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

Monday 06 February 2023

Date	Action	Responsibility	Due date	Progress to date
31/01/2022	Assess whether further outputs are required in the topic of the impact of the microbiome, and whether it needs to be considered as a treatment add-on.	Dina Halai, Head of Policy	Ongoing	This will be assessed as part of an agenda item at the June 2023 SCAAC meeting. This has been amended from the SCAAC workplan due to internal resourcing restrictions.
06/06/2022	Following discussions and decisions regarding the application of the addition of Androgen supplementation as a treatment add-on. Members expressed concern over language used within the treatment add-ons eligibility criteria. With the Authority decisions on changes to both the evidence base and how evidence is presented, members requested for the decision to be reviewed and presented to the Committee at a future meeting.	Dina Halai, Head of Policy	Ongoing	The Executive will amend the treatment add-ons application form decision tree in line with the evolving treatment add-ons rating system. This will be presented at the June SCAAC meeting.
03/10/2022	Consider including information for patients on the HFEA website about additional risks of treatment related to hypertension in pregnancy following frozen embryo transfer in medicated cycles of fertility treatment.	Victoria Askew, Head of Policy	Complete	Information has been added to the HFEA website page that discusses risks of treatment

03/10/2022	Consider a framework for assessing AI technologies which fall within the regulatory remit of the HFEA. Publish a Clinic Focus article for the sector on developments in the regulation of AI.	Annabel Salisbury, Policy Manager	Ongoing	AI will be next discussed by the SCAAC in October 2023. In the interim, the Executive will develop a report detailing the uses of AI in clinics that fall within HFEA's regulatory remit and publish a clinic focus as part of that work.

Horizon scanning and priortisation of issues

Details about this paper

Area of strategy this paper relates to:	Shaping the future
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	5
Paper number:	HFEA (06/02/2023) 005
Meeting date:	6 February 2023
Author:	Zoe Constable, Policy Manager
Annexes	Annex A: Briefing on key issues identified during horizon scanning Annex B: Horizon scanning reference list Annex C: Committee workplan 2023/24

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to: • note the issues identified as high, medium and low priority through the horizon scanning process; • consider the high, medium and low priority issues and work recommendations; and • consider whether advice from additional external advisors would help in achieving the work recommendations.
Resource implications:	Subject to committee recommendations
Implementation date:	As per Committee workplan for 2023/24 (Annex C)
Communication(s):	NA
Organisational risk:	Low

1. Background

1.1. The Authority established a horizon scanning function in 2004 to identify issues that could have an impact on the field of assisted reproduction or embryo research. By identifying these issues, the Authority can be aware of potential license applications and prepare, if necessary, a policy position or relevant patient information.

1.2. Issues are identified from journal articles, conferences, and contact with experts who are invited to the Authority's Horizon Scanning meetings (an international panel of experts who meet annually to discuss developing and future technologies within the fertility sector).

1.3. The horizon scanning process is an annual cycle that feeds into the business planning of the Executive, the Scientific and Clinical Advances Advisory Committee (SCAAC), and the Authority's consideration of scientific and ethical issues and standards.

2. Prioritisation process

2.1. A full list of papers identified during the 2023 horizon scanning process can be found in Annex B to this paper.

2.2. To help with the business planning process, it is important for the Executive to be fully aware of which issues members consider to be high priority. Issues that have been identified this year have been categorised as high, medium, or low priority using the following criteria:

a) Within the HFEA's remit
b) Timescale for likely introduction (2-3 years)
c) High patient demand/clinical use if it were to be introduced
d) Technically feasible
e) Ethical issues raised or public interest

2.3. Issues are high priority if they are within the HFEA's remit and meet at least two other criteria. Issues are medium priority if they are within the HFEA's remit and meet one other criterion, or are outside of HFEA remit but meet at least two other criteria. Low priority issues are those outside of HFEA's remit and unlikely to impact on research or treatment in the near future. Published studies in these areas will continue to be collected and considered as part of the horizon scanning process.

2.4. High priority categorisation is also given to established techniques or issues which fall within the HFEA's remit that require ongoing monitoring or provision of patient information.

3. High priority issues

3.1. The Executive considers the following topics to be high priority (in no particular order) for 2023/24.

a) Treatment add-ons
b) New technologies in embryo and gamete testing
c) Genome editing
d) Mitochondrial donation

e) Alternative methods to derive embryonic and embryonic-like stem cells
f) Artificial intelligence (AI), robotics and automation
g) Scientific considerations relevant to the '14-day rule'
h) Impact of long-term cryopreservation of gametes and embryos

3.2. Based on this year's horizon scanning findings, key developments on some of these high priority issues can be found in Annex A. Briefings have not been written for all prioritised issues, as these topics are either standing items that are considered by the Committee every year, or they have been considered by the Committee recently.

3.3. One new topic has been included in the high priority list, the impact of long-term cryopreservation of gametes and embryos.

3.4. The Executive has recommended the addition of robotics and automation to the topic 'Artificial Intelligence'.

3.5. Existing topics were reviewed according to the prioritisation criteria above and three topics were deprioritised from high to medium priority issues. These topics are health outcomes in children conceived by ART, in vitro derived gametes, and synthetic embryo-like entities.

Annual review of treatment add-ons

3.6. The Authority currently undertakes an annual evidence review for treatment add-ons. Evidence for treatment add-ons that the HFEA provides information on is reviewed by an expert in systematic reviews and evidence assessment. They carry out an independent assessment of the quality of evidence using the GRADE methodology[1] for each treatment add-on. The SCAAC considers the quality of new evidence for each treatment add-on based on the independent assessor's findings and recommends updates to the HFEA's **treatment add-ons information**.

3.7. As part of this horizon scanning process, the Executive have identified wider research investigating treatments that claim to increase live birth rate that are not currently part of the HFEA's **treatment add-ons information**. A briefing on these can be found at Annex A.

4. Medium priority issue

4.1. The Executive considers the following topics to be medium priority for consideration in 2023/24.

a) Health outcomes in children conceived by ART (including the impact of culture media)
b) In vitro derived gametes
c) Synthetic embryo-like entities
d) The impact of the microbiome on fertility and fertility treatment outcomes

4.2. Existing topics were reviewed according to the prioritisation criteria above and two topics were deprioritised from medium to low priority issues. These topics are the impact of stress on fertility treatment outcomes and artificial wombs for early or whole gestation (ectogenesis).

[1] GRADE is an approach for grading the quality of evidence and the strength of recommendations. It was developed by the Grading of Recommendations, Assessment, Development and Evaluation Working Group.

4.3. The Executive has recommended the removal of COVID-19 from the horizon scanning topic list. This is in line with the discussion at the **June 2022** SCAAC meeting where it was agreed that limited new information is emerging regarding the effects of COVID-19 on fertility.

5. Low priority issue

5.1. The Executive considers the following topics to be low priority for consideration in 2023/24.

a) The impact of stress on fertility treatment outcomes
b) Artificial wombs for early or whole gestation (ectogenesis)

6. Recommendations

6.1. Members are asked to:

- note the issues identified as high, medium, and low priority through the horizon scanning process;
- consider the high, medium, and low priority issues and work recommendations; and
- consider whether advice from additional external advisors would help in achieving the work recommendations.

Annex A Briefing on key issues identified during horizon scanning

7. Treatment add-ons

Background

7.1. Since the introduction of the HFEA's traffic light rated list of **treatment add-ons**, other organisations and research groups have published their own lists of what they would classify as treatment add-ons. These lists contain some treatments that the HFEA does not currently provide information on. Some of these potential treatment add-ons are summarised below.

Summary of developments

7.2. ESHRE are developing a **good practice recommendation paper** which outlines a set of treatment add-on tests and treatments, describes their rationale and any evidence of their efficacy and safety, and provides a recommendation for clinical practice. This is currently a draft paper and the recommendations therein have not been considered by our expert reviewer but may be of interest to the committee.

7.3. Add-ons featured in this paper that are not on the HFEA list are:

- Screening hysteroscopy
- Mitochondrial replacement therapy (to improve oocyte quality) – this is not currently rated as an add-on, but information on mitochondrial donation to avoid mitochondrial diseases is provided on the HFEA **website**
- In vitro activation of dormant follicles (IVA)
- In vitro maturation (IVM)
- Sperm DNA damage testing – this is not currently rated as an add-on, but information is provided on the HFEA **website**
- Artificial sperm activation
- Magnetic-activated cell sorting (MACS) and microfluidics – SCAAC have previously agreed that MACS should not be included on the HFEA add-ons list due to low clinical use in **October 2018**
- Growth factor-supplemented embryo culture medium – this falls under MRHA's remit but is monitored through the horizon scanning and was last discussed by SCAAC in **February 2021**
- Non-invasive pre-implantation genetic testing (niPGT) and mitochondria DNA load measurement
- Platelet rich plasma (PRP) – this falls under MRHA's remit
- Duostim
- Adjuncts during ovarian stimulation – this includes androgen supplementation e.g., dehydroepiandrosterone and testosterone. SCAAC agreed that androgen supplementation did not meet the add-on criteria for a HFEA rating in **June 2022**

- Flushing of the uterus
- Stem cell mobilisation
- Antioxidant therapy – this is not rated as an add-on, but information is provided on the HFEA website
- Complementary and alternative medicine – this is not rated as an add-on, but information is provided on the HFEA website

Level of work recommendation

7.4. Applications can be made to the HFEA to propose a new treatment for inclusion in the HFEA's traffic-light rated list of add-ons. The application form is being reviewed so that it aligns with the criteria agreed at the July 2022 Authority meeting, but will re-open for applications in 2023. If a new add-on is accepted, the evidence base for that treatment would then be reviewed in line with the process for review of treatment add-ons conducted by the Executive and the Committee.

8. Impact of long-term cryopreservation of gametes and embryos

Background

8.1. From 1 July 2022**,** all patients can store their eggs, sperm and embryos for their own treatment for up to 55 years, providing they reconsent every ten years. This change may increase the number of gametes and embryos in long-term storage; thus, it is important to be aware of any safety or viability concerns of the long-term storage changes. Therefore, the impact of long-term cryopreservation of gametes and embryos has been proposed as a new topic to monitor in the annual horizon scanning process.

Summary of developments

Embryos

8.2. Previous case reports have demonstrated that live births can be achieved after long-term (>10 years) embryo cryostorage (Yuan et al., 2019). However, there remains to be some uncertainty around the impact of embryo cryostorage duration on clinical outcomes, with many existing studies having a limited sample size and methodological differences, making comparison challenging.

8.3. Many of the studies identified through the horizon scanning process found no significant impact of cryostorage duration on clinical outcomes for both slow-freezing (Canosa et al., 2022, Liu et al., 2014) and vitrification of embryos (Ueno et al., 2018, Wirleitner et al., 2013). A recent observational study of 2688 vitrified-warmed blastocyst transfers found no significant difference in live birth rate across seven groups of different cryostorage duration ranging from <60 days to >1080 days (Cimadomo et al., 2022). No significant differences were reported across the groups for any of the secondary outcomes that included miscarriage rate, obstetric and perinatal issues.

8.4. Mao et al., 2022, compared the pregnancy and neonatal outcomes across five groups of embryo vitrification duration ranging from 1-90 days to >731 days (n = 31,143). The highest clinical pregnancy rate was identified in the group with the shortest embryo storage duration,

but no significant impact was found on neonatal outcomes. Cui et al., 2022, conducted a similar study with longer storage durations to compare pregnancy outcomes across groups ranging from <3 months to >5 years (n = 9806). No significant difference was observed between groups stored for <5 years. However, a significantly reduced clinical pregnancy and live birth rate was identified when using propensity score matching to compare 171 cycles stored for >5 years to cycles stored <1 year. These studies suggest that long-term storage of embryos may negatively impact pregnancy outcomes, but this is unlikely to result in negative neonatal impacts. However, the results should be interpreted cautiously due to the small sample size, particularly in the longer storage duration groups.

8.5. Other studies have identified a relationship between embryo cryostorage duration and treatment outcomes. Hu et al., 2022, analysed storage duration as a continuous variable and identified an inverted U-shaped relationship between embryo cryostorage time and treatment success in women with high ovarian response in freeze-all cycles (n = 14,928). This suggested that treatment success is reduced after >6 months of embryo cryostorage. Previous studies have found similar relationships between longer storage duration and reduced clinical pregnancy and live birth rates (Zhang et al., 2021). However, it should be noted that these studies only include a maximum storage duration of four years and most of the embryo transfers occur within one year of storage. Therefore, the findings should be used cautiously when considering the likely impact of long-term storage of >10 years under the new storage regulations.

Oocytes

8.6. Various case reports have demonstrated the potential of long-term cryopreserved oocytes (>10 years) to result in a live birth (Urquiza et al., 2014, Dinh et al., 2022). Cobo et al., 2015, investigated the impact of storage duration of cryopreserved oocytes on clinical outcomes across eight categories ranging from <6 months to >5 years. No significant differences in survival rates of oocytes or clinical outcomes, such as ongoing pregnancy rate, were identified between the groups. This suggests that oocyte cryostorage duration does not impact clinical outcomes.

Stigliani et al., 2015, found that the length of storage time had no impact on the gene expression of cryopreserved human metaphase II oocytes when comparing oocytes cryostored for three years (n = 32) and six years (n = 36). This finding was supported by a more recent study that found no difference in gene expression between oocytes cryostored for 1-, 2-, 3- or 12-months (n = 16, Huo et al., 2021). Both studies identified a difference in gene expression between cryopreserved and non-cryopreserved oocytes. The findings of the publications suggest that cryostorage duration does not have an impact on oocyte gene transcription and supports the safety of long-term cryostorage of oocytes.

Sperm

8.7. The impact of long-term cryostorage of sperm on clinical outcomes has also been investigated. Huang et al., 2019, compared the clinical outcomes from sperm samples that had been cryopreserved for 0.5–5, 6–10 and 11–15 years (n = 119,558). No significant impact on clinical outcomes, such as live birth rates, were observed between the groups. However, a decrease in sperm quality was observed after 5-years of cryopreservation.

8.8. The studies identified through the horizon scanning process provide support for the safety of long-term cryopreservation of gametes and embryos. However, many of the studies have small sample sizes, are observational and have shorter timescales than the new storage law's 55-year limit. Therefore, this topic will continue to be monitored for new developments as part of the annual horizon scanning process.

Level of work recommendation

8.9. The Committee will be asked to monitor any further developments in the scientific and clinical literature relating to long-term impacts of gamete and embryo storage as part of the committee's workplan for 2023/24. The Authority will continue to monitor any developments as part of the annual horizon scanning.

References

- Canosa, S., Cimadomo, D., Conforti, A., Maggiulli, R., Giancani, A., Tallarita, A., Golia, F., Fabozzi, G., Vaiarelli, A., Gennarelli, G., Revelli, A., Bongioanni, F., Alviggi, C., Ubaldi, F.M., Rienzi, L., 2022. The effect of extended cryo-storage following vitrification on embryo competence: a systematic review and meta-analysis. J Assist Reprod Genet 39, 873–882. https://doi.org/10.1007/S10815-022-02405-3
- Cimadomo, D., Fabozzi, G., Dovere, L., Maggiulli, R., Albricci, L., Innocenti, F., Soscia, D., Giancani, A., Vaiarelli, A., Guido, M., Ubaldi, F.M., Rienzi, L., 2022. Clinical, obstetric and perinatal outcomes after vitrified-warmed euploid blastocyst transfer are independent of cryo-storage duration. Reprod Biomed Online 44, 221–227. https://doi.org/10.1016/J.RBMO.2021.09.027
- Cobo, A., Garrido, N., Pellicer, A., Remohí, J., 2015. Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate. Fertil Steril 104, 1426-1434.e8. https://doi.org/10.1016/J.FERTNSTERT.2015.08.020
- Cui, M., Dong, X., Lyu, S., Zheng, Y., Ai, J., 2021. The Impact of Embryo Storage Time on Pregnancy and Perinatal Outcomes and the Time Limit of Vitrification: A Retrospective Cohort Study. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.724853
- Dinh, T., Son, W.-Y., Demirtas, E., Dahan, M.H., 2022. How long can oocytes be frozen with vitrification and still produce competent embryos? A series of six cases. Obstet Gynecol Sci. https://doi.org/10.5468/OGS.20344
- Hu, K.L., Hunt, S., Zhang, D., Li, R., Mol, B.W., 2022. The association between embryo storage time and treatment success in women undergoing freeze-all embryo transfer. Fertil Steril 118, 513–521. https://doi.org/10.1016/J.FERTNSTERT.2022.06.003
- Huang, C., Lei, L., Wu, H.L., Gan, R.X., Yuan, X.B., Fan, L.Q., Zhu, W.B., 2019. Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes. Fertil Steril 112, 663-669.e1. https://doi.org/10.1016/J.FERTNSTERT.2019.06.008
- Huo, Y., Yuan, P., Qin, Q., Yan, Z., Yan, L., Liu, P., Li, R., Yan, J., Qiao, J., 2021. Effects of vitrification and cryostorage duration on single-cell RNA-Seq profiling of vitrified-thawed human metaphase II oocytes. Front Med 15, 144–154. https://doi.org/10.1007/S11684-020-0792-7
- Liu, Q., Lian, Y., Huang, J., Ren, X., Li, M., Lin, S., Liu, P., Qiao, J., 2014. The safety of long-term cryopreservation on slow-frozen early cleavage human embryos. J Assist Reprod Genet 31, 471–475. https://doi.org/10.1007/S10815-014-0197-0
- Mao, Y., Tang, N., Luo, Y., Yin, P., Li, L., 2022. Effects of vitrified cryopreservation duration on IVF and neonatal outcomes. J Ovarian Res 15. https://doi.org/10.1186/S13048-022-01035-8

- Stigliani, S., Moretti, S., Anserini, P., Casciano, I., Venturini, P.L., Scaruffi, P., 2015. Storage time does not modify the gene expression profile of cryopreserved human metaphase II oocytes. Hum Reprod 30, 2519–2526. https://doi.org/10.1093/HUMREP/DEV232
- Ueno, S., Uchiyama, K., Kuroda, T., Yabuuchi, A., Ezoe, K., Okimura, T., Okuno, T., Kobayashi, T., Kato, K., 2018. Cryostorage duration does not affect pregnancy and neonatal outcomes: a retrospective single-centre cohort study of vitrified-warmed blastocysts. Reprod Biomed Online 36, 614–619. https://doi.org/10.1016/J.RBMO.2018.03.008
- Urquiza, M.F., Carretero, I., Cano Carabajal, P.R., Pasqualini, R.A., Felici, M.M., Pasqualini, R.S., Quintans, C.J., 2014. Successful live birth from oocytes after more than 14 years of cryopreservation. J Assist Reprod Genet 31, 1553–1555. https://doi.org/10.1007/S10815-014-0318-9
- Wirleitner, B., Vanderzwalmen, P., Bach, M., Baramsai, B., Neyer, A., Schwerda, D., Schuff, M., Spitzer, D., Stecher, A., Zintz, M., Zech, N.H., 2013. The time aspect in storing vitrified blastocysts: its impact on survival rate, implantation potential and babies born. Hum Reprod 28, 2950–2957. https://doi.org/10.1093/HUMREP/DET361
- Yuan, Y., Mai, Q., Ma, J., Deng, M., Xu, Y., Zhuang, G., Zhou, C., 2019. What was the fate of human embryos following long-term cryopreservation (≥12 years) and frozen embryo transfer? Hum Reprod 34, 52–55. https://doi.org/10.1093/HUMREP/DEY350
- Zhang, X., Wu, S., Hao, G., Wu, X., Ren, H., Zhang, Yinfeng, Yang, A., Bi, X., Bai, L., Zhang, Yunshan, Tan, J., 2021. Prolonged Cryopreservation Negatively Affects Embryo Transfer Outcomes Following the Elective Freeze-All Strategy: A Multicenter Retrospective Study. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.709648

Annex C Committee work plan 2023/2024

Priority topic	Item	Possible speaker(s)	Last discussed	Next meeting
In vitro derived gametes	Literature review	Academic	June 2020	June 2023
Health outcomes (inc. culture media)	Literature review	Internal	February 2020	June 2023
Impact of the microbiome on fertility treatment outcomes	Literature review	Internal	February 2019	June 2023
Artificial intelligence	Literature review	Internal	October 2022	October 2023
Genome editing	Literature review	Internal	October 2020	October 2023
Ectogenesis	Literature review	Academic	Added February 2022	October 2023
New technologies in embryo and gamete testing	Literature review	Internal	October 2021	February 2024
Treatment add-ons	Literature review and external speaker	Expert reviewer	October 2022	February 2024
Impacts of long-term cryopreservation	Literature review	Academic	Added February 2023	June 2024
Mitochondrial donation	Literature review	Academic	February 2022	June 2024
Alternative methods to derive embryonic stem cells	Literature review	Internal	February 2022	June 2024

Human Fertilisation & Embryology Authority

Horizon scanning prioritisation of issues.

