence of a borderline ovarian tumour from initial date of infertility evaluation until a date of migration, death or 31 December 2006, whichever occurred first. The analysis included 142 women with borderline ovarian tumours (cases) and 1328 randomly selected sub-cohort members identified in the cohort during the follow-up through 2006. Cases were identified by linkage to the Danish Cancer Register and Danish Register of Pathology. Cohort analyses showed that the overall risk for borderline ovarian tumours was not associated with any fertility drug use or of gonadotrophins, CC, human chorionic gonadotrophins or gonadotrophin-releasing hormone analogues. Furthermore, no associations were observed between the risk for borderline ovarian tumours and these groups of fertility drugs according to the number of cycles of use, length of follow-up or parity. In contrast, progesterone use increased the risk for borderline ovarian tumours, particularly serous tumours. Statistically significantly increased risks of serous tumours were observed with any progesterone use among women treated with ≥4 cycles of progesterone and for all women followed up for ≥4 years after their first progesterone treatment.

2.57. A systematic review by (Lerner-Geva et al., 2010) assessed the association between infertility and cancer development, with an emphasis on the influence of fertility treatment. Results on the possible association of infertility, ovulation induction medications and invasive ovarian cancer show no increased risk and are reassuring. However, results for increased risk for BC and endometrial cancer following exposure to ovarian stimulation medications are inconclusive.

2.58. A retrospective population-based cohort study by (Lindquist et al., 2022) assessed the incidence of thyroid cancer following fertility drug use in a cohort of 146,024 infertile women aged 20-45 years and living in Denmark in 1995-2017. The women were followed from cohort entry (i.e. date of first infertility diagnosis) until the occurrence of thyroid cancer or any other cancer (except non-melanoma skin cancer), death, emigration, total thyroidectomy or follow-up end (31 December 2018), whichever occurred first. The median length of follow-up was 11.3 years. In total, 167 women were diagnosed with thyroid cancer during the follow-up period. Information on the use of specific fertility drugs (clomiphene citrate (CC), gonadotropins, hCGs, GnRH receptor modulators and progesterone), thyroid cancer, covariates and vital status was obtained from the Danish Infertility Cohort and various Danish national registers. After adjustment for the calendar year of infertility diagnosis, the highest obtained level of education, parity status, obesity or thyroid disease and mutual adjustment for other registered fertility drugs, no marked associations were observed between the use of CC, hCG, gonadotropins or GnRH receptor modulators and risk of overall or papillary thyroid cancer. However, every use of progesterone was associated with an increased rate of both overall and papillary thyroid cancer after mutual adjustment for other specific fertility drugs. For most specific fertility drugs, a tendency toward higher associations with thyroid cancer within the first 5 years after the start of drug use was observed than after 5 years from the start of use. Although it's shown that there is no strong link between the use of fertility drugs and thyroid cancer incidence, a modest increase in thyroid cancer incidence after the use of progesterone was noted.

2.59. A review by (Farhud et al., 2021) summarises the use of IVF medication, such as gonadotrophins and clomiphene citrate (CC) on breast cancer (BC) risk from 100 studies. The results presented conflicting findings, with some studies reporting a slight increase in cancer risk for hormone sensitive cancers including breast cancers. Authors conclude that long-term use can increase oestrogen hormones and cause excessive expression of genes, resulting in an increased risk of BC. The risk of BC may also be increased in women with a positive family history and related inherited genes.

3. Conclusions

3.1. The literature identified suggests an increased risk for adverse obstetric outcomes such as, pre-eclampsia and hypertension, in patients who undergo ART in comparison to spontaneous pregnancies, which may be even higher when the treatment involves multiple pregnancies or frozen embryo transfer. However, it remains unclear to what extent these associations might be related to the underlying cause(s) of infertility. A couple of studies also suggest a link between ART and cardiovascular disease, although not conclusive.

3.2. Conflicting evidence remains on the association between ART using autologous oocytes and the risk of reproductive cancers such as, breast and ovarian cancer. Other studies looked at the effects of embryo developmental stage and cryopreservation method, cross border reproductive care and advanced maternal age on obstetric outcomes and kidney dysfunction.