Annex B: Horizon Scanning Reference List

Alternative methods of deriving embryonic and embryonic-like stem cells

Castel, G., David, L., 2022. Induction of human trophoblast stem cells. Nat Protoc 17. https://doi.org/10.1038/S41596-022-00744-0

Cesare, E., Urciuolo, A., Stuart, H.T., Torchio, E., Gesualdo, A., Laterza, C., Gagliano, O., Martewicz, S., Cui, M., Manfredi, A., di Filippo, L., Sabatelli, P., Squarzoni, S., Zorzan, I., Betto, R.M., Martello, G., Cacchiarelli, D., Luni, C., Elvassore, N., 2022. 3D ECM-rich environment sustains the identity of naive human iPSCs. Cell Stem Cell 29, 1703-1717.e7. https://doi.org/10.1016/J.STEM.2022.11.011

Cui, G., Xu, Y., Cao, S., Shi, K., 2022. Inducing somatic cells into pluripotent stem cells is an important platform to study the mechanism of early embryonic development. Mol Reprod Dev 89, 70–85. https://doi.org/10.1002/MRD.23559

Fischer, L.A., Khan, S.A., Theunissen, T.W., 2022. Induction of Human Naïve Pluripotency Using 5i/L/A Medium. Methods Mol Biol 2416, 13–28. https://doi.org/10.1007/978-1-0716-1908-7_2

Gao, X., Yourick, J.J., Sprando, R.L., 2022. Generation of Human Induced Pluripotent Stem Cells Using Endothelial Progenitor Cells Derived from Umbilical Cord Blood and Adult Peripheral Blood. Methods Mol Biol 2454, 381–396. https://doi.org/10.1007/7651_2021_372

Guan, J., Wang, G., Wang, J., Zhang, Z., Fu, Y., Cheng, L., Meng, G., Lyu, Y., Zhu, J., Li, Y., Wang, Y., Liuyang, S., Liu, B., Yang, Z., He, H., Zhong, X., Chen, Q., Zhang, X., Sun, S., Lai, W., Shi, Y., Liu, L., Wang, L., Li, C., Lu, S., Deng, H., 2022. Chemical reprogramming of human somatic cells to pluripotent stem cells. Nature 605, 325–331. https://doi.org/10.1038/S41586-022-04593-5

Haraguchi, D., Nakamura, T., 2022. Pramef12 enhances reprogramming into naïve iPS cells. Biochem Biophys Rep 30. https://doi.org/10.1016/J.BBREP.2022.101267

Kristiansen, C.K., Chen, A., Høyland, L.E., Ziegler, M., Sullivan, G.J., Bindoff, L.A., Liang, K.X., 2022. Comparing the mitochondrial signatures in ESCs and iPSCs and their neural derivations. Cell Cycle 21, 2206–2221. https://doi.org/10.1080/15384101.2022.2092185

Kunitomi, A., Hirohata, R., Arreola, V., Osawa, M., Kato, T.M., Nomura, M., Kawaguchi, J., Hara, H., Kusano, K., Takashima, Y., Takahashi, K., Fukuda, K., Takasu, N., Yamanaka, S., 2022. Improved Sendai viral system for reprogramming to naive pluripotency. Cell reports methods 2, 100317. https://doi.org/10.1016/J.CRMETH.2022.100317

Mashinchian, O., de Franceschi, F., Nassiri, S., Michaud, J., Migliavacca, E., Aouad, P., Metairon, S., Pruvost, S., Karaz, S., Fabre, P., Molina, T., Stuelsatz, P., Hegde, N., le Moal, E., Dammone, G., Dumont, N.A., Lutolf, M.P., Feige, J.N., Bentzinger, C.F., 2022. An engineered multicellular stem cell niche for the 3D derivation of human myogenic progenitors from iPSCs. EMBO J 41. https://doi.org/10.15252/EMBJ.2022110655

Moauro, A., Kruger, R.E., O'Hagan, D., Ralston, A., 2022. Fluorescent Reporters Distinguish Stem Cell Colony Subtypes During Somatic Cell Reprogramming. Cell Reprogram 24. https://doi.org/10.1089/CELL.2022.0071

Moauro, A., Ralston, A., 2022. Distinguishing Between Endodermal and Pluripotent Stem Cell Lines During Somatic Cell Reprogramming. Methods Mol Biol 2429, 41–55. https://doi.org/10.1007/978-1-0716-1979-7_4

Mouka, A., Arkoun, B., Moison, P., Drévillon, L., Jarray, R., Brisset, S., Mayeur, A., Bouligand, J., Boland-Auge, A., Deleuze, J.F., Yates, F., Lemonnier, T., Callier, P., Duffourd, Y., Nitschke, P., Ollivier, E., Bourdin, A., de Vos, J., Livera, G., Tachdjian, G., Maouche-Chrétien, L., Tosca, L., 2022. iPSCs derived from infertile men carrying complex genetic abnormalities can generate primordial germ-like cells. Sci Rep 12. https://doi.org/10.1038/S41598-022-17337-2

Neeman-Egozi, S., Baskin, P., Binah, O., 2022. Methods for Isolation and Reprogramming of Various Somatic Cell Sources into iPSCs. Methods Mol Biol 2454, 643–655. https://doi.org/10.1007/7651_2021_387

Onfray, C., Tan, J.P., Kilens, S., Liu, X., Polo, J., David, L., 2022. Induction of Human Naïve Pluripotent Stem Cells from Somatic Cells. Methods Mol Biol 2416, 39–51. https://doi.org/10.1007/978-1-0716-1908-7_4

Romayor, I., Herrera, L., Burón, M., Martin-Inaraja, M., Prieto, L., Etxaniz, J., Inglés-Ferrándiz, M., Pineda, J.R., Eguizabal, C., 2022. A Comparative Study of Cell Culture Conditions during Conversion from Primed to Naive Human Pluripotent Stem Cells. Biomedicines 10. https://doi.org/10.3390/BIOMEDICINES10061358

Sevinç, K., Sevinç, G.G., Cavga, A.D., Philpott, M., Kelekçi, S., Can, H., Cribbs, A.P., Yıldız, A.B., Yılmaz, A., Ayar, E.S., Arabacı, D.H., Dunford, J.E., Ata, D., Sigua, L.H., Qi, J., Oppermann, U., Onder, T.T., 2022. BRD9-containing non-canonical BAF complex maintains somatic cell transcriptome and acts as a barrier to human reprogramming. Stem Cell Reports 17. https://doi.org/10.1016/J.STEMCR.2022.10.005

Sharp, B., Rallabandi, R., Devaux, P., 2022. Advances in RNA Viral Vector Technology to Reprogram Somatic Cells: The Paramyxovirus Wave. Mol Diagn Ther 26, 353–367. https://doi.org/10.1007/S40291-022-00599-X

Shukla, A.K., Gao, G., Kim, B.S., 2022. Applications of 3D Bioprinting Technology in Induced Pluripotent Stem Cells-Based Tissue Engineering. Micromachines (Basel) 13. https://doi.org/10.3390/MI13020155

Sokka, J., Yoshihara, M., Kvist, J., Laiho, L., Warren, A., Stadelmann, C., Jouhilahti, E.M., Kilpinen, H., Balboa, D., Katayama, S., Kyttälä, A., Kere, J., Otonkoski, T., Weltner, J., Trokovic, R., 2022. CRISPR activation enables high-fidelity reprogramming into human pluripotent stem cells. Stem Cell Reports 17, 413–426. https://doi.org/10.1016/J.STEMCR.2021.12.017

Sun, R., Gong, T., Liu, H., Shen, J., Wu, B., Jiang, Q., Wang, Q., Zhang, Y., Duan, L., Hu, J., Li, Q., Lei, L., Shan, Z., 2022. Identification of microRNAs related with neural germ layer lineage-specific progenitors during reprogramming. J Mol Histol 53, 623–634. https://doi.org/10.1007/S10735-022-10082-W

Tan, J.P., Liu, X., Polo, J.M., 2022. Establishment of human induced trophoblast stem cells via reprogramming of fibroblasts. Nat Protoc 17. https://doi.org/10.1038/S41596-022-00742-2

Thanaskody, K., Jusop, A.S., Tye, G.J., Wan Kamarul Zaman, W.S., Dass, S.A., Nordin, F., 2022. MSCs vs. iPSCs: Potential in therapeutic applications. Front Cell Dev Biol 10. https://doi.org/10.3389/FCELL.2022.1005926

Tian, P., Elefanty, A., Stanley, E.G., Durnall, J.C., Thompson, L.H., Elwood, N.J., 2022. Creation of GMP-Compliant iPSCs From Banked Umbilical Cord Blood. Front Cell Dev Biol 10. https://doi.org/10.3389/FCELL.2022.835321

Wang, Z., Zheng, J., Pan, R., Chen, Y., 2021. Current status and future prospects of patient-derived induced pluripotent stem cells. Hum Cell 34, 1601–1616. https://doi.org/10.1007/S13577-021-00592-2

Wille, C.K., Sridharan, R., 2022. DOT1L inhibition enhances pluripotency beyond acquisition of epithelial identity and without immediate suppression of the somatic transcriptome. Stem Cell Reports 17, 384–396. https://doi.org/10.1016/J.STEMCR.2021.12.004

Zorzan, I., Gagliano, O., Elvassore, N., Martello, G., 2022. Using Microfluidics to Generate Human Naïve and Primed Pluripotent Stem Cells. Methods Mol Biol 2416, 53–71. https://doi.org/10.1007/978-1-0716-1908-7_5

Artificial Intelligence

Abdullah, K.A.L., Atazhanova, T., Chavez-Badiola, A., Shivhare, S.B., 2022. Automation in ART: Paving the Way for the Future of Infertility Treatment. Reprod Sci. https://doi.org/10.1007/S43032-022-00941-Y

Afnan, M., Afnan, M.A.M., Liu, Y., Savulescu, J., Mishra, A., Conitzer, V., Rudin, C., 2022. Data solidarity for machine learning for embryo selection: a call for the creation of an open access repository of embryo data. Reprod Biomed Online 45, 10–13. https://doi.org/10.1016/J.RBMO.2022.03.015

Amitai, T., Kan-Tor, Y., Or, Y., Shoham, Z., Shofaro, Y., Richter, D., Har-Vardi, I., Ben-Meir, A., Srebnik, N., Buxboim, A., 2022. Embryo classification beyond pregnancy: early prediction of first trimester miscarriage using machine learning. J Assist Reprod Genet. https://doi.org/10.1007/S10815-022-02619-5

Arsalan, M., Haider, A., Cho, S.W., Kim, Y.H., Park, K.R., 2022a. Human Blastocyst Components Detection Using Multiscale Aggregation Semantic Segmentation Network for Embryonic Analysis. Biomedicines 10. https://doi.org/10.3390/BIOMEDICINES10071717

Arsalan, M., Haider, A., Choi, J., Park, K.R., 2022b. Detecting Blastocyst Components by Artificial Intelligence for Human Embryological Analysis to Improve Success Rate of In Vitro Fertilization. J Pers Med 12. https://doi.org/10.3390/JPM12020124

Bardet, L., Excoffier, J.B., Salaun-Penquer, N., Ortala, M., Pasquier, M., Mathieu d'Argent, E., Massin, N., 2022. Comparison of predictive models for cumulative live birth rate after treatment with ART. Reprod Biomed Online 45, 246–255. https://doi.org/10.1016/J.RBMO.2022.03.020

Barreto, N.C.N., Castro, G.Z., Pereira, R.G., Pereira, F.A.N., Reis, F.M., Junior, W.M., Cavallo, I.K.D., Gomes, K.B., 2022. Predicting in vitro fertilization success in the Brazilian public health system: a machine learning approach. Med Biol Eng Comput 60, 1851–1861. https://doi.org/10.1007/S11517-022-02569-1

Benchaib, M., Labrune, E., Giscard d'Estaing, S., Salle, B., Lornage, J., 2022. Shallow artificial networks with morphokinetic time-lapse parameters coupled to ART data allow to predict live birth. Reprod Med Biol 21. https://doi.org/10.1002/RMB2.12486

Berntsen, J., Rimestad, J., Lassen, J.T., Tran, D., Kragh, M.F., 2022. Robust and generalizable embryo selection based on artificial intelligence and time-lapse image sequences. PLoS One 17. https://doi.org/10.1371/JOURNAL.PONE.0262661

Bhaskar, D., Chang, T.A., Wang, S., 2022. Current trends in artificial intelligence in reproductive endocrinology. Curr Opin Obstet Gynecol 34, 159–163. https://doi.org/10.1097/GCO.0000000000000796

Bori, L., Meseguer, F., Valera, M.A., Galan, A., Remohi, J., Meseguer, M., 2022. The higher the score, the better the clinical outcome: retrospective evaluation of automatic embryo grading as a support tool for embryo selection in IVF laboratories. Hum Reprod 37, 1148–1160. https://doi.org/10.1093/HUMREP/DEAC066

Bori, L., Meseguer, M., 2021. Will the introduction of automated ART laboratory systems render the majority of embryologists redundant? Reprod Biomed Online 43, 979–981. https://doi.org/10.1016/J.RBMO.2021.10.002

C. N. Barreto, N., Castro, G.Z., Pereira, R.G., Pereira, F.A.N., Reis, F.M., Junior, W.M., Cavallo, I.K.D., Gomes, K.B., 2022. Predicting in vitro fertilization success in the Brazilian public health system: a machine learning approach. Med Biol Eng Comput 60, 1851–1861. https://doi.org/10.1007/S11517-022-02569-1

Chen, F., Chen, Y., Mai, Q., 2022. Multi-Omics Analysis and Machine Learning Prediction Model for Pregnancy Outcomes After Intracytoplasmic Sperm Injection- in vitro Fertilization. Front Public Health 10. https://doi.org/10.3389/FPUBH.2022.924539

Chen, L., Li, W., Liu, Y., Peng, Z., Cai, L., Zhang, N., Xu, J., Wang, L., Teng, X., Yao, Y., Zou, Y., Ma, M., Liu, J., Lu, S., Sun, H., Yao, B., 2022. Non-invasive embryo selection strategy for clinical IVF to avoid wastage of potentially competent embryos. Reprod Biomed Online 45, 26–34. https://doi.org/10.1016/J.RBMO.2022.03.006

Cheredath, A., Uppangala, S., Asha, C.S., Jijo, A., Vani Lakshmi, R., Kumar, P., Joseph, D., Nagana, N.G., Kalthur, G., Adiga, S.K., 2022. Combining Machine Learning with Metabolomic and Embryologic Data Improves Embryo Implantation Prediction. Reprod Sci. https://doi.org/10.1007/S43032-022-01071-1

Cherouveim, P., Jiang, V.S., Kanakasabapathy, M.K., Thirumalaraju, P., Souter, I., Dimitriadis, I., Bormann, C.L., Shafiee, H., 2022. Quality assurance (QA) for monitoring the performance of assisted reproductive technology (ART) staff using artificial intelligence (AI). J Assist Reprod Genet. https://doi.org/10.1007/S10815-022-02649-Z

Cimadomo, D., Marconetto, A., Trio, S., Chiappetta, V., Innocenti, F., Albricci, L., Erlich, I., Ben-Meir, A., Har-Vardi, I., Kantor, B., Sakov, A., Coticchio, G., Borini, A., Ubaldi, F.M., Rienzi, L., 2022. Human blastocyst spontaneous collapse is associated with worse morphological quality and higher degeneration and aneuploidy rates: a comprehensive analysis standardized through artificial intelligence. Hum Reprod 37, 2291–2306. https://doi.org/10.1093/HUMREP/DEAC175

Correa, N., Cerquides, J., Arcos, J.L., Vassena, R., 2022. Supporting first FSH dosage for ovarian stimulation with machine learning. Reprod Biomed Online 45, 1039–1045. https://doi.org/10.1016/J.RBMO.2022.06.010

Danardono, G.B., Erwin, A., Purnama, J., Handayani, N., Polim, A.A., Boediono, A., Sini, I., 2022. A Homogeneous Ensemble of Robust Pre-defined Neural Network Enables Automated Annotation of Human Embryo Morphokinetics. J Reprod Infertil 23. https://doi.org/10.18502/JRI.V23I4.10809

de Santiago, I., Polanski, L., 2022. Data-Driven Medicine in the Diagnosis and Treatment of Infertility. J Clin Med 11, 6426. https://doi.org/10.3390/JCM11216426

Diakiw, Sonya M., Hall, J.M.M., VerMilyea, M., Lim, A.Y.X., Quangkananurug, W., Chanchamroen, S., Bankowski, B., Stones, R., Storr, A., Miller, A., Adaniya, G., van Tol, R.A., Hanson, R., Aizpurua, J., Giardini, L., Johnston, A., van Nguyen, T., Dakka, M.A., Perugini, D., Perugini, M., 2022. An artificial intelligence model correlated with morphological and genetic features of blastocyst quality improves ranking of viable embryos. Reprod Biomed Online 45. https://doi.org/10.1016/J.RBMO.2022.07.018

Diakiw, S. M., Hall, J.M.M., VerMilyea, M.D., Amin, J., Aizpurua, J., Giardini, L., Briones, Y.G., Lim, A.Y.X., Dakka, M.A., Nguyen, T. v., Perugini, D., Perugini, M., 2022. Development of an artificial intelligence model for predicting the likelihood of human embryo euploidy based on blastocyst images from multiple imaging systems during IVF. Hum Reprod 37, 1746–1759. https://doi.org/10.1093/HUMREP/DEAC131

Dimitriadis, I., Zaninovic, N., Badiola, A.C., Bormann, C.L., 2022. Artificial intelligence in the embryology laboratory: a review. Reprod Biomed Online 44, 435–448. https://doi.org/10.1016/J.RBMO.2021.11.003

Enatsu, N., Miyatsuka, I., An, L.M., Inubushi, M., Enatsu, K., Otsuki, J., Iwasaki, T., Kokeguchi, S., Shiotani, M., 2022. A novel system based on artificial intelligence for predicting blastocyst viability and visualizing the explanation. Reprod Med Biol 21. https://doi.org/10.1002/RMB2.12443

Ezoe, K., Shimazaki, K., Miki, T., Takahashi, T., Tanimura, Y., Amagai, A., Sawado, A., Akaike, H., Mogi, M., Kaneko, S., Okimura, T., Kato, K., 2022. Association between a deep learning-based scoring system with morphokinetics and morphological alterations in human embryos. Reprod Biomed Online 45. https://doi.org/10.1016/J.RBMO.2022.08.098

Fanton, M., Nutting, V., Rothman, A., Maeder-York, P., Hariton, E., Barash, O., Weckstein, L., Sakkas, D., Copperman, A.B., Loewke, K., 2022. An interpretable machine learning model for individualized gonadotrophin starting dose selection during ovarian stimulation. Reprod Biomed Online 45. https://doi.org/10.1016/J.RBMO.2022.07.010

Fordham, D.E., Rosentraub, D., Polsky, A.L., Aviram, T., Wolf, Y., Perl, O., Devir, A., Rosentraub, S., Silver, D.H., Gold Zamir, Y., Bronstein, A.M., Lara Lara, M., ben Nagi, J., Alvarez, A., Munné, S., 2022. Embryologist agreement when assessing blastocyst implantation probability: is data-driven prediction the solution to embryo assessment subjectivity? Hum Reprod. https://doi.org/10.1093/HUMREP/DEAC171

Gardner, D.K., Sakkas, D., 2022. Making and selecting the best embryo in the laboratory. Fertil Steril. https://doi.org/10.1016/J.FERTNSTERT.2022.11.007

Gatimel, N., Moreau, J., Bettiol, C., Parinaud, J., Léandri, R.D., 2021. Semi-automated versus manual embryo vitrification: inter-operator variability, time-saving, and clinical outcomes. J Assist Reprod Genet 38, 3213–3222. https://doi.org/10.1007/S10815-021-02346-3

Hammer, K.C., Jiang, V.S., Kanakasabapathy, M.K., Thirumalaraju, P., Kandula, H., Dimitriadis, I., Souter, I., Bormann, C.L., Shafiee, H., 2022. Using artificial intelligence to avoid human error in identifying embryos: a retrospective cohort study. J Assist Reprod Genet 39, 2343–2348. https://doi.org/10.1007/S10815-022-02585-Y

Hernández-González, J., Valls, O., Torres-Martín, A., Cerquides, J., 2022. Modeling three sources of uncertainty in assisted reproductive technologies with probabilistic graphical models. Comput Biol Med 150. https://doi.org/10.1016/J.COMPBIOMED.2022.106160

Hua, L., Zhe, Y., Jing, Y., Fujin, S., Jiao, C., Liu, L., 2022. Prediction model of gonadotropin starting dose and its clinical application in controlled ovarian stimulation. BMC Pregnancy Childbirth 22. https://doi.org/10.1186/S12884-022-05152-6

Jeong, H.G., Kim, S.K., Lee, J.R., Jee, B.C., 2022. Correlation of oocyte number with serum anti-Müllerian hormone levels measured by either Access or Elecsys in fresh in vitro fertilization cycles. Clin Exp Reprod Med 49, 202–209. https://doi.org/10.5653/CERM.2022.05211

Jiang, X., Cai, J., Liu, L., Liu, Z., Wang, W., Chen, J., Yang, C., Geng, J., Ma, C., Ren, J., 2022. Does conventional morphological evaluation still play a role in predicting blastocyst formation? Reprod Biol Endocrinol 20. https://doi.org/10.1186/S12958-022-00945-Y

Joshi, A.S., Alegria, A.D., Auch, B., Khosla, K., Mendana, J.B., Liu, K., Bischof, J., Gohl, D.M., Kodandaramaiah, S.B., 2021. Multiscale, multi-perspective imaging assisted robotic microinjection of 3D biological structures. Annu Int Conf IEEE Eng Med Biol Soc 2021, 4844–4850. https://doi.org/10.1109/EMBC46164.2021.9630858

Kato, K., Ueno, S., Berntsen, J., Kragh, M.F., Okimura, T., Kuroda, T., 2022. Does embryo categorization by existing artificial intelligence, morphokinetic or morphological embryo selection models correlate with blastocyst euploidy rates? Reprod Biomed Online. https://doi.org/10.1016/J.RBMO.2022.09.010

Khodabandelu, S., Basirat, Z., Khaleghi, S., Khafri, S., Montazery Kordy, H., Golsorkhtabaramiri, M., 2022. Developing machine learning-based models to predict intrauterine insemination (IUI) success by address modeling challenges in imbalanced data and providing modification solutions for them. BMC Med Inform Decis Mak 22. https://doi.org/10.1186/S12911-022-01974-8

Kragh, M.F., Rimestad, J., Lassen, J.T., Berntsen, J., Karstoft, H., 2022. Predicting Embryo Viability Based on Self-Supervised Alignment of Time-Lapse Videos. IEEE Trans Med Imaging 41, 465–475. https://doi.org/10.1109/TMI.2021.3116986

la Marca, A., Capuzzo, M., Longo, M., Imbrogno, M.G., Spedicato, G.A., Fiorentino, F., Spinella, F., Greco, P., Minasi, M.G., Greco, E., 2022. The number and rate of euploid blastocysts in women undergoing IVF/ICSI cycles are strongly dependent on ovarian reserve and female age. Hum Reprod. https://doi.org/10.1093/HUMREP/DEAC191

Lee, R., Witherspoon, L., Robinson, M., Lee, J.H., Duffy, S.P., Flannigan, R., Ma, H., 2022. Automated rare sperm identification from low-magnification microscopy images of dissociated microsurgical testicular sperm extraction samples using deep learning. Fertil Steril 118, 90–99. https://doi.org/10.1016/J.FERTNSTERT.2022.03.011

Letterie, G., MacDonald, A., Shi, Z., 2022. An artificial intelligence platform to optimize workflow during ovarian stimulation and IVF: process improvement and outcome-based predictions. Reprod Biomed Online 44, 254–260. https://doi.org/10.1016/J.RBMO.2021.10.006

Liang, X., Liang, J., Zeng, F., Lin, Y., Li, Y., Cai, K., Ni, D., Chen, Z., 2022. Evaluation of oocyte maturity using artificial intelligence quantification of follicle volume biomarker by three-dimensional ultrasound. Reprod Biomed Online 45. https://doi.org/10.1016/J.RBMO.2022.07.012

Liu, G., Shi, H., Zhang, H., Zhou, Y., Sun, Y., Li, W., Huang, X., Jiang, Y., Fang, Y., Yang, G., 2022. Fast Noninvasive Morphometric Characterization of Free Human Sperms Using Deep Learning. Microsc Microanal 28, 1767–1779. https://doi.org/10.1017/S1431927622012132

Liu, Y., Cui, M., Zhang, Y., Zhao, Xiangfei, Sun, M., Zhao, Xin, 2022. Oocyte Penetration Speed Optimization Based on Intracellular Strain. Micromachines (Basel) 13. https://doi.org/10.3390/MI13020309

Loewke, K., Cho, J.H., Brumar, C.D., Maeder-York, P., Barash, O., Malmsten, J.E., Zaninovic, N., Sakkas, D., Miller, K.A., Levy, M., VerMilyea, M.D., 2022. Characterization of an artificial intelligence model for ranking static images of blastocyst stage embryos. Fertil Steril 117, 528–535. https://doi.org/10.1016/J.FERTNSTERT.2021.11.022

Malin-Mayor, C., Hirsch, P., Guignard, L., McDole, K., Wan, Y., Lemon, W.C., Kainmueller, D., Keller, P.J., Preibisch, S., Funke, J., 2022. Automated reconstruction of whole-embryo cell lineages by learning from sparse annotations. Nat Biotechnol. https://doi.org/10.1038/S41587-022-01427-7

Marsh, P., Radif, D., Rajpurkar, P., Wang, Z., Hariton, E., Ribeiro, S., Simbulan, R., Kaing, A., Lin, W., Rajah, A., Rabara, F., Lungren, M., Demirci, U., Ng, A., Rosen, M., 2022. A proof of concept for a deep learning system that can aid embryologists in predicting blastocyst survival after thaw. Sci Rep 12. https://doi.org/10.1038/S41598-022-25062-Z

Mehrjerd, A., Rezaei, H., Eslami, S., Khadem Ghaebi, N., 2022. Determination of Cut Off for Endometrial Thickness in Couples with Unexplained Infertility: Trustable AI. Stud Health Technol Inform 294, 264–268. https://doi.org/10.3233/SHTI220450

Miao, S., Jiang, Z., Luo, J., Zhong, F., Wei, H., Sun, X., Jiang, X., Jiang, M., Liu, Y., 2022. A Robotic System With Embedded Open Microfluidic Chip for Automatic Embryo Vitrification. IEEE Trans Biomed Eng 69. https://doi.org/10.1109/TBME.2022.3171628

Mokhtare, A., Davaji, B., Xie, P., Yaghoobi, M., Rosenwaks, Z., Lal, A., Palermo, G., Abbaspourrad, A., 2022. Non-contact ultrasound oocyte denudation. Lab Chip 22, 777–792. https://doi.org/10.1039/D1LC00715G

Mushtaq, A., Mumtaz, M., Raza, A., Salem, N., Yasir, M.N., 2022. Artificial Intelligence-Based Detection of Human Embryo Components for Assisted Reproduction by In Vitro Fertilization. Sensors (Basel) 22. https://doi.org/10.3390/S22197418

Oktay, K., Marin, L., Bedoschi, G., Pacheco, F., Sugishita, Y., Kawahara, T., Taylan, E., Acosta, C., Bang, H., 2022. Ovarian transplantation with robotic surgery and a neovascularizing human extracellular matrix scaffold: a case series in comparison to meta-analytic data. Fertil Steril 117, 181–192. https://doi.org/10.1016/J.FERTNSTERT.2021.08.034

Ortiz, J.A., Morales, R., Lledó, B., Vicente, J.A., González, J., García-Hernández, E.M., Cascales, A., Ten, J., Bernabeu, A., Bernabeu, R., 2022. Application of machine learning to predict aneuploidy and mosaicism in embryos from in vitro fertilization cycles. AJOG global reports 2, 100103. https://doi.org/10.1016/J.XAGR.2022.100103

Ozer, G., Akca, A., Yuksel, B., Duzguner, I., Pehlivanli, A.C., Kahraman, S., 2022. Prediction of risk factors for first trimester pregnancy loss in frozen-thawed good-quality embryo transfer cycles using machine learning algorithms. J Assist Reprod Genet. https://doi.org/10.1007/S10815-022-02645-3

Payá, E., Bori, L., Colomer, A., Meseguer, M., Naranjo, V., 2022b. Automatic characterization of human embryos at day 4 post-insemination from time-lapse imaging using supervised contrastive learning and inductive transfer learning techniques. Comput Methods Programs Biomed 221. https://doi.org/10.1016/J.CMPB.2022.106895

Sayadi, N., Monji-Azad, S., Mirroshandel, S.A., Ghasemian, F., 2022. Morphometric evaluation of two-pronucleus zygote images using image-processing techniques. Zygote 30, 1–11. https://doi.org/10.1017/S0967199422000326

Sfakianoudis, K., Maziotis, E., Grigoriadis, S., Pantou, A., Kokkini, G., Trypidi, A., Giannelou, P., Zikopoulos, A., Angeli, I., Vaxevanoglou, T., Pantos, K., Simopoulou, M., 2022. Reporting on the Value of Artificial Intelligence in Predicting the Optimal Embryo for Transfer: A Systematic Review including Data Synthesis. Biomedicines 10. https://doi.org/10.3390/BIOMEDICINES10030697

Shen, L., Zhang, Y., Chen, W., Yin, X., 2022. The Application of Artificial Intelligence in Predicting Embryo Transfer Outcome of Recurrent Implantation Failure. Front Physiol 13. https://doi.org/10.3389/FPHYS.2022.885661

Srivastava, D., Gupta, S., Kudavelly, S., Venkata Suryanarayana, K., Ga, R., 2021. Unsupervised Deep Learning based Longitudinal Follicular Growth Tracking during IVF Cycle using 3D Transvaginal Ultrasound in Assisted Reproduction. Annu Int Conf IEEE Eng Med Biol Soc 2021, 3209–3212. https://doi.org/10.1109/EMBC46164.2021.9630495

Sun, S., Miller, M., Wang, Y., Tyc, K.M., Cao, X., Scott, R.T., Tao, X., Bromberg, Y., Schindler, K., Xing, J., 2022. Predicting embryonic aneuploidy rate in IVF patients using whole-exome sequencing. Hum Genet 141, 1615–1627. https://doi.org/10.1007/S00439-022-02450-Z

Tokuoka, Y., Yamada, T.G., Mashiko, D., Ikeda, Z., Kobayashi, T.J., Yamagata, K., Funahashi, A., 2022. An explainable deep learning-based algorithm with an attention mechanism for predicting the live birth potential of mouse embryos. Artif Intell Med 134, 102432. https://doi.org/10.1016/J.ARTMED.2022.102432

Toporcerová, S., Špaková, I., Šoltys, K., Klepcová, Z., Kľoc, M., Bohošová, J., Trachtová, K., Peterová, L., Mičková, H., Urdzík, P., Mareková, M., Slabý, O., Rabajdová, M., 2022. Small Non-Coding RNAs as New Biomarkers to Evaluate the Quality of the Embryo in the IVF Process. Biomolecules 12. https://doi.org/10.3390/BIOM12111687

Tu, F., Bhat, M., Benson, J.D., 2022. Real-time computer assisted measurement of oocyte and embryo volume for assessment of transport parameters. Cryobiology 108, 19–26. https://doi.org/10.1016/J.CRYOBIOL.2022.08.005

Ueno, S., Berntsen, J., Ito, M., Okimura, T., Kato, K., 2022. Correlation between an annotation-free embryo scoring system based on deep learning and live birth/neonatal outcomes after single vitrified-warmed blastocyst transfer: a single-centre, large-cohort retrospective study. J Assist Reprod Genet. https://doi.org/10.1007/s10815-022-02562-5

Wang, C.W., Kuo, C.Y., Chen, C.H., Hsieh, Y.H., Su, E.C.Y., 2022. Predicting clinical pregnancy using clinical features and machine learning algorithms in in vitro fertilization. PLoS One 17. https://doi.org/10.1371/JOURNAL.PONE.0267554

Wen, J.Y., Liu, C.F., Chung, M.T., Tsai, Y.C., 2022. Artificial intelligence model to predict pregnancy and multiple pregnancy risk following in vitro fertilization-embryo transfer (IVF-ET). Taiwan J Obstet Gynecol 61, 837–846. https://doi.org/10.1016/J.TJOG.2021.11.038

Yagi, N., Tsuji, H., Morimoto, T., Maekawa, T., Mizuta, S., Ishikawa, T., Hata, Y., 2022. Rupture Prediction for Microscopic Oocyte Images of Piezo Intracytoplasmic Sperm Injection by Principal Component Analysis. J Clin Med 11, 6546. https://doi.org/10.3390/JCM11216546

Yiğit, P., Bener, A., Karabulut, S., 2022. Comparison of machine learning classification techniques to predict implantation success in an IVF treatment cycle. Reprod Biomed Online 45, 923–934. https://doi.org/10.1016/J.RBMO.2022.06.022

Zhai, R., Shan, G., Dai, C., Hao, M., Zhu, J., Ru, C., Sun, Y., 2022. Automated Denudation of Oocytes. Micromachines (Basel) 13. https://doi.org/10.3390/MI13081301

Zhang, Y., Shen, L., Yin, X., Chen, W., 2022. Live-Birth Prediction of Natural-Cycle In Vitro Fertilization Using 57,558 Linked Cycle Records: A Machine Learning Perspective. Front Endocrinol (Lausanne) 13. https://doi.org/10.3389/FENDO.2022.838087

Zinchenko, V., Chetverikov, S., Akhmad, E., Arzamasov, K., Vladzymyrskyy, A., Andreychenko, A., Morozov, S., 2022. Changes in software as a medical device based on artificial intelligence technologies. Int J Comput Assist Radiol Surg 17. https://doi.org/10.1007/S11548-022-02669-1

Zou, Y., Pan, Y., Ge, N., Xu, Y., Gu, R., Li, Z., Fu, J., Gao, J., Sun, X., Sun, Y., 2022. Can the combination of time-lapse parameters and clinical features predict embryonic ploidy status or implantation? Reprod Biomed Online 45, 643–651. https://doi.org/10.1016/J.RBMO.2022.06.007

Artificial wombs (ectogenesis)

de Bie, F.R., Kim, S.D., Bose, S.K., Nathanson, P., Partridge, E.A., Flake, A.W., Feudtner, C., 2022. Ethics Considerations Regarding Artificial Womb Technology for the Fetonate. Am J Bioeth. https://doi.org/10.1080/15265161.2022.2048738

Flake, A.W., 2022. A supportive physiologic environment for the extreme premature infant: Improving life outside the womb. J Pediatr Surg 57, 167–171. https://doi.org/10.1016/J.JPEDSURG.2021.10.025

Hooton, V., Romanis, E.C., 2022. Artificial womb technology, pregnancy, and EU employment rights. J Law Biosci 9. https://doi.org/10.1093/JLB/LSAC009

Larson, A.C., de Bie, F.R., Chang, J., Davey, M.G., Flake, A.W., 2022. The EXTrauterine Environment for Neonatal Development: Present and Future. Pediatr Dev Pathol 25, 253–262. https://doi.org/10.1177/10935266211067110

Romanis, E.C., 2022. Assisted gestative technologies. J Med Ethics 48, 439–446. https://doi.org/10.1136/MEDETHICS-2021-107769

Segers, S., Romanis, E.C., 2022. Ethical, Translational, and Legal Issues Surrounding the Novel Adoption of Ectogestative Technologies. Risk Manag Healthc Policy 15, 2207–2220. https://doi.org/10.2147/RMHP.S358553

Spencer, B.L., Mychaliska, G.B., 2022a. Updates in Neonatal Extracorporeal Membrane Oxygenation and the Artificial Placenta. Clin Perinatol 49, 873–891. https://doi.org/10.1016/J.CLP.2022.07.002

Spencer, B.L., Mychaliska, G.B., 2022b. Milestones for clinical translation of the artificial placenta. Semin Fetal Neonatal Med 27. https://doi.org/10.1016/J.SINY.2022.101408

Verweij, E.J., de Proost, L., van Laar, J.O.E.H., Frank, L., Obermann-Borstn, S.A., Vermeulen, M.J., van Baalen, S., van der Hout-van der Jagt, M.B., Kingma, E., 2021. Ethical Development of Artificial Amniotic Sac and Placenta Technology: A Roadmap. Front Pediatr 9. https://doi.org/10.3389/FPED.2021.793308

Werner, K.M., Mercurio, M.R., 2022. Ethical considerations in the use of artificial womb/placenta technology. Semin Perinatol 46. https://doi.org/10.1016/J.SEMPERI.2021.151521

Wozniak, P.S., 2022. Clinical challenges to the concept of ectogestation. J Med Ethics medethics-2021-107892. https://doi.org/10.1136/MEDETHICS-2021-107892

Zaami, S., Gullo, G., Varone, M.C., Ronchi, F.U., Vergallo, G.M., 2021. From the maternal uterus to the “uterus device”? Ethical and scientific considerations on partial ectogenesis. Eur Rev Med Pharmacol Sci 25, 7354–7362. https://doi.org/10.26355/EURREV_202112_27429

Extension to the 14-day rule

Anifandis, G., Sutovsky, P., Turek, P.J., Chavez, S.L., Kunej, T., Messini, C.I., Schon, S.B., Mavroforou, A., Adashi, E.Y., Krawetz, S.A., 2022. Bioethics in human embryology: the double-edged sword of embryo research. Syst Biol Reprod Med 68, 169–179. https://doi.org/10.1080/19396368.2022.2052771

Matthews, K.R.W., Morali, D., 2022. Can we do that here? An analysis of US federal and state policies guiding human embryo and embryoid research. J Law Biosci 9. https://doi.org/10.1093/JLB/LSAC014

Nuffield Council on Bioethics, 2017. *Discussions concerning the statutory time limit for maintaining human embryos in culture in the light of some recent scientific developments*. Available at: https://www.nuffieldbioethics.org/assets/pdfs/Human-Embryo-Culture.pdf (Accessed: 27/06/2022)

Peng, Y., Lv, J., Xiao, Z., Ding, L., Zhou, Q., 2022. A framework for the responsible reform of the 14-day rule in human embryo research. Protein Cell 13, 552–558. https://doi.org/10.1007/S13238-022-00907-5

Tarazi, S., Aguilera-Castrejon, A., Joubran, C., Ghanem, N., Ashouokhi, S., Roncato, F., Wildschutz, E., Haddad, M., Oldak, B., Gomez-Cesar, E., Livnat, N., Viukov, S., Lokshtanov, D., Naveh-Tassa, S., Rose, M., Hanna, S., Raanan, C., Brenner, O., Kedmi, M., Keren-Shaul, H., Lapidot, T., Maza, I., Novershtern, N., Hanna, J.H., 2022. Post-gastrulation synthetic embryos generated ex utero from mouse naive ESCs. Cell 185, 3290-3306.e25. https://doi.org/10.1016/J.CELL.2022.07.028

Tyser, R.C.V., Srinivas, S., 2022. Recent advances in understanding cell types during human gastrulation. Semin Cell Dev Biol 131. https://doi.org/10.1016/J.SEMCDB.2022.05.004

Xue, Y., Shang, L., 2022. Are we ready for the revision of the 14-day rule? Implications from Chinese legislations guiding human embryo and embryoid research. Front Cell Dev Biol 10. https://doi.org/10.3389/FCELL.2022.1016988