3.3. Studies also show that compared to the use of autologous oocytes, the risk for adverse obstetric outcomes such as, pre-eclampsia and hypertension is especially high in patients who undergo ART using donated gametes or embryos, which again increases further when treatment involves multiple pregnancies. This may be due to the unrelated embryo and gestational carrier having

fewer matching genes and a higher number of HLA mismatches. The evidence on increased risk for cancer-related morbidity in the decade following delivery and impact on kidney function are inconclusive.

3.4. Two studies investigating the risk of pre-eclampsia between IVF with donor sperm vs. partner sperm found there to be no difference.

3.5. Studies which investigate the risks for egg donors only report on the short-term complications of the procedure, including OHSS and pain. They also evaluate psychological issues including anxiety amongst egg donors. Current evidence on breast cancer incidence in egg donors is inconclusive and conflicting with no clear established link. Studies investigating egg donors are conducted mostly via surveys completed by egg donors, rather than studies comparing complications experienced by egg donors to the general population.

3.6. In surrogates who are gestational carriers, studies demonstrate an increased risk for adverse obstetric outcomes (for example hypertensive disorders, postpartum haemorrhage, and gestational diabetes) in comparison to non-surrogates who conceived with and without assistance. Furthermore, one study investigating the impact of twin pregnancies concluded that the incidence of gestational diabetes was higher among surrogates than other non-surrogate twin pregnancies, while other complications such as, pre-eclampsia and hypertension were lower. However, another study concluded that severe maternal morbidity and mortality was rare among gestational carriers.

3.7. The brief review and opinion by Professor Stuart Campbell (Annex A) summarises that not all studies investigating the risk of gestational surrogates find an increase in maternal morbidity, with three good research papers showing no significant effect. Those that do report complications that are similar to those found in donor oocyte cycles (with the exception of one paper (Velez et al., 2024)) appear to be less severe. It is also concluded that of the thousands of surrogate pregnancies now reported there have been no reports of maternal death.

3.8. Research investigating a link between the use of ovulation stimulating drugs and increased ovarian and thyroid cancer risk are conflicting.

3.9. Overall, many of the identified studies are retrospective cohort or population-based studies. Though a few of the studies were large, national and register based, limitations include, absence of complete data, short follow-up times, selection bias and control of confounding factors. Additional long term follow-up studies and linkage studies are needed for more robust data.

4. Recommendations

4.1. Members are asked to:

- Advise the Executive if they are aware of any other recent development.
- Consider the research findings and the quality of the evidence and draw conclusions on risks for patients undergoing ART, including for gestational surrogates and egg donors.
- Review whether any outputs from the HFEA are required addressing health outcomes for ART patients (including gestational surrogates and egg donors).

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6. Annex A – A brief review and opinion of the evidence base on risks to gestational surrogates by Professor Stuart Campbell

6.1.

The first thing is to ascertain whether increased maternal morbidity (MM) is increased in Gestation Carrier (GC) pregnancies. (We will leave Fetal Morbidity (FM) as the results from the papers you have referenced are inconsistent and mainly reassuring).