Germline genome editing

Bekaert, B., Boel, A., Cosemans, G., de Witte, L., Menten, B., Heindryckx, B., 2022. CRISPR/Cas gene editing in the human germline. Semin Cell Dev Biol 131, 93–107. https://doi.org/10.1016/J.SEMCDB.2022.03.012

Benston, S., 2022. Walking a Fine Germline: Synthesizing Public Opinion and Legal Precedent to Develop Policy Recommendations for Heritable Gene-Editing. J Bioeth Inq 19, 421–431. https://doi.org/10.1007/S11673-022-10186-8

Dang, L., Zhou, X., Zhong, X., Yu, W., Huang, S., Liu, H., Chen, Y., Zhang, W., Yuan, L., Li, L., Huang, X., Li, G., Liu, J., Tong, G., 2022. Correction of the pathogenic mutation in TGM1 gene by adenine base editing in mutant embryos. Molecular Therapy 30, 175–183. https://doi.org/10.1016/j.ymthe.2021.05.007

Labude, M.K., Xafis, V., Lai, P.S., Mills, C., 2022. Vulnerability and the Ethics of Human Germline Genome Editing. CRISPR J 5, 358–363. https://doi.org/10.1089/CRISPR.2021.0053

Lasemi, M.V., Sahebkar, A., 2022. Non-surgical in vivo germ cell-mediated gene editing by CRISPR mutagenic chain reaction with the aid of magnetic nanoparticles. Curr Med Chem 29. https://doi.org/10.2174/0929867329666220722113832

M. Farrell, R., Malek, J., Thomas Scott, C., 2021. Beyond babies: Implications of human genome editing for women, children, and families. https://doi.org/10.1080/08989621.2021.1899824 29, 67–76. https://doi.org/10.1080/08989621.2021.1899824

Mattar, C.N.Z., Labude, M.K., Lee, T.N., Lai, P.S., 2021. Ethical considerations of preconception and prenatal gene modification in the embryo and fetus. Hum Reprod 36, 3018–3027. https://doi.org/10.1093/HUMREP/DEAB222

Peng, Y., Lv, J., Ding, L., Gong, X., Zhou, Q., 2022. Responsible governance of human germline genome editing in China†. Biol Reprod 107, 261–268. https://doi.org/10.1093/BIOLRE/IOAC114

Petri, K., Zhang, W., Ma, J., Schmidts, A., Lee, H., Horng, J.E., Kim, D.Y., Kurt, I.C., Clement, K., Hsu, J.Y., Pinello, L., Maus, M. v., Joung, J.K., Yeh, J.R.J., 2022. CRISPR prime editing with ribonucleoprotein complexes in zebrafish and primary human cells. Nat Biotechnol 40, 189–193. https://doi.org/10.1038/S41587-021-00901-Y

Stamatiadis, P., Cosemans, G., Boel, A., Menten, B., de Sutter, P., Stoop, D., Chuva de Sousa Lopes, S.M., Lluis, F., Coucke, P., Heindryckx, B., 2022. TEAD4 regulates trophectoderm differentiation upstream of CDX2 in a GATA3-independent manner in the human preimplantation embryo. Hum Reprod 37, 1760–1773. https://doi.org/10.1093/HUMREP/DEAC138

Zhang, X., Li, T., Ou, J., Huang, J., Liang, P., 2022. Homology-based repair induced by CRISPR-Cas nucleases in mammalian embryo genome editing. Protein Cell 13, 316–335. https://doi.org/10.1007/S13238-021-00838-7

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

Aoki, S., Hashimoto, K., Ogawa, K., Horikawa, R., Sago, H., 2022. Developmental outcomes in Japanese preschool-age children conceived through assisted reproductive technology. J Obstet Gynaecol Res 48. https://doi.org/10.1111/JOG.15386

Arendt, L.H., Gaml-Sørensen, A., Ernst, A., Brix, N., Toft, G., Tøttenborg, S.S., Hougaard, K.S., Bonde, J.P.E., Ramlau-Hansen, C.H., 2022. Semen quality and reproductive hormones in sons of subfertile couples: a cohort study. Fertil Steril 118. https://doi.org/10.1016/J.FERTNSTERT.2022.06.035

Basso, O., Shapiro, G.D., Twardowski, S.E., Monnier, P., Buckett, W., Tamblyn, R., 2022. The influence of regulation of medically assisted reproduction on the risk of hospitalization in the first 2 years of life. Hum Reprod 37, 2143–2153. https://doi.org/10.1093/HUMREP/DEAC158

Borgmann-Staudt, A., Michael, S., Sommerhaeuser, G., Fernández-González, M.J., Friedrich, L.A., Klco-Brosius, S., Kepak, T., Kruseova, J., Michel, G., Panasiuk, A., Schmidt, S., Lotz, L., Balcerek, M., 2022. The Use of Assisted Reproductive Technology by European Childhood Cancer Survivors. Curr Oncol 29, 5748–5762. https://doi.org/10.3390/CURRONCOL29080453

Catford, Sarah R., Halliday, J., Lewis, S., O'Bryan, M.K., Handelsman, D.J., Hart, R.J., McBain, J., Rombauts, L., Amor, D.J., Saffery, R., McLachlan, R.I., 2022. Reproductive function in men conceived with in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 117, 727–737. https://doi.org/10.1016/J.FERTNSTERT.2021.12.026

Catford, S R, Halliday, J., Lewis, S., O'Bryan, M.K., Handelsman, D.J., Hart, R.J., McBain, J., Rombauts, L., Amor, D.J., Saffery, R., McLachlan, R.I., 2022. The metabolic health of young men conceived using intracytoplasmic sperm injection. Hum Reprod. https://doi.org/10.1093/HUMREP/DEAC212

Cristea, O., Mohora, R., Nastase, L., Diaconu, A., Stoicescu, S.M., 2022. Respiratory pathology in late preterm infants conceived by in vitro fertilization. J Med Life 15, 1047–1051. https://doi.org/10.25122/JML-2022-0194

Cui, L., Zhao, M., Zhang, Z., Zhou, W., Lv, J., Hu, J., Ma, J., Fang, M., Yang, L., Magnussen, C.G., Xi, B., Chen, Z.J., 2021. Assessment of Cardiovascular Health of Children Ages 6 to 10 Years Conceived by Assisted Reproductive Technology. JAMA Netw Open 4. https://doi.org/10.1001/JAMANETWORKOPEN.2021.32602

Elhakeem, A., Taylor, A.E., Inskip, H.M., Huang, J., Tafflet, M., Vinther, J.L., Asta, F., Erkamp, J.S., Gagliardi, L., Guerlich, K., Halliday, J., Harskamp-Van Ginkel, M.W., He, J.R., Jaddoe, V.W.V., Lewis, S., Maher, G.M., Manios, Y., Mansell, T., McCarthy, F.P., McDonald, S.W., Medda, E., Nisticò, L., de Moira, A.P., Popovic, M., Reiss, I.K.M., Rodrigues, C., Salika, T., Smith, A., Stazi, M.A., Walker, C., Wu, M., Åsvold, B.O., Barros, H., Brescianini, S., Burgner, D., Chan, J.K.Y., Charles, M.A., Eriksson, J.G., Gaillard, R., Grote, V., Håberg, S.E., Heude, B., Koletzko, B., Morton, S., Moschonis, G., Murray, D., O'Mahony, D., Porta, D., Qiu, X., Richiardi, L., Rusconi, F., Saffery, R., Tough, S.C., Vrijkotte, T.G.M., Nelson, S.M., Nybo Andersen, A.M., Magnus, M.C., Lawlor, D.A., 2022. Association of Assisted Reproductive Technology With Offspring Growth and Adiposity From Infancy to Early Adulthood. JAMA Netw Open 5, E2222106. https://doi.org/10.1001/JAMANETWORKOPEN.2022.22106

Gao, J., Zhang, Y., Cui, L., Zhang, T., Wu, B., Gao, S., Chen, Z.J., 2022. "Double Frozen Transfer" Could Influence the Perinatal and Children's Growth: A Nested Case-Control Study of 6705 Live Birth Cycles. Front Endocrinol (Lausanne) 13. https://doi.org/10.3389/FENDO.2022.878929

Gullo, G., Scaglione, M., Cucinella, G., Chiantera, V., Perino, A., Greco, M.E., Laganà, A.S., Marinelli, E., Basile, G., Zaami, S., 2022. Neonatal Outcomes and Long-Term Follow-Up of Children Born from Frozen Embryo, a Narrative Review of Latest Research Findings. Medicina (Kaunas) 58. https://doi.org/10.3390/MEDICINA58091218

Gulrajani, N.B., Montes, S., McGough, D., Wimberly, C.E., Khattab, A., Semmes, E.C., Towry, L., Cohen, J.L., Hurst, J.H., Landi, D., Hill, S.N., Walsh, K.M., 2022. Assisted reproductive technology and association with childhood cancer subtypes. Cancer Med. https://doi.org/10.1002/CAM4.5114

Hart, R.J., Penova-Veselinovic, B., Wijs, L.A., Yovich, J.L., Burton, P., 2022. Cohort profile: The Growing Up Healthy Study (GUHS)-A prospective and observational cohort study investigating the long-term health outcomes of offspring

conceived after assisted reproductive technologies. PLoS One 17. https://doi.org/10.1371/JOURNAL.PONE.0272064

Jiang, Y., Zhu, H., Chen, H., Yu, Y.-C., Xu, Y.-T., Liu, F., He, S.-N., Sagnelli, M., Zhu, Y.-M., Luo, Q., 2022. Elevated Expression of lncRNA MEG3 Induces Endothelial Dysfunction on HUVECs of IVF Born Offspring via Epigenetic Regulation. Front Cardiovasc Med 8. https://doi.org/10.3389/FCVM.2021.717729

Lisonkova, S., Ukah, U.V., John, S., Yearwood, L., Muraca, G.M., Razaz, N., Sabr, Y., Yong, P.J., Bedaiwy, M.A., 2022. Racial and Ethnic Disparities in the Perinatal Health of Infants Conceived by ART. Pediatrics 150. https://doi.org/10.1542/PEDS.2021-055855

Lloyd, D.T., Skinner, H.G., Maguire, R., Murphy, S.K., Motsinger-Reif, A.A., Hoyo, C., House, J.S., 2022. Clomifene and Assisted Reproductive Technology in Humans Are Associated with Sex-Specific Offspring Epigenetic Alterations in Imprinted Control Regions. Int J Mol Sci 23. https://doi.org/10.3390/IJMS231810450

Luke, B., Brown, M.B., Wantman, E., Schymura, M.J., Browne, M.L., Fisher, S.C., Forestieri, N.E., Rao, C., Nichols, H.B., Yazdy, M.M., Gershman, S.T., Sacha, C.R., Williams, M., Ethen, M.K., Canfield, M.A., Doody, K.J., Eisenberg, M.L., Baker, V.L., Williams, C., Sutcliffe, A.G., Richard, M.A., Lupo, P.J., 2022. The risks of birth defects and childhood cancer with conception by assisted reproductive technology. Hum Reprod 37. https://doi.org/10.1093/HUMREP/DEAC196

Mitter, V.R., Håberg, S.E., Magnus, M.C., 2022. Early childhood respiratory tract infections according to parental subfertility and conception by assisted reproductive technologies. Hum Reprod 37, 2113–2125. https://doi.org/10.1093/HUMREP/DEAC162

Mizrak, I., Asserhøj, L.L., Lund, M.A.V., Kielstrup, L.R., Greisen, G., Clausen, T.D., Main, K.M., Jensen, R.B., Vejlstrup, N.G., Madsen, P.L., Pinborg, A., 2022. Cardiovascular function in 8- to 9-year-old singletons born after ART with frozen and fresh embryo transfer. Hum Reprod 37, 600–611. https://doi.org/10.1093/HUMREP/DEAB284

Noda, A., Ishikuro, M., Obara, T., Murakami, K., Ueno, F., Matsuzaki, F., Onuma, T., Watanabe, Z., Shiga, N., Iwama, N., Hirotaka, H., Otsuka, T., Tachibana, M., Tomita, H., Saito, M., Sugawara, J., Kure, S., Yaegashi, N., Kuriyama, S., 2022. Association between maternal infertility treatment and child neurodevelopment: findings from the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study in Miyagi and Iwate Prefectures, Japan. BMJ Open 12. https://doi.org/10.1136/BMJOPEN-2022-060944

Perros, P., Psarris, A., Antsaklis, P., Theodora, M., Syndos, M., Koutras, A., Ntounis, T., Fasoulakis, Z., Rodolakis, A., Daskalakis, G., 2022. Neurodevelopmental Outcomes of Pregnancies Resulting from Assisted Reproduction: A Review of the Literature. Children (Basel) 9. https://doi.org/10.3390/CHILDREN9101511

Pettersson, M.L., Bladh, M., Nedstrand, E., Svanberg, A.S., Lampic, C., Sydsjö, G., 2022. Maternal advanced age, single parenthood, and ART increase the risk of child morbidity up to five years of age. BMC Pediatr 22. https://doi.org/10.1186/S12887-021-03103-2

Pohjonen, E.M., Söderström-Anttila, V., Bergh, C., Loft, A., Magnusson, Pinborg, A., Oldereid, N.B., Petzold, M., Romundstad, L.B., Laivuori, H., 2022. Obstetric and perinatal risks after the use of donor sperm: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol 274, 210–228. https://doi.org/10.1016/J.EJOGRB.2022.05.031

Polinski, K.J., Stevens, D.R., Mendola, P., Lin, T.C., Sundaram, R., Bell, E., Yeung, E.H., 2022. Infertility treatment associated with childhood asthma and atopy. Hum Reprod 37, 1609–1618. https://doi.org/10.1093/HUMREP/DEAC070

Sanders, J.N., Simonsen, S.E., Porucznik, C.A., Hammoud, A.O., Smith, K.R., Stanford, J.B., 2022. Fertility treatments and the risk of preterm birth among women with subfertility: a linked-data retrospective cohort study. Reprod Health 19. https://doi.org/10.1186/S12978-022-01363-4

Schroeder, M., Badini, G., Sferruzzi-Perri, A.N., Albrecht, C., 2022. The Consequences of Assisted Reproduction Technologies on the Offspring Health Throughout Life: A Placental Contribution. Front Cell Dev Biol 10. https://doi.org/10.3389/FCELL.2022.906240

Sciorio, R., Esteves, S.C., 2022. Contemporary Use of ICSI and Epigenetic Risks to Future Generations. J Clin Med 11. https://doi.org/10.3390/JCM11082135

Sutcliffe, A.G., Purkayastha, M., Brison, D.R., Nelson, S.M., Roberts, S.A., Lawlor, D.A., 2022. General health in a cohort of children conceived after assisted reproductive technology in the United Kingdom: a population-based record-linkage study. Am J Obstet Gynecol. https://doi.org/10.1016/J.AJOG.2022.07.032

Wang, C., Johansson, A.L.V., Rodriguez-Wallberg, K.A., Landén, M., Almqvist, C., Hernández-Díaz, S., Oberg, A.S., 2022. Long-term Follow-up of Psychiatric Disorders in Children and Adolescents Conceived by Assisted Reproductive Techniques in Sweden. JAMA Psychiatry 79, 133–142. https://doi.org/10.1001/JAMAPSYCHIATRY.2021.3647

Wang, L., Chen, M., Yan, G., Zhao, S., 2021. DNA Methylation Differences Between Zona Pellucida-Bound and Manually Selected Spermatozoa Are Associated With Autism Susceptibility. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.774260

Wang, R., Shi, Q., Jia, B., Zhang, W., Zhang, H., Shan, Y., Qiao, L., Chen, G., Chen, C., 2022. Association of Preterm Singleton Birth With Fertility Treatment in the US. JAMA Netw Open 5. https://doi.org/10.1001/JAMANETWORKOPEN.2021.47782

Weng, S.S., Chien, L.Y., Huang, Yi Ting, Huang, Yen Tsung, Chang, M., 2022a. Why does mode of conception affect early breastfeeding outcomes? A retrospective cohort study. PLoS One 17. https://doi.org/10.1371/JOURNAL.PONE.0265776

Weng, S.S., Huang, Yen Tsung, Huang, Yi Ting, Li, Y.P., Chien, L.Y., 2022b. Assisted Reproductive Technology and Risk of Childhood Cancers. JAMA Netw Open 5, E2230157. https://doi.org/10.1001/JAMANETWORKOPEN.2022.30157

Wijs, L.A., Doherty, D.A., Keelan, J.A., Burton, P., Yovich, J.L., Beilin, L., Mori, T.A., Huang, R.C., Adams, L.A., Olynyk, J.K., Ayonrinde, O.T., Penova-Veselinovic, B., Hart, R.J., 2022. Comparison of the cardiometabolic profiles of adolescents conceived through ART with those of a non-ART cohort. Hum Reprod 37, 1880–1895. https://doi.org/10.1093/HUMREP/DEAC122

Wijs, L.A., Doherty, D.A., Keelan, J.A., Burton, P., Yovich, J.L., Robinson, M., Hart, R.J., 2022. Mental health and behavioural problems in adolescents conceived after ART. Hum Reprod. https://doi.org/10.1093/HUMREP/DEAC214

Wijs, Laura A., Doherty, D.A., Keelan, J.A., Penova-Veselinovic, B., Burton, P., Yovich, J.L., Hall, G.L., Sly, P.D., Holt, P.G., Hart, R.J., 2022. Asthma and allergies in a cohort of adolescents conceived with ART. Reprod Biomed Online. https://doi.org/10.1016/J.RBMO.2022.07.007

Wijs, L.A., Fusco, M.R., Doherty, D.A., Keelan, J.A., Hart, R.J., 2021. Asthma and allergies in offspring conceived by ART: a systematic review and meta-analysis. Hum Reprod Update 28, 132–148. https://doi.org/10.1093/HUMUPD/DMAB031

Xie, Q., Jiang, W., Ji, H., Li, X., Zhou, Y., Zhao, C., Zhang, J., Lu, J., Ling, X., 2022. Perinatal outcomes of singletons born after blastocyst or cleavage-stage embryo transfer in FET cycles. Eur J Obstet Gynecol Reprod Biol 271, 265–270. https://doi.org/10.1016/J.EJOGRB.2022.03.001

Xiong, Y., Zang, X., Xie, T., Yang, C., Jiang, X., Chen, M., 2022. Additional Adverse Perinatal Outcomes With No Effect on Neonatal Mortality and Birth Defects in Pregnancies Conceived by Assisted Reproductive Technology. Front Pediatr 10. https://doi.org/10.3389/FPED.2022.809259

Yeung, E.H., Mendola, P., Sundaram, R., Lin, T.C., Broadney, M.M., Putnick, D.L., Robinson, S.L., Polinski, K.J., Wactawski-Wende, J., Ghassabian, A., O'Connor, T.G., Gore-Langton, R.E., Stern, J.E., Bell, E., 2022. Conception by fertility treatment and cardiometabolic risk in middle childhood. Fertil Steril 118, 349–359. https://doi.org/10.1016/J.FERTNSTERT.2022.04.030

Yu, H., Liang, Z., Cai, R., Jin, S., Xia, T., Wang, C., Kuang, Y., 2022. Association of adverse birth outcomes with in vitro fertilization after controlling infertility factors based on a singleton live birth cohort. Sci Rep 12. https://doi.org/10.1038/S41598-022-08707-X

Zhang, C.X., Xue, J.L., Zhao, W., Wu, Y.Q., Liu, X.Y., Wang, S.W., Li, L.H., Gu, S.M., Li, J.Q., Zhang, Y.Y., Zhang, F.H., Yang, Y.Z., Wang, Y.M., Zhu, Y.M., Xing, L.F., Qian, Y.L., Zhang, D., 2022. Embryo morphologic quality in relation to the metabolic and cognitive development of singletons conceived by in vitro fertilization and intracytoplasmic sperm injection: a matched cohort study. Am J Obstet Gynecol 227, 479.e1-479.e23. https://doi.org/10.1016/J.AJOG.2022.05.019

Impact of long-term cryopreservation of gametes and embryos

Albertini, D.F., Olsen, R., 2013. Effects of fertility preservation on oocyte genomic integrity. Adv Exp Med Biol 761, 19–27. https://doi.org/10.1007/978-1-4614-8214-7_3

Canosa, S., Cimadomo, D., Conforti, A., Maggiulli, R., Giancani, A., Tallarita, A., Golia, F., Fabozzi, G., Vaiarelli, A., Gennarelli, G., Revelli, A., Bongioanni, F., Alviggi, C., Ubaldi, F.M., Rienzi, L., 2022. The effect of extended cryo-storage following vitrification on embryo competence: a systematic review and meta-analysis. J Assist Reprod Genet 39, 873–882. https://doi.org/10.1007/S10815-022-02405-3

Cimadomo, D., Fabozzi, G., Dovere, L., Maggiulli, R., Albricci, L., Innocenti, F., Soscia, D., Giancani, A., Vaiarelli, A., Guido, M., Ubaldi, F.M., Rienzi, L., 2022. Clinical, obstetric and perinatal outcomes after vitrified-warmed euploid blastocyst transfer are independent of cryo-storage duration. Reprod Biomed Online 44, 221–227. https://doi.org/10.1016/J.RBMO.2021.09.027

Cobo, A., Garrido, N., Pellicer, A., Remohí, J., 2015. Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate. Fertil Steril 104, 1426-1434.e8. https://doi.org/10.1016/J.FERTNSTERT.2015.08.020

Cui, M., Dong, X., Lyu, S., Zheng, Y., Ai, J., 2021. The Impact of Embryo Storage Time on Pregnancy and Perinatal Outcomes and the Time Limit of Vitrification: A Retrospective Cohort Study. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.724853

Dinh, T., Son, W.-Y., Demirtas, E., Dahan, M.H., 2022. How long can oocytes be frozen with vitrification and still produce competent embryos? A series of six cases. Obstet Gynecol Sci. https://doi.org/10.5468/OGS.20344

Fang, Y., Zeng, S., Fu, X., Jia, B., Li, S., An, X., Chen, Y., Zhu, S., 2014. Developmental competence in vitro and in vivo of bovine IVF blastocyst after 15 years of vitrification. Cryo Letters 35, 232–238.

Hu, K.L., Hunt, S., Zhang, D., Li, R., Mol, B.W., 2022. The association between embryo storage time and treatment success in women undergoing freeze-all embryo transfer. Fertil Steril 118, 513–521. https://doi.org/10.1016/J.FERTNSTERT.2022.06.003

Huang, C., Lei, L., Wu, H.L., Gan, R.X., Yuan, X.B., Fan, L.Q., Zhu, W.B., 2019. Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes. Fertil Steril 112, 663-669.e1. https://doi.org/10.1016/J.FERTNSTERT.2019.06.008

Huo, Y., Yuan, P., Qin, Q., Yan, Z., Yan, L., Liu, P., Li, R., Yan, J., Qiao, J., 2021. Effects of vitrification and cryostorage duration on single-cell RNA-Seq profiling of vitrified-thawed human metaphase II oocytes. Front Med 15, 144–154. https://doi.org/10.1007/S11684-020-0792-7

Kably-Ambe, A., Carballo-Mondragón, E., Roque-Sánchez, A.M., Durán-Monterosas, L., Amaro-Hernández, E.A., 2016. Evaluation of semen parameters in long term cryopreserved samples for over 10 years. Ginecol Obstet Mex 84.

Li, J., Yin, M., Wang, B., Lin, J., Chen, Q., Wang, N., Lyu, Q., Wang, Y., Kuang, Y., Zhu, Q., 2020. The effect of storage time after vitrification on pregnancy and neonatal outcomes among 24 698 patients following the first embryo transfer cycles. Hum Reprod 35, 1675–1684. https://doi.org/10.1093/HUMREP/DEAA136

Lin, R., Zhou, H., Wang, C., Chen, H., Shu, J., Gan, X., Xu, K., Zhao, X., 2021. Does longer storage of blastocysts with equal grades in a cryopreserved state affect the perinatal outcomes? Cryobiology 103, 87–91. https://doi.org/10.1016/J.CRYOBIOL.2021.09.003

Liu, Q., Lian, Y., Huang, J., Ren, X., Li, M., Lin, S., Liu, P., Qiao, J., 2014. The safety of long-term cryopreservation on slow-frozen early cleavage human embryos. J Assist Reprod Genet 31, 471–475. https://doi.org/10.1007/S10815-014-0197-0

Mao, Y., Tang, N., Luo, Y., Yin, P., Li, L., 2022. Effects of vitrified cryopreservation duration on IVF and neonatal outcomes. J Ovarian Res 15. https://doi.org/10.1186/S13048-022-01035-8

Rodriguez-Wallberg, K.A., 2015. Clinical aspects and perinatal outcomes after cryopreservation of embryos and gametes. Minerva Ginecol 67, 207–215.

Stigliani, S., Moretti, S., Anserini, P., Casciano, I., Venturini, P.L., Scaruffi, P., 2015. Storage time does not modify the gene expression profile of cryopreserved human metaphase II oocytes. Hum Reprod 30, 2519–2526. https://doi.org/10.1093/HUMREP/DEV232

Ueno, S., Uchiyama, K., Kuroda, T., Yabuuchi, A., Ezoe, K., Okimura, T., Okuno, T., Kobayashi, T., Kato, K., 2018. Cryostorage duration does not affect pregnancy and neonatal outcomes: a retrospective single-centre cohort study of vitrified-warmed blastocysts. Reprod Biomed Online 36, 614–619. https://doi.org/10.1016/J.RBMO.2018.03.008

Urquiza, M.F., Carretero, I., Cano Carabajal, P.R., Pasqualini, R.A., Felici, M.M., Pasqualini, R.S., Quintans, C.J., 2014. Successful live birth from oocytes after more than 14 years of cryopreservation. J Assist Reprod Genet 31, 1553–1555. https://doi.org/10.1007/S10815-014-0318-9

Wirleitner, B., Vanderzwalmen, P., Bach, M., Baramsai, B., Neyer, A., Schwerda, D., Schuff, M., Spitzer, D., Stecher, A., Zintz, M., Zech, N.H., 2013. The time aspect in storing vitrified blastocysts: its impact on survival rate, implantation potential and babies born. Hum Reprod 28, 2950–2957. https://doi.org/10.1093/HUMREP/DET361

Yuan, Y., Mai, Q., Ma, J., Deng, M., Xu, Y., Zhuang, G., Zhou, C., 2019. What was the fate of human embryos following long-term cryopreservation (≥12 years) and frozen embryo transfer? Hum Reprod 34, 52–55. https://doi.org/10.1093/HUMREP/DEY350

Zhang, X., Wu, S., Hao, G., Wu, X., Ren, H., Zhang, Yinfeng, Yang, A., Bi, X., Bai, L., Zhang, Yunshan, Tan, J., 2021. Prolonged Cryopreservation Negatively Affects Embryo Transfer Outcomes Following the Elective Freeze-All Strategy: A Multicenter Retrospective Study. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.709648

Impact of the microbiome on fertility and fertility treatment outcomes

Doroftei, B., Ilie, O.D., Dabuleanu, A.M., Hutanu, D., Vaduva, C.C., 2022. A Retrospective Narrative Mini-Review Regarding the Seminal Microbiota in Infertile Male. Medicina (Kaunas) 58. https://doi.org/10.3390/MEDICINA58081067

Ersahin, S., Ersahin, A., Gungor, N.D., Gungor, K., Yalçin, D., Ersahin, C., Celik, N., 2022. High serum AMH inhibits pathological growth of the low biomass endometrial microbiome. Eur Rev Med Pharmacol Sci 26, 7600–7604. https://doi.org/10.26355/EURREV_202210_30035

Fabozzi, G., Rebuzzini, P., Cimadomo, D., Allori, M., Franzago, M., Stuppia, L., Garagna, S., Ubaldi, F.M., Zuccotti, M., Rienzi, L., 2022. Endocrine-Disrupting Chemicals, Gut Microbiota, and Human (In)Fertility-It Is Time to Consider the Triad. Cells 11, 3335. https://doi.org/10.3390/CELLS11213335

Gao, X.S., Laven, J., Louwers, Y., Budding, A., Schoenmakers, S., 2022. Microbiome as a predictor of implantation. Curr Opin Obstet Gynecol 34, 122–132. https://doi.org/10.1097/GCO.0000000000000782

Gunderson, S., Eskew, A.M., Stoutenburg, D., Riley, J.K., Stout, M.J., Schrimpf, J., Jungheim, E.S., Wylie, K.M., 2022. Association of the human semen DNA virome with successful in vitro fertilization. F S Sci 3, 2–9. https://doi.org/10.1016/J.XFSS.2021.10.005

Hao, Y., Feng, Y., Yan, X., Chen, L., Ma, X., Tang, X., Zhong, R., Sun, Z., Agarwal, M., Zhang, H., Zhao, Y., 2022a. Gut Microbiota-Testis Axis: FMT Mitigates High-Fat Diet-Diminished Male Fertility via Improving Systemic and Testicular Metabolome. Microbiol Spectr 10. https://doi.org/10.1128/SPECTRUM.00028-22

Hao, Y., Feng, Y., Yan, X., Chen, L., Zhong, R., Tang, X., Shen, W., Sun, Q., Sun, Z., Ren, Y., Zhang, H., Zhao, Y., 2022b. Gut microbiota-testis axis: FMT improves systemic and testicular micro-environment to increase semen quality in type 1 diabetes. Mol Med 28. https://doi.org/10.1186/S10020-022-00473-W

Hashem, N.M., Gonzalez-Bulnes, A., 2022. The Use of Probiotics for Management and Improvement of Reproductive Eubiosis and Function. Nutrients 14. https://doi.org/10.3390/NU14040902

Hong, X., Yin, J., Wang, W., Zhao, F., Ding, X., Yu, H., Zhang, X., Wang, B., 2022. The associations between low abundance of Mycoplasma hominis and female fecundability: a pregnancy-planning cohort study. BMC Microbiol 22. https://doi.org/10.1186/S12866-022-02545-7

Karaer, A., Doğan, B., Günal, S., Tuncay, G., Arda Düz, S., Ünver, T., Tecellioğlu, N., 2021. The vaginal microbiota composition of women undergoing assisted reproduction: a prospective cohort study. BJOG 128, 2101–2109. https://doi.org/10.1111/1471-0528.16782

Keburiya, L.K., Smolnikova, V.Y., Priputnevich, T. v., Muravieva, V. v., Gordeev, A.B., Trofimov, D.Y., Shubina, E.S., Kochetkova, T.O., Rogacheva, M.S., Kalinina, E.A., Sukhikh, G.T., 2022. Does the uterine microbiota affect the reproductive outcomes in women with recurrent implantation failures? BMC Womens Health 22. https://doi.org/10.1186/S12905-022-01750-W

Koedooder, R., Maghdid, D.M., Beckers, N.G.M., Schoenmakers, S., Kok, D.J., Laven, J.S.E., 2021. Dynamics of the urinary microbiome in pregnancy and the coincidental predictive value of the microbiota for IVF/IVF-ICSI outcome. Reprod Biomed Online 43, 871–879. https://doi.org/10.1016/J.RBMO.2021.07.018

Liu, F.-T., Yang, S., Yang, Z., Zhou, P., Peng, T., Yin, J., Ye, Z., Shan, H., Yu, Y., Li, R., 2022. An Altered Microbiota in the Lower and Upper Female Reproductive Tract of Women with Recurrent Spontaneous Abortion. Microbiol Spectr 10. https://doi.org/10.1128/SPECTRUM.00462-22

Lledo, B., Fuentes, A., Lozano, F.M., Cascales, A., Morales, R., Hortal, M., Sellers, F., Palacios-Marques, A., Bermejo, R., Quereda, F., Martínez-Escoriza, J.C., Bernabeu, R., Bernabeu, A., 2022. Identification of vaginal microbiome associated with IVF pregnancy. Sci Rep 12. https://doi.org/10.1038/S41598-022-10933-2

Lokken, E.M., Jisuvei, C., Hughes, J.P., Mandaliya, K., Manhart, L.E., Mwinyikai, K., Muller, C.H., Jaoko, W., Kinuthia, J., McClelland, R.S., 2022. Cultivable vaginal Lactobacillus is not associated with fecundability in Kenyan women attempting to conceive. Fertil Steril 117, 603–611. https://doi.org/10.1016/J.FERTNSTERT.2021.11.032

Lüll, K., Saare, M., Peters, M., Kakhiani, E., Zhdanova, A., Salumets, A., Boyarsky, K., Org, E., 2022. Differences in microbial profile of endometrial fluid and tissue samples in women with in vitro fertilization failure are driven by Lactobacillus abundance. Acta Obstet Gynecol Scand 101, 212–220. https://doi.org/10.1111/AOGS.14297

Morawiec, E., Czerwiński, M., Czerwińska, A.B., Wiczkowski, A., 2022. Semen dysbiosis-just a male problem? Front Cell Infect Microbiol 12. https://doi.org/10.3389/FCIMB.2022.815786

Moreno, I., Garcia-Grau, I., Perez-Villaroya, D., Gonzalez-Monfort, M., Bahçeci, M., Barrionuevo, M.J., Taguchi, S., Puente, E., Dimattina, M., Lim, M.W., Meneghini, G., Aubuchon, M., Leondires, M., Izquierdo, A., Perez-Olgiati, M., Chavez, A., Seethram, K., Bau, D., Gomez, C., Valbuena, D., Vilella, F., Simon, C., 2022. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. Microbiome 10. https://doi.org/10.1186/S40168-021-01184-W

Muzil, L., di Tucci, C., Galati, G., Mattei, G., Pietrangeli, D., di Donato, V., Perinola, G., Palaia, I., Benedetti Panici, P., 2022. The role of microbiota in female fertility and infertility. Minerva obstetrics and gynecology 74. https://doi.org/10.23736/S2724-606X.22.04915-6

Puerta Suárez, J., Hernandez, J.C., Cardona Maya, W.D., 2022. Molecular analysis of microorganisms in the semen and their impact on semen parameters. Arch Ital Urol Androl 94, 199–205. https://doi.org/10.4081/AIUA.2022.2.199

Reschini, M., Benaglia, L., Ceriotti, F., Borroni, R., Ferrari, S., Castiglioni, M., Guarneri, D., Porcaro, L., Vigano', P., Somigliana, E., Uceda Renteria, S., 2022. Endometrial microbiome: sampling, assessment, and possible impact on embryo implantation. Sci Rep 12. https://doi.org/10.1038/S41598-022-12095-7

Rodriguez Paris, V., Wong, X.Y.D., Solon-Biet, S.M., Edwards, M.C., Aflatounian, A., Gilchrist, R.B., Simpson, S.J., Handelsman, D.J., Kaakoush, N.O., Walters, K.A., 2022. The interplay between PCOS pathology and diet on gut microbiota in a mouse model. Gut Microbes 14. https://doi.org/10.1080/19490976.2022.2085961

Salliss, M.E., Farland, L. v., Mahnert, N.D., Herbst-Kralovetz, M.M., 2021. The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain. Hum Reprod Update 28, 92–131. https://doi.org/10.1093/HUMUPD/DMAB035

Sehring, J., Jeelani, R., 2022. Human implantation: The complex interplay between endometrial receptivity, inflammation, and the microbiome. Placenta 117, 179–186. https://doi.org/10.1016/J.PLACENTA.2021.12.015

Simón, C., 2022. Endometrial factor testing is a useful tool in clinical infertility management. Reprod Biomed Online 44, 953–960. https://doi.org/10.1016/J.RBMO.2021.12.014

Toson, B., Simon, C., Moreno, I., 2022. The Endometrial Microbiome and Its Impact on Human Conception. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23010485

van den Tweel, M.M., van der Struijs, S., van den Munckhof, E.H.A., Boers, K.E., 2022. The relationship between vaginal pH and bacterial vaginosis as diagnosed using qPCR in an asymptomatic subfertile population. Arch Gynecol Obstet 306, 1787–1793. https://doi.org/10.1007/S00404-022-06764-1

Vanstokstraeten, R., Mackens, S., Callewaert, E., Blotwijk, S., Emmerechts, K., Crombé, F., Soetens, O., Wybo, I., Vandoorslaer, K., Mostert, L., de Geyter, D., Muyldermans, A., Blockeel, C., Piérard, D., Demuyser, T., 2022. Culturomics to Investigate the Endometrial Microbiome: Proof-of-Concept. Int J Mol Sci 23. https://doi.org/10.3390/IJMS232012212

Venneri, M.A., Franceschini, E., Sciarra, F., Rosato, E., D'Ettorre, G., Lenzi, A., 2022. Human genital tracts microbiota: dysbiosis crucial for infertility. J Endocrinol Invest 45, 1151–1160. https://doi.org/10.1007/S40618-022-01752-3

Ventolini, G., Vieira-Baptist, P., de Seta, F., Verstraelen, H., Lonnee-Hoffman, R., Lev-Sagi, A., 2022. The Vaginal Microbiome: IV. The Role of Vaginal Microbiome in Reproduction and in Gynecologic Cancers. J Low Genit Tract Dis 26, 93–98. https://doi.org/10.1097/LGT.0000000000000646

Vitale, S.G., Ferrari, F., Ciebiera, M., Zgliczyńska, M., Rapisarda, A.M.C., Vecchio, G.M., Pino, A., Angelico, G., Knafel, A., Riemma, G., de Franciscis, P., Cianci, S., 2021. The Role of Genital Tract Microbiome in Fertility: A Systematic Review. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23010180

Wang, Qing, Wang, Qi, Zhao, L., Bin, Y., Wang, Li, Wang, Lei, Zhang, K., Li, Q., 2022. Blood Bacterial 16S rRNA Gene Alterations in Women With Polycystic Ovary Syndrome. Front Endocrinol (Lausanne) 13. https://doi.org/10.3389/FENDO.2022.814520

Zhang, M., Hu, R., Huang, Y., Zhou, F., Li, F., Liu, Z., Geng, Y., Dong, H., Ma, W., Song, K., Song, Y., 2022. Present and Future: Crosstalks Between Polycystic Ovary Syndrome and Gut Metabolites Relating to Gut Microbiota. Front Endocrinol (Lausanne) 13. https://doi.org/10.3389/FENDO.2022.933110

Zhao, Z., Ji, X., Zhang, T., Li, Q., Marcella, C., Wen, Q., Cui, B., Zhang, F., Ji, Y., 2022. Washed microbiota transplantation improves the fertility of patients with inflammatory bowel disease. Chin Med J (Engl) 135, 1489–1491. https://doi.org/10.1097/CM9.0000000000002284

Impact of stress on fertility treatment outcomes

Bian, Y., Liu, F., Wang, Y., Wang, X., Chen, R., 2022. Effects of Heart Rate Variability (HRV) Biofeedback for Women Undergoing First-time In Vitro Fertilization and Embryo Transfer. Altern Ther Health Med.