No Increase in MM

1. Dar et al (1) found no increase in MM in a large case series with 333 consecutive intended parent (IP) cycles 178 pregnancies achieved. The overall maternal complication rate was only 9.8% (13/133). Of these, 12 were relatively minor and only one was major and there were no maternal deaths. Surprisingly there were no significant differences between Donor and non-Donor Cycles.
2. Soderstrom-Anttila et al (2) carried out a large systematic review of 75 Surrogacy papers from PubMed, Cochrane and Embase databases which met very stringent inclusion criteria. This is a very detailed review, and I will only quote a few relevant sentences" Only five studies have looked into the risks of pregnancy complications; four of these involved gestational and one traditional surrogacy. ***The incidence of HDP was between 4.3 and 10% in singleton gestational carrier pregnancies compared to between 16 and 40% usually reported in OD pregnancies*.** The high frequency of HDP noted in OD pregnancies has been associated with the fact that the oocyte recipient is immunologically unrelated to the donor. In theory, the same situation occurs in gestational surrogacy, as in such cases the entire fetal genome is allogeneic to the carrier. Based on the few reports on GC outcome available, there has been speculation that a healthy carrier with a normal reproductive background might somehow compensate for atypical immunological reactions related to a foreign embryo ,and that the surrogates might have a more hospitable uterine environment than infertile oocyte recipients"
3. Swanson et al (3) performed a population-based study comparing gestational carrier pregnancies to non-surrogate pregnancies. This paper was on your list of papers showing increased MM in surrogacy patients. However, the risks demonstrated are not strong. 361 gestational carrier pregnancies and 509,015 other pregnancies which resulted in live births were compared. Severe morbidity was less common among gestational carrier pregnancies than IVF pregnancies (1.7% versus 5.5%) and was not different when compared to all other pregnancies (1.0%,). Caesarean delivery (CD) was less common among gestational carrier pregnancies than IVF pregnancies, but not different than all other pregnancies or matched controls. Hypertensive disorders of pregnancy were lower among gestational carrier pregnancies than IVF pregnancies, it was higher than all other women who delivered and comparable to matched controls. The overall conclusion of the authors was: ***Women who are gestational carriers are at lower risk of morbidity and CD than others who conceive through IVF and do not appear to be at increased risk compared to matched controls***.

Increase in MM

1. Peters et al (4) performed a retrospective cohort study which reported all data of gestational surrogacy treatment in the VU University Medical Centre in Amsterdam over a period of 10 years. Data was collected from 60 intended parents and 63 gestational carriers, including reproductive and obstetric outcomes. All intended mothers had a medical

indication for gestational surrogacy and used autologous oocytes, and semen of the intended father. *Pregnancy was complicated in 20.6% by a hypertensive disorder. Labour was induced in 52.9%, and the Caesarean section rate was 8.8%. None of the pregnancies was complicated by preterm birth. Postpartum haemorrhage (PPH) (>500 ml) occurred in 23.5%.* This is relatively small study. For example, although the incidence of hypertension was 20%, it amounted to 7 patients only 2 of which had preeclampsia. The incidence of PPH fell to 8.8% when losses >1000ml were considered.

2. Woo et al (5) is an interesting paper as it compared perinatal outcomes between live births achieved via ART and gestational surrogacy versus spontaneously conceived pregnancies in the same woman. They identified 124 gestational surrogates who achieved a total of 494 pregnancies and had obstetric data on 103 surrogate pregnancies versus 249 spontaneous. Surrogate births had significantly higher obstetrical complications, including gestational diabetes, hypertension, use of amniocentesis, placenta previa, antibiotic requirement during labour, and caesarean section. The numbers again were small to draw too many inferences. For example, the incidence of hypertension in the GS group was 6.8%, pre-eclampsia 1.9%, and gestational diabetes 6.8% which would be regarded as normal compared to that occurring in the general obstetric population.
3. Pavlovic et al (6) identified GC singleton pregnancies from a database of 895 commissioned cycles from a large fertility centre. 78 commissioned cycles met inclusion and exclusion criteria and were compared with 71 spontaneous cycles by the same GCs. Commissioned cycles were significantly associated with adverse perinatal outcomes (25.6% vs. 9.9%; $p = 0.02$) and lower average gestational age (38.7 ± 1.5 vs. 39.4 ± 0.9; $p < 0.001$) compared with spontaneous cycles. *There was no significant difference in the incidence of gestational hypertension, gestational diabetes, abruptio, post-partum haemorrhage or Caesarean Section*.
4. Velez et al (7) In a large retrospective study Velez investigated all singleton births at more than 20 weeks from 2012-2021 in Ontario. They compared gestational carriage (main exposure), unassisted conception (comparison group 1), and in vitro fertilization (IVF) (comparison group 2). Main outcomes were a composite for Severe Maternal Morbidity (SMM) and Severe Neonatal morbidity (SNM).
   Of all eligible singleton births, 846124 (97.6%) were by unassisted conception, 16087 (1.8%) by IVF, and 806 (0.1%) by gestational carriage. Respective risks for SMM were 2.3%, 4.3%, and 7.8%.
   The main conclusion was that the study suggests that, among singleton births, gestational carriage has a higher associated risk for pre-eclampsia, and postpartum haemorrhage compared with women who conceive with and without assistance. Although gestational carriage was associated with preterm birth at less than 37 weeks, there was less clear evidence of a higher risk for SNM. After adjusting for age, gestational diabetes, obesity and chronic hypertension each risk factors for pre-eclampsia. The actual incidence of severe preeclampsia was 1.86%
5. Lahl et al (8). A cohort of 96 women who had GS pregnancies were interviewed. Advertisements were placed on social media—such as Facebook and Instagram—to recruit women who met inclusion criteria. Respondents to the advertisement were first screened using the inclusion criteria which required women to: (1) be 21 years old or older; (2) have acted as a gestational surrogate at least once; (3) be able to give verbal informed consent for the study; (4) not be employed by a fertility clinic; (5) reside in the U.S.; and (6) have the ability to speak English. Those women who met inclusion criteria were interviewed online by way of a secure online video platform.
   The survey found that Gestational Surrogate pregnancies were significantly more likely to be high-risk, deliver earlier, and require a caesarean section for delivery, than spontaneous