Jaiswal, P., Mahey, R., Singh, S., Vanamail, P., Gupta, M., Cheluvaraju, R., Sharma, J.B., Bhatla, N., 2022. Psychological impact of suspension/postponement of fertility treatments on infertile women waiting during COVID pandemic. Obstet Gynecol Sci 65, 197–206. https://doi.org/10.5468/OGS.21254

Lawson, A.K., Mcqueen, D.B., Swanson, A.C., Confino, R., Feinberg, E.C., Pavone, M.E., 2023. Psychological distress and postponed fertility care during the COVID-19 pandemic. https://doi.org/10.1007/s10815-020-02023-x/Published

Marom Haham, L., Youngster, M., Kuperman Shani, A., Yee, S., Ben-Kimhy, R., Medina-Artom, T.R., Hourvitz, A., Kedem, A., Librach, C., 2021. Suspension of fertility treatment during the COVID-19 pandemic: views, emotional reactions and psychological distress among women undergoing fertility treatment. Reprod Biomed Online 42, 849. https://doi.org/10.1016/J.RBMO.2021.01.007

Peaston, G., Subramanian, V., Brunckhorst, O., Sarris, I., Ahmed, K., 2022. The impact of emotional health on assisted reproductive technology outcomes: a systematic review and meta-analysis. Hum Fertil 25, 410–421. https://doi.org/10.1080/14647273.2020.1832262

Peng, M., Wen, M., Jiang, T., Jiang, Y., Lv, H., Chen, T., Ling, X., Li, H., Meng, Q., Huang, B., Tao, S., Huang, L., Liu, C., Xu, X., Lu, Q., Liu, X., Xu, B., Han, X., Zhou, K., Chen, J., Lin, Y., Ma, H., Xia, Y., Shen, H., Hu, Z., Chen, F., Du, J., Jin, G., 2021. Stress, anxiety, and depression in infertile couples are not associated with a first IVF or ICSI treatment outcome. BMC Pregnancy Childbirth 21, 725. https://doi.org/10.1186/s12884-021-04202-9

Sallem, A., Essoussi, H., Mustapha, H. ben, Zaouali, M., Ajina, M., 2021. Impact of psychological stress on the outcomes of assisted reproduction in Tunisian infertile women. Pan African Medical Journal 40. https://doi.org/10.11604/pamj.2021.40.250.32207

Wedner-Ross, S., Schippert, C., von Versen-Höynck, F., 2022. The impact of the COVID-19 pandemic on women seeking fertility treatment: the patient's perspective. Arch Gynecol Obstet. https://doi.org/10.1007/S00404-021-06379-Y

In vitro gametogenesis

Amirkhani, Z., Movahedin, M., Baheiraei, N., Ghiaseddin, A., 2022. Mini Bioreactor Can Support In Vitro Spermatogenesis of Mouse Testicular Tissue. Cell J 24, 277–284. https://doi.org/10.22074/CELLJ.2022.8053

Delessard, M., Stalin, L., Rives-Feraille, A., Moutard, L., Saulnier, J., Dumont, L., Rives, N., Rondanino, C., 2022. Achievement of complete in vitro spermatogenesis in testicular tissues from prepubertal mice exposed to mono- or polychemotherapy. Sci Rep 12. https://doi.org/10.1038/S41598-022-11286-6

Diao, L., Turek, P.J., John, C.M., Fang, F., Reijo Pera, R.A., 2022. Roles of Spermatogonial Stem Cells in Spermatogenesis and Fertility Restoration. Front Endocrinol (Lausanne) 13. https://doi.org/10.3389/FENDO.2022.895528

Eyni, H., Ghorbani, S., Nazari, H., Hajialyani, M., Razavi Bazaz, S., Mohaqiq, M., Ebrahimi Warkiani, M., Sutherland, D.S., 2021. Advanced bioengineering of male germ stem cells to preserve fertility. J Tissue Eng 12, 20417314211060590. https://doi.org/10.1177/20417314211060590

Hayashi, M., Zywitza, V., Naitou, Y., Hamazaki, N., Goeritz, F., Hermes, R., Holtze, S., Lazzari, G., Galli, C., Stejskal, J., Diecke, S., Hildebrandt, T.B., Hayashi, K., 2022. Robust induction of primordial germ cells of white rhinoceros on the brink of extinction. Sci Adv 8. https://doi.org/10.1126/SCIADV.ABP9683

Hu, X., Wang, H., Tian, G.G., Hou, C., Xu, B., Zhao, X., Zhao, Y., Fang, Q., Li, X., He, L., Chen, X., Li, S., Wu, J., 2022. Offspring production of haploid spermatid-like cells derived from mouse female germline stem cells with chromatin condensation. Cell Biosci 12. https://doi.org/10.1186/S13578-021-00697-Z

Kanbar, M., de Michele, F., Poels, J., van Loo, S., Giudice, M.G., Gilet, T., Wyns, C., 2022. Microfluidic and Static Organotypic Culture Systems to Support Ex Vivo Spermatogenesis From Prepubertal Porcine Testicular Tissue: A Comparative Study. Front Physiol 13. https://doi.org/10.3389/FPHYS.2022.884122

Khampang, S., Cho, I.K., Punyawai, K., Gill, B., Langmo, J.N., Nath, S., Greeson, K.W., Symosko, K.M., Fowler, K.L., Tian, S., Statz, J.P., Steves, A.N., Parnpai, R., White, M.A., Hennebold, J.D., Orwig, K.E., Simerly, C.R., Schatten, G., Easley, C.A., 2021. Blastocyst development after fertilization with in vitro spermatids derived from nonhuman primate embryonic stem cells. F S Sci 2, 365–375. https://doi.org/10.1016/J.XFSS.2021.09.001

Li, L., Yuan, Y., Sha, J., 2022. Potential clinical value of in vitro spermatogenesis†. Biol Reprod 107, 95–100. https://doi.org/10.1093/BIOLRE/IOAC076

Mizuta, K., Katou, Y., Nakakita, B., Kishine, A., Nosaka, Y., Saito, S., Iwatani, C., Tsuchiya, H., Kawamoto, I., Nakaya, M., Tsukiyama, T., Nagano, M., Kojima, Y., Nakamura, T., Yabuta, Y., Horie, A., Mandai, M., Ohta, H., Saitou, M., 2022. Ex vivo reconstitution of fetal oocyte development in humans and cynomolgus monkeys. EMBO J 41. https://doi.org/10.15252/EMBJ.2022110815

Oikawa, M., Kobayashi, H., Sanbo, M., Mizuno, N., Iwatsuki, K., Takashima, T., Yamauchi, K., Yoshida, F., Yamamoto, T., Shinohara, T., Nakauchi, H., Kurimoto, K., Hirabayashi, M., Kobayashi, T., 2022. Functional primordial germ cell-like cells from pluripotent stem cells in rats. Science 376. https://doi.org/10.1126/SCIENCE.ABL4412

Önen, S., Köse, S., Yersal, N., Korkusuz, P., 2022. Mesenchymal stem cells promote spermatogonial stem/progenitor cell pool and spermatogenesis in neonatal mice in vitro. Sci Rep 12. https://doi.org/10.1038/S41598-022-15358-5

Oqani, R.K., So, S., Lee, Y., Ko, J.J., Kang, E., 2022. Artificial Oocyte: Development and Potential Application. Cells 11. https://doi.org/10.3390/CELLS11071135

Ramsoomair, C.K., Alver, C.G., Flannigan, R., Ramasamy, R., Agarwal, A., 2022. Spermatogonial Stem Cells and In Vitro Spermatogenesis: How Far Are We from a Human Testis on a Chip? Eur Urol Focus. https://doi.org/10.1016/J.EUF.2022.11.006

Rolfes, V., Bittner, U., Kruessel, J.S., Fehm, T., Fangerau, H., 2022. In vitro gametogenesis: A benefit for women at advanced and very advanced age? An ethical perspective. Eur J Obstet Gynecol Reprod Biol 272, 247–250. https://doi.org/10.1016/J.EJOGRB.2022.03.038

Sanou, I., van Maaren, J., Eliveld, J., Lei, Q., Meißner, A., de Melker, A.A., Hamer, G., van Pelt, A.M.M., Mulder, C.L., 2022. Spermatogonial Stem Cell-Based Therapies: Taking Preclinical Research to the Next Level. Front Endocrinol (Lausanne) 13. https://doi.org/10.3389/FENDO.2022.850219

Saulnier, J., Chalmel, F., Delessard, M., Moutard, L., Pereira, T., Fraissinet, F., Dumont, L., Rives-Feraille, A., Rondanino, C., Rives, N., 2022a. Understanding the Underlying Molecular Mechanisms of Meiotic Arrest during In Vitro Spermatogenesis in Rat Prepubertal Testicular Tissue. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23115893

Saulnier, J., Soirey, M., Kébir, N., Delessard, M., Rives-Feraille, A., Moutard, L., Dumont, L., Rives, N., Rondanino, C., 2022b. Complete meiosis in rat prepubertal testicular tissue under in vitro sequential culture conditions. Andrology 11. https://doi.org/10.1111/ANDR.13325

Severino, J., Bauer, M., Mattimoe, T., Arecco, N., Cozzuto, L., Lorden, P., Hamada, N., Nosaka, Y., Nagaoka, S.I., Audergon, P., Tarruell, A., Heyn, H., Hayashi, K., Saitou, M., Payer, B., 2022. Controlled X-chromosome dynamics defines meiotic potential of female mouse in vitro germ cells. EMBO J 41. https://doi.org/10.15252/EMBJ.2021109457

Shuchat, S., Yossifon, G., Huleihel, M., 2022. Perfusion in Organ-on-Chip Models and Its Applicability to the Replication of Spermatogenesis In Vitro. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23105402

Tran, K.T.D., Valli-Pulaski, H., Colvin, A., Orwig, K.E., 2022. Male fertility preservation and restoration strategies for patients undergoing gonadotoxic therapies†. Biol Reprod 107, 382–405. https://doi.org/10.1093/BIOLRE/IOAC072

Wang, D., Hildorf, S., Ntemou, E., Mamsen, L.S., Dong, L., Pors, S.E., Fedder, J., Clasen-Linde, E., Cortes, D., Thorup, J., Andersen, C.Y., 2022. Organotypic Culture of Testicular Tissue from Infant Boys with Cryptorchidism. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23147975

Yao, C., Yao, R., Luo, H., Shuai, L., 2022. Germline specification from pluripotent stem cells. Stem Cell Res Ther 13. https://doi.org/10.1186/S13287-022-02750-1

Yoshimatsu, S., Kisu, I., Qian, E., Noce, T., 2022. A New Horizon in Reproductive Research with Pluripotent Stem Cells: Successful In Vitro Gametogenesis in Rodents, Its Application to Large Animals, and Future In Vitro Reconstitution of Reproductive Organs Such as “Uteroid” and “Oviductoid.” Biology (Basel) 11. https://doi.org/10.3390/BIOLOGY11070987

Mitochondrial donation

Dziadek, M.A., Sue, C.M., 2022. Mitochondrial donation: is Australia ready? Med J Aust 216, 118–121. https://doi.org/10.5694/MJA2.51309

Komesaroff, P.A., Kerridge, I., Norman, R.J., 2022. Mitochondrial donation is now possible: science must now ensure that it is safe. Intern Med J 52, 1663–1665. https://doi.org/10.1111/IMJ.15925

Koplin, J., Lestrell, E., 2022. Toward ethical regulation of mitochondrial donation. Med J Aust 216, 158. https://doi.org/10.5694/MJA2.51396

Koplin, J., Lestrell, E., Mills, C., Degeling, C., Kneebone, E., Ludlow, K., Newson, A., Sparrow, R., Sutton, L., Warren, N., 2022. Ethical implementation of mitochondrial donation in Australia. Australian and New Zealand Journal of Obstetrics and Gynaecology. https://doi.org/10.1111/AJO.13603

Nicol, D., Rudge, C., Paxton, R., Niemeyer, S., 2022. How Should We Regulate Heritable Human Genome Editing in Australia? J Law Med 29, 322–336.

Noohi, F., Ravitsky, V., Knoppers, B.M., Joly, Y., 2022. Mitochondrial Replacement Therapy: In Whose Interests? The Journal of Law, Medicine & Ethics 50, 597. https://doi.org/10.1017/JME.2022.98

O’Sullivan, G.M., Philips, J.G., Mitchell, H.J., Dornbusch, M., Rasko, J.E.J., 2022. 20 Years of Legislation - How Australia Has Responded to the Challenge of Regulating Genetically Modified Organisms in the Clinic. Front Med (Lausanne) 9. https://doi.org/10.3389/FMED.2022.883434

Pennings, G., 2022. Enucleated oocyte donation: first for infertility treatment, then for mitochondrial diseases. J Assist Reprod Genet 39, 605–608. https://doi.org/10.1007/S10815-022-02428-W

New technologies in gamete and embryo testing

Anagnostopoulou, C., Maldonado Rosas, I., Singh, N., Gugnani, N., Chockalingham, A., Singh, K., Desai, D., Darbandi, M., Manoharan, M., Darbandi, S., Leonardi Diaz, S.I., Gupta, S., Henkel, R., Sallam, H.N., Boitrelle, F., Wirka, K.A., Agarwal, A., 2022. Oocyte quality and embryo selection strategies: a review for the embryologists, by the embryologists. Panminerva Med 64, 171–184. https://doi.org/10.23736/S0031-0808.22.04680-8

Aydın, Ş., Bulgan Kılıçdağ, E., Çağlar Aytaç, P., Çok, T., Şimşek, E., Haydardedeoğlu, B., 2022. Prospective randomized controlled study of a microfluidic chip technology for sperm selection in male infertility patients. Andrologia 54. https://doi.org/10.1111/AND.14415

Baldini, D., Ferri, D., Baldini, G.M., Lot, D., Catino, A., Vizziello, D., Vizziello, G., 2021. Sperm Selection for ICSI: Do We Have a Winner? Cells 10. https://doi.org/10.3390/CELLS10123566

Barratt, C.L.R., Wang, C., Baldi, E., Toskin, I., Kiarie, J., Lamb, D.J., 2022. What advances may the future bring to the diagnosis, treatment, and care of male sexual and reproductive health? Fertil Steril 117, 258–267. https://doi.org/10.1016/J.FERTNSTERT.2021.12.013

Campbell, J.M., Mahbub, S.B., Bertoldo, M.J., Habibalahi, A., Goss, D.M., Ledger, W.L., Gilchrist, R.B., Wu, L.E., Goldys, E.M., 2022. Multispectral autofluorescence characteristics of reproductive aging in old and young mouse oocytes. Biogerontology 23, 237–249. https://doi.org/10.1007/S10522-022-09957-Y

Chen, L., Li, W., Liu, Y., Peng, Z., Cai, L., Zhang, N., Xu, J., Wang, L., Teng, X., Yao, Y., Zou, Y., Ma, M., Liu, J., Lu, S., Sun, H., Yao, B., 2022. Non-invasive embryo selection strategy for clinical IVF to avoid wastage of potentially competent embryos. Reprod Biomed Online 45, 26–34. https://doi.org/10.1016/J.RBMO.2022.03.006

Dahdouh, E.M., Mourad, A.M., Balayla, J., Sylvestre, C., Brezina, P.R., Kutteh, W.H., Piccehetta, L., Capalbo, A., Garcia-Velasco, J.A., 2022. Update on preimplantation genetic testing for aneuploidy and outcomes of embryos with mosaic results. Minerva obstetrics and gynecology. https://doi.org/10.23736/S2724-606X.22.05166-1

de Witte, L., Raman, L., Baetens, M., de Koker, A., Callewaert, N., Symoens, S., Tilleman, K., Meerschaut, F. vanden, Dheedene, A., Menten, B., 2022. GENType: all-in-one preimplantation genetic testing by pedigree haplotyping and copy number profiling suitable for third-party reproduction. Hum Reprod 37, 1678–1691. https://doi.org/10.1093/HUMREP/DEAC088

Farkouh, A., Salvio, G., Kuroda, S., Saleh, R., Vogiatzi, P., Agarwal, A., 2022. Sperm DNA integrity and male infertility: a narrative review and guide for the reproductive physicians. Transl Androl Urol 11, 1023–1044. https://doi.org/10.21037/TAU-22-149/COIF)

Garcia-Segura, S., del Rey, J., Closa, L., Garcia-Martínez, I., Hobeich, C., Castel, A.B., Vidal, F., Benet, J., Ribas-Maynou, J., Oliver-Bonet, M., 2022. Seminal Microbiota of Idiopathic Infertile Patients and Its Relationship With Sperm DNA Integrity. Front Cell Dev Biol 10. https://doi.org/10.3389/FCELL.2022.937157

Gianzo, M., Urizar-Arenaza, I., Muñoa-Hoyos, I., Labaka, G., Larreategui, Z., Garrido, N., Irazusta, J., Subirán, N., 2022. Sperm aminopeptidase N identifies the potential for high-quality blastocysts and viable embryos in oocyte-donation cycles. Hum Reprod 37. https://doi.org/10.1093/HUMREP/DEAC170

Gil Juliá, M., Hervás, I., Navarro-Gomezlechon, A., Quintana, F., Amorós, D., Pacheco, A., González-Ravina, C., Rivera-Egea, R., Garrido, N., 2022. Cumulative live birth rates in donor oocyte ICSI cycles are not improved by magnetic-activated cell sorting sperm selection. Reprod Biomed Online 44, 677–684. https://doi.org/10.1016/J.RBMO.2021.09.024

Gómez-Torres, M.J., Huerta-Retamal, N., Sáez-Espinosa, P., Robles-Gómez, L., Avilés, M., Aizpurua, J., 2022. Molecular Chaperone HSPA2 Distribution During Hyaluronic Acid Selection in Human Sperm. Reprod Sci. https://doi.org/10.1007/S43032-022-01031-9

González-Ravina, C., Santamaría-López, E., Pacheco, A., Ramos, J., Carranza, F., Murria, L., Ortiz-Vallecillo, A., Fernández-Sánchez, M., 2022. Effect of Sperm Selection by Magnetic-Activated Cell Sorting in D-IUI: A Randomized Control Trial. Cells 11. https://doi.org/10.3390/CELLS11111794

Hernández-Silva, G., Caballero-Campo, P., Chirinos, M., 2022. Sperm mRNAs as potential markers of male fertility. Reprod Biol 22. https://doi.org/10.1016/J.REPBIO.2022.100636

Itoi, F., Miyamoto, T., Himaki, T., Honnma, H., Sano, M., Ueda, J., 2022. Importance of real-time measurement of sperm head morphology in intracytoplasmic sperm injection. Zygote 30. https://doi.org/10.1017/S0967199421000307

Janiszewska, E., Kokot, I., Kmieciak, A., Stelmasiak, Z., Gilowska, I., Faundez, R., Kratz, E.M., 2022. The Association between Clusterin Sialylation Degree and Levels of Oxidative-Antioxidant Balance Markers in Seminal Plasmas and Blood Sera of Male Partners with Abnormal Sperm Parameters. Int J Mol Sci 23. https://doi.org/10.3390/IJMS231810598

Karabulut, S., Camcı, İ.Y., Atambay, C., Yiğit, P., Keskin, İ., 2022. Sperm selection with Annexin-V coated polystrene bead technique (APB-Tech): A novel and reliable method for the microscopic selection of viable and non-apoptotic sperm to be used for intracytoplasmic sperm injection. Theriogenology 194. https://doi.org/10.1016/J.THERIOGENOLOGY.2022.09.028

Kasak, L., Lillepea, K., Nagirnaja, L., Aston, K.I., Schlegel, P.N., Gonçalves, J., Carvalho, F., Moreno-Mendoza, D., Almstrup, K., Eisenberg, M.L., Jarvi, K.A., O'Bryan, M.K., Lopes, A.M., Conrad, D.F., Punab, M., Laan, M., 2022. Actionable secondary findings following exome sequencing of 836 non-obstructive azoospermia cases and their value in patient management. Hum Reprod 37, 1652–1663. https://doi.org/10.1093/HUMREP/DEAC100

Keskin, M., Pabuçcu, E.G., Arslanca, T., Demirkıran, Ö.D., Pabuçcu, R., 2022. Does Microfluidic Sperm Sorting Affect Embryo Euploidy Rates in Couples with High Sperm DNA Fragmentation? Reprod Sci 29, 1801–1808. https://doi.org/10.1007/S43032-021-00784-Z

Kocur, O.M., Xie, P., Souness, S., Cheung, S., Rosenwaks, Z., Palermo, G.D., 2022. Assessing male gamete genome integrity to ameliorate poor assisted reproductive technology clinical outcome. F S Sci. https://doi.org/10.1016/J.XFSS.2022.08.001

Kulmann, M.I.R., Riboldi, M., Martello, C., Bos-Mikich, A., Frantz, G., Dutra, C., Donatti, L.M., Oliveira, N., Frantz, N., 2021. First Baby Born in Brazil after Simultaneous Diagnosis through Non-Invasive and Conventional PGT-A. Rev Bras Ginecol Obstet 43, 878–882. https://doi.org/10.1055/S-0041-1736302

Kumar, A., Im, K., Banjevic, M., Ng, P.C., Tunstall, T., Garcia, G., Galhardo, L., Sun, J., Schaedel, O.N., Levy, B., Hongo, D., Kijacic, D., Kiehl, M., Tran, N.D., Klatsky, P.C., Rabinowitz, M., 2022. Whole-genome risk prediction of common diseases in human preimplantation embryos. Nat Med 28, 513–516. https://doi.org/10.1038/S41591-022-01735-0

Lara-Cerrillo, S., Urda Muñoz, C., de la Casa Heras, M., Camacho Fernández-Pacheco, S., Gijón de la Santa, J., Lacruz-Ruiz, T., Rosado-Iglesias, C., Gonçalves-Aponte, V., Badajoz Liébana, V., García-Peiró, A., 2022. Microfluidic sperm sorting improves ICSI outcomes in patients with increased values of Double-Strand Breaks in sperm DNA. Rev Int Androl. https://doi.org/10.1016/J.ANDROL.2021.10.003

Lencz, T., Sabatello, M., Docherty, A., Peterson, R.E., Soda, T., Austin, J., Bierut, L., Crepaz-Keay, D., Curtis, D., Degenhardt, F., Huckins, L., Lazaro-Munoz, G., Mattheisen, M., Meiser, B., Peay, H., Rietschel, M., Walss-Bass, C., Davis, L.K., 2022. Concerns about the use of polygenic embryo screening for psychiatric and cognitive traits. Lancet Psychiatry 9, 838–844. https://doi.org/10.1016/S2215-0366(22)00157-2

Martínez-Moro, Á., Lamas-Toranzo, I., González-Brusi, L., Pérez-Gómez, A., Padilla-Ruiz, E., García-Blanco, J., Bermejo-Álvarez, P., 2022. mtDNA content in cumulus cells does not predict development to blastocyst or implantation. Hum Reprod Open 2022. https://doi.org/10.1093/HROPEN/HOAC029

Mendizabal-Ruiz, G., Chavez-Badiola, A., Aguilar Figueroa, I., Martinez Nuño, V., Flores-Saiffe Farias, A., Valencia-Murilloa, R., Drakeley, A., Garcia-Sandoval, J.P., Cohen, J., 2022. Computer software (SiD) assisted real-time single sperm selection associated with fertilization and blastocyst formation. Reprod Biomed Online 45. https://doi.org/10.1016/J.RBMO.2022.03.036

Minhas, S., Bettocchi, C., Boeri, L., Capogrosso, P., Carvalho, J., Cilesiz, N.C., Cocci, A., Corona, G., Dimitropoulos, K., Gül, M., Hatzichristodoulou, G., Jones, T.H., Kadioglu, A., Martínez Salamanca, J.I., Milenkovic, U., Modgil, V.,

Russo, G.I., Serefoglu, E.C., Tharakan, T., Verze, P., Salonia, A., 2021. European Association of Urology Guidelines on Male Sexual and Reproductive Health: 2021 Update on Male Infertility. Eur Urol 80, 603–620. https://doi.org/10.1016/J.EURURO.2021.08.014

Mirsanei, J.S., Sheibak, N., Zandieh, Z., Mehdizadeh, M., Aflatoonian, R., Tabatabaei, M., Mousavi, A.S., Amjadi, F., 2022. Microfluidic chips as a method for sperm selection improve fertilization rate in couples with fertilization failure. Arch Gynecol Obstet 306. https://doi.org/10.1007/S00404-022-06618-W

Molka, B., Gwladys, B., Dorian, B., Lucie, M., Mustapha, B., Rosalie, C., Brigitte, G., Hafida, K.C., Moncef, B., 2022. Follicular Fluid Growth Factors and Interleukin Profiling as Potential Predictors of IVF Outcomes. Front Physiol 13. https://doi.org/10.3389/FPHYS.2022.859790

Nassir, M., Levi, M., Dardikman-Yoffe, G., Mirsky, S.K., Shaked, N.T., 2022. Prediction of Sperm Progression in Three Dimensions Using Rapid Optical Imaging and Dynamic Mechanical Modeling. Cells 11. https://doi.org/10.3390/CELLS11081319

Navarro-Sánchez, L., García-Pascual, C., Rubio, C., Simón, C., 2022. Non-invasive preimplantation genetic testing for aneuploidies: an update. Reprod Biomed Online 44, 817–828. https://doi.org/10.1016/J.RBMO.2022.01.012

Olatunji, O., More, A., 2022. A Review of the Impact of Microfluidics Technology on Sperm Selection Technique. Cureus 14. https://doi.org/10.7759/CUREUS.27369

Olcay, I.O., Akcay, B., Bahceci, M., Arici, A., Boynukalin, K., Yakicier, C., Ozpinar, A., Basar, M., 2022. Noninvasive amino acid turnover predicts human embryo aneuploidy. Gynecol Endocrinol 38, 461–466. https://doi.org/10.1080/09513590.2022.2068520

Omes, C., Tomasoni, V., de Amici, M., Testa, G., Torre, C., Nappi, R.E., 2022. Calprotectin as a novel diagnostic approach to screen male infertility risk: A pilot study. Immunobiology 227. https://doi.org/10.1016/J.IMBIO.2022.152291

Ou, Z., Deng, Y., Liang, Y., Chen, Z., Sun, L., 2022. Improved Non-Invasive Preimplantation Genetic Testing for Beta-Thalassemia Using Spent Embryo Culture Medium Containing Blastocoelic Fluid. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.793821

Pereira, S., Carmi, S., Altarescu, G., Austin, J., Barlevy, D., Hershlag, A., Juengst, E., Kostick-Quenet, K., Kovanci, E., Lathi, R.B., Mukherjee, M., van den Veyver, I., Zuk, O., Lázaro-Muñoz, G., Lencz, T., 2022. Polygenic embryo screening: four clinical considerations warrant further attention. Hum Reprod 37, 1375–1378. https://doi.org/10.1093/HUMREP/DEAC110

Polyakov, A., Amor, D.J., Savulescu, J., Gyngell, C., Georgiou, E.X., Ross, V., Mizrachi, Y., Rozen, G., 2022. Polygenic risk score for embryo selection-not ready for prime time. Hum Reprod 37, 2229–2236. https://doi.org/10.1093/HUMREP/DEAC159

Pujol, A., García-Peiró, A., Ribas-Maynou, J., Lafuente, R., Mataró, D., Vassena, R., 2022. A microfluidic sperm-sorting device reduces the proportion of sperm with double-stranded DNA fragmentation. Zygote 30, 200–205. https://doi.org/10.1017/S0967199421000484

Quinn, M.M., Ribeiro, S., Juarez-Hernandez, F., Simbulan, R.K., Jalalian, L., Cedars, M.I., Rosen, M.P., 2022. Microfluidic preparation of spermatozoa for ICSI produces similar embryo quality to density-gradient centrifugation: a pragmatic, randomized controlled trial. Hum Reprod 37, 1406–1413. https://doi.org/10.1093/HUMREP/DEAC099

Rogers, A., Menezes, M., Kane, S.C., Zander-Fox, D., Hardy, T., 2021. Preimplantation Genetic Testing for Monogenic Conditions: Is Cell-Free DNA Testing the Next Step? Mol Diagn Ther 25, 683–690. https://doi.org/10.1007/S40291-021-00556-0

Sáez-Espinosa, P., Robles-Gómez, L., Ortega-López, L., Aizpurua, J., Gómez-Torres, M.J., 2022. Immunofluorescence and High-Resolution Microscopy Reveal New Insights in Human Globozoospermia. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23031729

Scaruffi, P., Bovis, F., Casciano, I., Maccarini, E., de Leo, C., Gazzo, I., Massarotti, C., Sozzi, F., Stigliani, S., Anserini, P., 2022. Hyaluronic acid-sperm selection significantly improves the clinical outcome of couples with previous ICSI cycles failure. Andrology 10, 677–685. https://doi.org/10.1111/ANDR.13155

Schardein, J.N., Fendereski, K., Hotaling, J.M., 2023. Evolution of the basic semen analysis and processing sperm. Curr Opin Urol 33. https://doi.org/10.1097/MOU.0000000000001054

Sciorio, R., Miranian, D., Smith, G.D., 2022. Non-invasive oocyte quality assessment. Biol Reprod 106, 274–290. https://doi.org/10.1093/BIOLRE/IOAC009

Sengupta, P., Roychoudhury, S., Nath, M., Dutta, S., 2022. Oxidative Stress and Idiopathic Male Infertility. Adv Exp Med Biol 1358, 181–204. https://doi.org/10.1007/978-3-030-89340-8_9

Shah, J.S., Venturas, M., Sanchez, T.H., Penzias, A.S., Needleman, D.J., Sakkas, D., 2022. Fluorescence lifetime imaging microscopy (FLIM) detects differences in metabolic signatures between euploid and aneuploid human blastocysts. Hum Reprod 37, 400–410. https://doi.org/10.1093/HUMREP/DEAC016

Sialakouma, A., Karakasiliotis, I., Ntala, V., Nikolettos, N., Asimakopoulos, B., 2021. Embryonic Cell-free DNA in Spent Culture Medium: A Non-invasive Tool for Aneuploidy Screening of the Corresponding Embryos. In Vivo 35, 3449–3457. https://doi.org/10.21873/INVIVO.12645

Silva, J.R.V., Barroso, P.A.A., Nascimento, D.R., Figueira, C.S., Azevedo, V.A.N., Silva, B.R., Santos, R.P. dos, 2021. Benefits and challenges of nanomaterials in assisted reproductive technologies. Mol Reprod Dev 88, 707–717. https://doi.org/10.1002/MRD.23536

Skowrońska, P., Kunicki, M., Pastuszek, E., Konieczna, L., Bączek, T., Męczekalski, B., Smolarczyk, R., Łukaszuk, K., 2022. Vitamin D and anti-Müllerian hormone concentration in human follicular fluid individually aspirated from all patient follicles. Gynecol Endocrinol 38, 28–32. https://doi.org/10.1080/09513590.2021.1933934

Sousa, L.N., Monteiro, P.B., 2022. Non-invasive preimplantation genetic testing: a literature review. JBRA Assist Reprod 26, 554–558. https://doi.org/10.5935/1518-0557.20210102

Srinivas, S., Donthi, S., Mettler, A.D., Tolani, A.D., Deenadayal, M., 2022. Does Choosing Microfluidics for Sperm Sorting Offer an Advantage to Improve Clinical Pregnancies in Donor Egg Recipients? J Hum Reprod Sci 15, 143–149. https://doi.org/10.4103/JHRS.JHRS_15_22

Tan, T.C.Y., Mahbub, S.B., Campbell, J.M., Habibalahi, A., Campugan, C.A., Rose, R.D., Chow, D.J.X., Mustafa, S., Goldys, E.M., Dunning, K.R., 2021. Non-invasive, label-free optical analysis to detect aneuploidy within the inner cell mass of the preimplantation embryo. Hum Reprod 37, 14–29. https://doi.org/10.1093/HUMREP/DEAB233

Tomic, M., Vrtacnik Bokal, E., Stimpfel, M., 2022. Non-Invasive Preimplantation Genetic Testing for Aneuploidy and the Mystery of Genetic Material: A Review Article. Int J Mol Sci 23. https://doi.org/10.3390/IJMS23073568

Tsai, N.C., Chang, Y.C., Su, Y.R., Lin, Y.C., Weng, P.L., Cheng, Y.H., Li, Y.L., Lan, K.C., 2022. Validation of Non-Invasive Preimplantation Genetic Screening Using a Routine IVF Laboratory Workflow. Biomedicines 10. https://doi.org/10.3390/BIOMEDICINES10061386

van Dijk, W., Derks, K., Drüsedau, M., Meekels, J., Koeck, R., Essers, R., Dreesen, J., Coonen, E., de Die-Smulders, C., Stevens, S.J.C., Brunner, H.G., van den Wijngaard, A., Paulussen, A.D.C., Zamani Esteki, M., 2022. Embryo tracking system for high-throughput sequencing-based preimplantation genetic testing. Hum Reprod 37, 2700–2708. https://doi.org/10.1093/HUMREP/DEAC208

Venturas, M., Shah, J.S., Yang, X., Sanchez, T.H., Conway, W., Sakkas, D., Needleman, D.J., 2022. Metabolic state of human blastocysts measured by fluorescence lifetime imaging microscopy. Hum Reprod 37, 411–427. https://doi.org/10.1093/HUMREP/DEAB283

Vickram, A.S., Anbarasu, K., Gulothungan, G., Thanigaivel, S., Nanmaran, R., Palanivelu, J., 2022. Characterization of human prostasomes protein Clusterin (macromolecule) - a novel biomarker for male infertility diagnosis and prognosis. J Biomol Struct Dyn 40, 3979–3988. https://doi.org/10.1080/07391102.2020.1852960

Wang, C., Fei, X., Zhang, H., Zhou, W., Cheng, Z., Feng, Y., 2022. Proteomic Analysis of the Alterations in Follicular Fluid Proteins During Oocyte Maturation in Humans. Front Endocrinol (Lausanne) 12. https://doi.org/10.3389/FENDO.2021.830691

Wang, J., Zhang, J., Sun, X., Lin, Y., Cai, L., Cui, Y., Liu, J., Liu, M., Yang, X., 2021. Novel bi-allelic variants in ACTL7A are associated with male infertility and total fertilization failure. Hum Reprod 36, 3161–3169. https://doi.org/10.1093/HUMREP/DEAB228

West, R., Coomarasamy, A., Frew, L., Hutton, R., Kirkman-Brown, J., Lawlor, M., Lewis, S., Partanen, R., Payne-Dwyer, A., Román-Montañana, C., Torabi, F., Tsagdi, S., Miller, D., 2022. Sperm selection with hyaluronic acid improved live birth outcomes among older couples and was connected to sperm DNA quality, potentially affecting all treatment outcomes. Hum Reprod 37, 1106–1125. https://doi.org/10.1093/HUMREP/DEAC058

Xie, P., Hu, X., Kong, L., Mao, Y., Cheng, D., Kang, K., Dai, J., Zhao, D., Zhang, Y., Lu, N., Wan, Z., Du, R., Xiong, B., Zhang, J., Tan, Y., Lu, G., Gong, F., Lin, G., Liang, B., Du, J., Hu, L., 2022. A novel multifunctional haplotyping-based preimplantation genetic testing for different genetic conditions. Hum Reprod 37, 2546–2559. https://doi.org/10.1093/HUMREP/DEAC190

Yang, L., Xu, Yan, Xia, J., Yan, H., Ding, C., Shi, Q., Wu, Y., Liu, P., Pan, J., Zeng, Y., Zhang, Y., Chen, F., Jiang, H., Xu, Yanwen, Li, W., Zhou, C., Gao, Y., 2022. Simultaneous detection of genomic imbalance in patients receiving preimplantation genetic testing for monogenic diseases (PGT-M). Front Genet 13. https://doi.org/10.3389/FGENE.2022.976131

Zhai, F., Wang, Yun, Li, H., Wang, Yuqian, Zhu, X., Kuo, Y., Guan, S., Li, J., Song, S., He, Q., An, J., Zhi, X., Lian, Y., Huang, J., Li, R., Qiao, J., Yan, L., Yan, Z., 2022. Preimplantation genetic testing for structural rearrangement based on low-coverage next-generation sequencing accurately discriminates between normal and carrier embryos for patients with translocations. Reprod Biomed Online 45, 473–480. https://doi.org/10.1016/J.RBMO.2022.05.012

Zhang, F.L., Qian, Y., Li, W.D., Cao, Q., Chu, H.T., Luk, A.C.S., Tsang, S.W., Lee, W.K., Tang, P.M.K., Chan, W.Y., Chow, K.L., Chan, D.Y.L., Li, T.C., Yip, K.Y.L., Shen, W., Lee, T.L., 2022. Parallel bimodal single-cell sequencing of transcriptome and methylome provides molecular and translational insights on oocyte maturation and maternal aging. Genomics 114. https://doi.org/10.1016/J.YGENO.2022.110379

Zhang, Z., Dai, C., Shan, G., Chen, X., Liu, H., Abdalla, K., Kuznyetsova, I., Moskovstev, S., Huang, X., Librach, C., Jarvi, K., Sun, Y., 2021. Quantitative selection of single human sperm with high DNA integrity for intracytoplasmic sperm injection. Fertil Steril 116, 1308–1318. https://doi.org/10.1016/J.FERTNSTERT.2021.06.016

Synthetic embryo like entities

Ai, Z., Yin, Y., Niu, B., Li, T., 2022. Deconstructing human peri-implantation embryogenesis based on embryos and embryoids†. Biol Reprod 107, 212–225. https://doi.org/10.1093/BIOLRE/IOAC096

Amadei, G., Handford, C.E., Qiu, C., de Jonghe, J., Greenfeld, H., Tran, M., Martin, B.K., Chen, D.Y., Aguilera-Castrejon, A., Hanna, J.H., Elowitz, M.B., Hollfelder, F., Shendure, J., Glover, D.M., Zernicka-Goetz, M., 2022. Embryo model completes gastrulation to neurulation and organogenesis. Nature 610, 143–153. https://doi.org/10.1038/S41586-022-05246-3

Amel, A., Rossouw, S., Goolam, M., 2022. Gastruloids: A Novel System for Disease Modelling and Drug Testing. Stem Cell Rev Rep. https://doi.org/10.1007/S12015-022-10462-5

Ankeny, R.A., Munsie, M.J., Leach, J., 2022. Developing a Reflexive, Anticipatory, and Deliberative Approach to Unanticipated Discoveries: Ethical Lessons from iBlastoids. Am J Bioeth 22, 36–45. https://doi.org/10.1080/15265161.2021.1974976

Arias, A.M., Marikawa, Y., Moris, N., 2022. Gastruloids: Pluripotent stem cell models of mammalian gastrulation and embryo engineering. Dev Biol 488, 35–46. https://doi.org/10.1016/J.YDBIO.2022.05.002

Bao, M., Cornwall-Scoones, J., Sanchez-Vasquez, E., Chen, D.Y., de Jonghe, J., Shadkhoo, S., Hollfelder, F., Thomson, M., Glover, D.M., Zernicka-Goetz, M., 2022a. Stem cell-derived synthetic embryos self-assemble by exploiting cadherin codes and cortical tension. Nat Cell Biol 24, 1341–1349. https://doi.org/10.1038/S41556-022-00984-Y

Bao, M., Cornwall-Scoones, J., Zernicka-Goetz, M., 2022b. Stem-cell-based human and mouse embryo models. Curr Opin Genet Dev 76, 101970. https://doi.org/10.1016/J.GDE.2022.101970

Cermola, F., Amoroso, F., Saracino, F., Ibello, E., de Cesare, D., Fico, A., Cobellis, G., Scalera, E., Casiraghi, C., D'Aniello, C., Patriarca, E.J., Minchiotti, G., 2022. Stabilization of cell-cell adhesions prevents symmetry breaking and locks in pluripotency in 3D gastruloids. Stem Cell Reports 17, 2548–2564. https://doi.org/10.1016/J.STEMCR.2022.09.013

Kagawa, H., Javali, A., Khoei, H.H., Sommer, T.M., Sestini, G., Novatchkova, M., Scholte op Reimer, Y., Castel, G., Bruneau, A., Maenhoudt, N., Lammers, J., Loubersac, S., Freour, T., Vankelecom, H., David, L., Rivron, N., 2022a. Human blastoids model blastocyst development and implantation. Nature 601, 600–605. https://doi.org/10.1038/S41586-021-04267-8

Kagawa, H., Javali, A., Khoei, H.H., Sommer, T.M., Sestini, G., Novatchkova, M., Scholte Op Reimer, Y., Rivron, N., 2022b. Protocol for Human Blastoids Modeling Blastocyst Development and Implantation. J Vis Exp 2022. https://doi.org/10.3791/63388

Langkabel, J., Horne, A., Bonaguro, L., Holsten, L., Hesse, T., Knaus, A., Riedel, Y., Becker, M., Händler, K., Elmzzahi, T., Bassler, K., Reusch, N., Yeghiazarian, L.H., Pecht, T., Saglam, A., Ulas, T., Aschenbrenner, A.C., Kaiser, F., Kubaczka, C., Schultze, J.L., Schorle, H., 2021. Induction of Rosette-to-Lumen stage embryoids using reprogramming paradigms in ESCs. Nature Communications 2021 12:1 12, 1–18. https://doi.org/10.1038/s41467-021-27586-w

Lau, K.Y.C., Rubinstein, H., Gantner, C.W., Hadas, R., Amadei, G., Stelzer, Y., Zernicka-Goetz, M., 2022. Mouse embryo model derived exclusively from embryonic stem cells undergoes neurulation and heart development. Cell Stem Cell 29, 1445-1458.e8. https://doi.org/10.1016/J.STEM.2022.08.013