pregnancies. We also found significant adverse consequences to both the mental and physical health and wellbeing of women following a surrogate pregnancy. However, the study found that women who are already more likely to experience poor health outcomes are those most likely to participate in gestational surrogacy, putting them at heightened risk for adverse health outcomes. No statistical controls were made for these demographic factors, so the data is not reliable for the effects of carrier status om MM.

Opinion

Not all of the studies on GS pregnancies show an increase in maternal morbidity. I have found 3 good papers that show no significant effect.

In most of the studies that find an increase in maternal morbidity the effect is not strong and the incidence of maternal complications not higher than we would expect in the general obstetric population. The exception is the large study of Velez et al which showed a statistically significant increase in severe pre-eclampsia and postpartum haemorrhage. The implication from Velez is that the donor oocytes were used in their GC group. To quote from Velez et al *"Furthermore, implantation of a nonautologous embryo could also contribute to the higher risk among gestational carriers, as evidence suggests oocyte donation increases the odds of hypertensive disorders of pregnancy"*. Thus, it is difficult to escape the coincidence that the complications presented in the GC cases described in these papers mirror the increase in MM found in Donor oocyte cycles. Of course, GS cycles are sometimes carried out with autologous oocytes, and it is not made clear in some of the studies whether the oocytes are autologous or donor.
I refer you to two good reviews on the outcomes of donor oocyte gestations (Savasi et al 2017 (9) and Silvestris et al 2023(10)). Both reviews record that there is an increase in pre-eclampsia and postpartum haemorrhage (PPH). Savasi *states:" Oocyte donation seems to be independently associated with a higher rate of pregnancy-induced hypertension and preeclampsia. An explanatory hypothesis is that an immunological maladaptation causes placenta-mediated disorders in oocyte donation pregnancies." Silvestris states "this practice (OD) seems to be associated with a higher rate of major risky events during pregnancy as recurrent miscarriage, infections and placental diseases including gestational hypertension, pre-eclampsia and post-partum haemorrhage, as well as several maternal–fetal complications due to gametes manipulation and immune system interaction.*
There is an increased risk of PPH in women who have PE which may explain the increase in PPH reported in some of the papers.

Much of the data for GC's comes from the USA which allows payment to the GC from the IP's. Demographic data were reported in one study of 204 women acting as GCs; their mean age was 33 years, more than 90% were non-Hispanic white, and more than 80% were married. In the UK commercial surrogacy is forbidden so the demographics will be different. Apart from Lahl et al in the papers studied there is no evidence that demographic factors affected the results and, in many studies, they were controlled for in the statistical analysis.

In summary, not all studies find an increase in maternal morbidity in GS gestations. Those that do report complications that are similar to those found in Donor oocyte cycles but (with the exception of one paper (Velez et al) appear to be less severe.

A final reassuring fact is that of the thousands of surrogate pregnancies now reported there has been no reports of maternal death.

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6. Pavlovic: Journal of Assisted Reproduction and Genetics (2020) 37:953–962
7. Velez: Ann Intern Med. doi:10.7326/M24-0417
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