Li, X., Kodithuwakku, S.P., Chan, R.W.S., Yeung, W.S.B., Yao, Y., Ng, E.H.Y., Chiu, P.C.N., Lee, C.L., 2022. Three-dimensional culture models of human endometrium for studying trophoblast-endometrium interaction during implantation. Reprod Biol Endocrinol 20. https://doi.org/10.1186/S12958-022-00973-8

Mazid, M.A., Ward, C., Luo, Z., Liu, C., Li, Y., Lai, Y., Wu, L., Li, J., Jia, W., Jiang, Y., Liu, H., Fu, L., Yang, Y., Ibañez, D.P., Lai, J., Wei, X., An, J., Guo, P., Yuan, Y., Deng, Q., Wang, Y., Liu, Y., Gao, F., Wang, J., Zaman, S., Qin, B., Wu, G., Maxwell, P.H., Xu, X., Liu, L., Li, W., Esteban, M.A., 2022. Rolling back human pluripotent stem cells to an eight-cell embryo-like stage. Nature 605, 315–324. https://doi.org/10.1038/S41586-022-04625-0

Niethammer, M., Burgdorf, T., Wistorf, E., Schönfelder, G., Kleinsorge, M., 2022. In vitro models of human development and their potential application in developmental toxicity testing. Development 149. https://doi.org/10.1242/DEV.200933

Oldak, B., Aguilera-Castrejon, A., Hanna, J.H., 2022. Recent insights into mammalian natural and synthetic ex utero embryogenesis. Curr Opin Genet Dev 77. https://doi.org/10.1016/J.GDE.2022.101988

Pham, T.X.A., Panda, A., Kagawa, H., To, S.K., Ertekin, C., Georgolopoulos, G., van Knippenberg, S.S.F.A., Allsop, R.N., Bruneau, A., Chui, J.S.H., Vanheer, L., Janiszewski, A., Chappell, J., Oberhuemer, M., Tchinda, R.S., Talon, I., Khodeer, S., Rossant, J., Lluis, F., David, L., Rivron, N., Balaton, B.P., Pasque, V., 2022. Modeling human extraembryonic mesoderm cells using naive pluripotent stem cells. Cell Stem Cell 29, 1346-1365.e10. https://doi.org/10.1016/J.STEM.2022.08.001

Pryzhkova, M. v., Boers, R., Jordan, P.W., 2022. Modeling Human Gonad Development in Organoids. Tissue Eng Regen Med 19. https://doi.org/10.1007/S13770-022-00492-Y

Rodriguez-Fraticelli, A.E., 2022. Synthetic Embryos Can Complete Gastrulation and Initiate Organogenesis Ex Utero. Cell Reprogram 24, 223–224. https://doi.org/10.1089/CELL.2022.0111

Rossi, G., Giger, S., Hübscher, T., Lutolf, M.P., 2022. Gastruloids as in vitro models of embryonic blood development with spatial and temporal resolution. Sci Rep 12. https://doi.org/10.1038/S41598-022-17265-1

Seong, J., Frias-Aldeguer, J., Holzmann, V., Kagawa, H., Sestini, G., Heidari Khoei, H., Scholte Op Reimer, Y., Kip, M., Pradhan, S.J., Verwegen, L., Vivié, J., Li, L., Alemany, A., Korving, J., Darmis, F., van Oudenaarden, A., ten Berge, D., Geijsen, N., Rivron, N.C., 2022. Epiblast inducers capture mouse trophectoderm stem cells in vitro and pattern blastoids for implantation in utero. Cell Stem Cell 29, 1102-1118.e8. https://doi.org/10.1016/J.STEM.2022.06.002

Shao, Y., Fu, J., 2022. Engineering multiscale structural orders for high-fidelity embryoids and organoids. Cell Stem Cell 29, 722–743. https://doi.org/10.1016/J.STEM.2022.04.003

Simunovic, M., Siggia, E.D., Brivanlou, A.H., 2022. In vitro attachment and symmetry breaking of a human embryo model assembled from primed embryonic stem cells. Cell Stem Cell 29, 962-972.e4. https://doi.org/10.1016/J.STEM.2022.05.001

Sozen, B., Conkar, D., Veenvliet, J. v., 2022. Carnegie in 4D? Stem-cell-based models of human embryo development. Semin Cell Dev Biol 131, 44–57. https://doi.org/10.1016/J.SEMCDB.2022.05.023

Tahmasbpour Marzouni, E., Stern, C., Henrik Sinclair, A., Tucker, E.J., 2022. Stem Cells and Organs-on-chips: New Promising Technologies for Human Infertility Treatment. Endocr Rev 43, 878–906. https://doi.org/10.1210/ENDREV/BNAB047

Tarazi, S., Aguilera-Castrejon, A., Joubran, C., Ghanem, N., Ashouokhi, S., Roncato, F., Wildschutz, E., Haddad, M., Oldak, B., Gomez-Cesar, E., Livnat, N., Viukov, S., Lokshtanov, D., Naveh-Tassa, S., Rose, M., Hanna, S., Raanan, C., Brenner, O., Kedmi, M., Keren-Shaul, H., Lapidot, T., Maza, I., Novershtern, N., Hanna, J.H., 2022. Post-gastrulation synthetic embryos generated ex utero from mouse naive ESCs. Cell 185, 3290-3306.e25. https://doi.org/10.1016/J.CELL.2022.07.028

Terhune, A.H., Bok, J., Sun, S., Fu, J., 2022. Stem cell-based models of early mammalian development. Development 149. https://doi.org/10.1242/DEV.201015

Veenvliet, J. v., Lenne, P.F., Turner, D.A., Nachman, I., Trivedi, V., 2021. Sculpting with stem cells: how models of embryo development take shape. Development 148. https://doi.org/10.1242/DEV.192914

Viukov, S., Shani, T., Bayerl, J., Aguilera-Castrejon, A., Oldak, B., Sheban, D., Tarazi, S., Stelzer, Y., Hanna, J.H., Novershtern, N., 2022. Human primed and naïve PSCs are both able to differentiate into trophoblast stem cells. Stem Cell Reports 17, 2484–2500. https://doi.org/10.1016/J.STEMCR.2022.09.008

Vrij, E.J., Scholte Op Reimer, Y.S., Fuentes, L.R., Guerreiro, I.M., Holzmann, V., Aldeguer, J.F., Sestini, G., Koo, B.K., Kind, J., van Blitterswijk, C.A., Rivron, N.C., 2022. A pendulum of induction between the epiblast and extra-embryonic endoderm supports post-implantation progression. Development 149. https://doi.org/10.1242/DEV.192310

Wehmeyer, A.E., Schüle, K.M., Conrad, A., Schröder, C.M., Probst, S., Arnold, S.J., 2022. Chimeric 3D-gastruloids – a versatile tool for studies of mammalian peri-gastrulation development. Development 149. https://doi.org/10.1242/DEV.200812/280536/AM/CHIMERIC-3D-GASTRULOIDS-A-VERSATILE-TOOL-FOR

Yamanaka, Y., Hamidi, S., Yoshioka-Kobayashi, K., Munira, S., Sunadome, K., Zhang, Y., Kurokawa, Y., Ericsson, R., Mieda, A., Thompson, J.L., Kerwin, J., Lisgo, S., Yamamoto, T., Moris, N., Martinez-Arias, A., Tsujimura, T., Alev, C., 2022. Reconstituting human somitogenesis in vitro. Nature 1–3. https://doi.org/10.1038/s41586-022-05649-2

Zhai, J., Xiao, Z., Wang, Y., Wang, H., 2022. Human embryonic development: from peri-implantation to gastrulation. Trends Cell Biol 32, 18–29. https://doi.org/10.1016/J.TCB.2021.07.008

Zhang, M., Reis, A.H., Simunovic, M., 2022. Human embryoids: A new strategy of recreating the first steps of embryonic development in vitro. Semin Cell Dev Biol. https://doi.org/10.1016/J.SEMCDB.2022.07.003

Zhang, Y., An, C., Yu, Yanhong, Lin, J., Jin, L., Li, C., Tan, T., Yu, Yang, Fan, Y., 2022. Epidermal growth factor induces a trophectoderm lineage transcriptome resembling that of human embryos during reconstruction of blastoids from extended pluripotent stem cells. Cell Prolif 55. https://doi.org/10.1111/CPR.13317

Treatment add-ons

ESHRE Add-ons working group. (2022). *Good practice recommendations for add-ons in reproductive medicine*. ESHRE. Available at: https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Guidelines-in-development/Addons (Accessed: 2 December 2022)

Treatment add-ons rating review – February 2023

Details about this paper

Area(s) of strategy:	The best care, the right information
Meeting:	Scientific and Clinical Advances Advisory Committee (SCAAC)
Agenda item:	6 and 7
Paper number:	HFEA (06/02/2023) 006 and 007
Meeting date:	06 February 2023
Author:	Zoe Constable, Policy Manager
Annexes	Annex A. Evidence decision tree for rating add-ons Annex B. Summary of current and recommended ratings Annex C. References of reviewed studies Annex D. Independent reviewer report (as a separate PDF)

Output from this paper

For information/ recommendation?	For recommendation
Recommendation:	Members asked to: • consider the quality of evidence for each treatment add-on based on the findings from an independent assessor at Annexes B and D; • consider the decision tree for rating add-ons at Annex A having applied it to the new ratings system for the first time; • agree and recommend ratings for each treatment add-on based on the outcome of live birth rate for the general population; and • agree and recommend ratings for each additional outcome(s) and population(s) relevant to specific treatment add-ons.
Resource implications:	In budget
Implementation date:	Recommendations will be implemented as soon as feasible
Communication(s):	Updates to the HFEA's website information on treatment add-ons and communication of updates to the sector, patients and public.
Organisational risk:	Low

1. Introduction

1.1. The Authority met in **July 2022** and agreed:

- the definition of treatment add-ons that the HFEA will provide information for
- to move to a five-category rating scale
- to rate additional outcomes, such as miscarriage, and outcomes for specific patient groups, such as male-factor infertility, in addition to live births for specific add-ons
- to expand the evidence base in line with SCAAC's recommendation that in the absence of high-quality RCTs or meta-analysis the evidence base should be expanded to non-RCTs

1.2. For information, ESHRE are developing a **good practice recommendation paper** which outlines a set of treatment add-on tests and treatments, describes their rationale and any evidence of their efficacy and safety, and provides a recommendation for clinical practice. This is currently a draft paper and the recommendations therein have not been considered by our expert reviewer but may be of interest to the committee.

1.3. To account for new evidence that arises from studies investigating treatment add-ons, the HFEA's add-ons list and their assigned ratings are reviewed annually. **The committee is asked for a view on how frequently the evidence base for add-ons and therefore the ratings should be reviewed.** For example, continuing the annual review, adjusting the length of time between reviews, or conducting ad-hoc reviews. If a significant study is published that could impact a rating, then the executive will aim to review the associated add-on as soon as possible in all cases.

2. Evolving the rating system

2.1. The Authority approved moving to a five category rating system with the following symbols/colours in **July 2022** and the SCAAC were updated in **October 2022**:

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

	On balance, the evidence from high quality studies shows that **this add-on has no effect on the treatment outcome**.
	**There are potential safety concerns** and/or, on balance, the evidence from high quality studies show that **this add-on may reduce treatment effectiveness.**

2.2. A summary table of the proposed ratings for each add-on from the independent reviewer can be found at Annex B.

3. Expanding the evidence base

3.1. The five-category rating system was also approved by the Authority to be applied to **additional outcomes**, such as miscarriage, and outcomes for **specific patient groups**, such as male-factor infertility, in addition to live births.

3.2. These additional outcomes/populations were selected by a review panel in November 2022. The panel consisted of:

- The Chair of SCAAC;
- One person from the HFEA who is either a member of the scientific policy team or is a member of the Register Research Panel;
- At least one member of SCAAC who is a clinician; and
- At least one member of SCAAC who is involved in clinical research/embryology.

3.3. At the Authority meeting in **July 2022** it was agreed that the add-ons rating system would consider non-RCTs where high quality RCTs are not available. This was applied where fewer than three RCTs could be identified for each add-on for live birth and each additional outcome/population. The Authority agreed that the next steps should be for a decision tree to be developed to determine how non-RCT evidence will be used by SCAAC when generating add-ons ratings and that this will be taken to SCAAC for their consideration.

- SCAAC agreed a decision tree (at annex A) in October 2022, the committee commented that the decision tree was good but ultimately skewed towards the grey category because at least three publications (NRSI or RCTs), which are considered at least medium quality, are required for a rating other than grey.
- The requirement for at least three publications, which are considered at least medium quality, is based on the current processes followed by other relevant organisations such as NICE that select the top three pieces of evidence prioritising:
    - Systematic reviews
    - Randomised control trials
    - Cohort/case-control/case series

      NICE rank upon a combination of the size/publication date/clarity of data/inclusion of an “active comparator” (effectively, a placebo option)/how representative the study population is of the relevant. When applying this to add-ons if none of the above can be identified, the intervention will be rated grey.

- For this review, the independent reviewer has made recommendations for ratings and has identified that the current decision tree leads to a significant number of the add-ons being rated grey. The independent reviewer has included comments where an alternative rating could be appropriate if the SCAAC were to consider evidence from at least one high quality study to be significant enough. For example, in the case of PICSI for which there is evidence from a well-designed trial (Miller 2019) that ruled out any major effect of PICSI in their population of couples using their own gametes, suggesting a black rating.
- Due to the concerns raised at the last SCAAC meeting about the decision tree being skewed towards the grey rating, and this now being apparent after applying the decision tree for the first time, **we ask the committee to consider whether any updates need to be made to the decision tree so that it fulfils the aim of producing transparent messaging for patients.**

4. Independent assessment of the quality of evidence

4.1. In order to categorise the treatment add-ons under consideration, it is necessary not only to identify the published evidence around each treatment add-on, but also to assess the quality of that evidence. For this reason, we seek advice from an expert in systematic reviews and evidence assessment to carry out an independent assessment of the quality of evidence (using the GRADE methodology[1]) for each treatment add-on.

4.2. The independent reviewer reassessed the traffic light ratings in light of the new five-category rating system and additional studies published since the last review (conducted in **October 2021**). New research (the published evidenced) in the form of RCTs were identified for nine of the 12 add-ons on the HFEA's traffic light rated list of add-ons. Three add-ons had fewer than three high quality RCTs so a search for non-RCTs in the last 10 years was also completed.

4.3. Additional outcomes/populations were identified for nine of the 12 add-ons by the review panel. Literature searches were conducted for each additional outcome and population, and where fewer than three RCTs were identified, the search was expanded to non-RCTs. Searches were limited to studies published in the last 10 years.

4.4. The critical review of studies included assessment of risk of bias from allocation method, blinding, selective reporting, unexplained attrition, unplanned interim analysis and other miscellaneous errors in the design, conduct or reporting of results.

4.5. The findings of this assessment for each add-on and the independent reviewer's recommended ratings can be found at Annex B, alongside the current traffic light rating agreed previously in consultation with the committee, last in October 2021.

4.6. The assessments made by the independent reviewer are from a methodological perspective without expertise in the clinical or scientific context. The independent reviewer's original report can be found as separate PDF at Annex D.

[1] GRADE is an approach for grading the quality of evidence and the strength of recommendations. It was developed by the Grading of Recommendations, Assessment, Development and Evaluation Working Group.

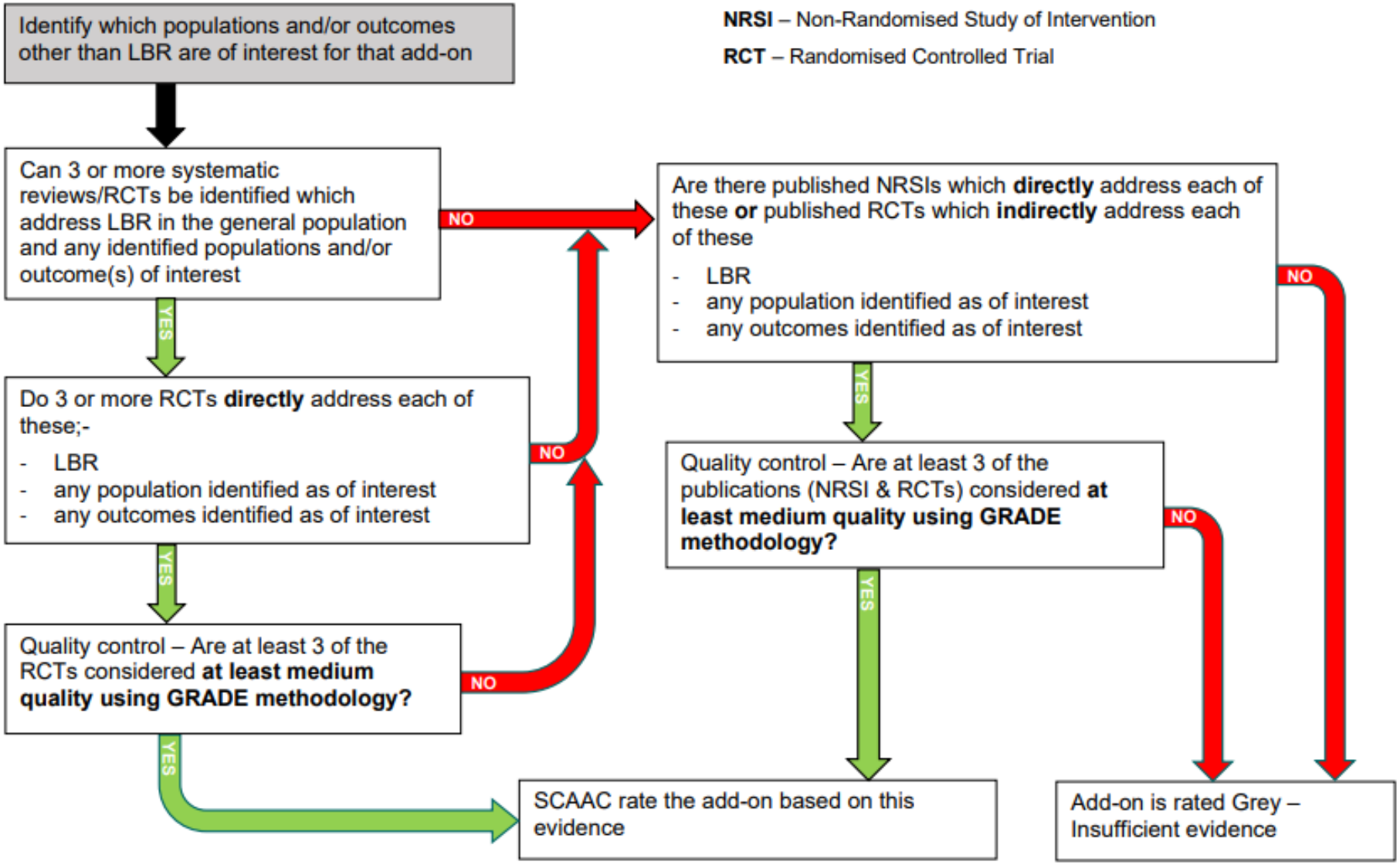
5. Recommendations

5.1.

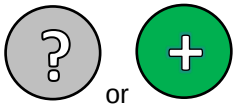
The committee is asked to:

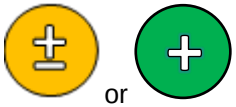
- **consider the quality of evidence for each treatment add-on based on the findings from an independent assessor at Annex B and D;**
- **consider the decision tree for rating add-ons at Annex A having applied it to the new ratings system for the first time;**
- **agree and recommend ratings for each treatment add-on based on the outcome of live birth rate for the general population; and**
- **agree and recommend ratings for each additional outcome and population relevant to specific treatment add-ons.**

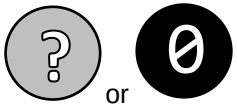
Annex A. Evidence decision tree for rating add-ons

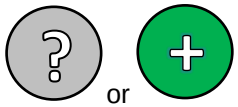
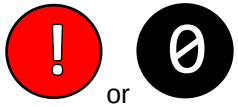
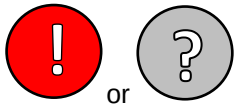


Annex B. Summary of treatment add-on rating review

Treatment add-on	Current rating	New rating recommended by the external reviewer	Studies reviewed
Artificial egg activation calcium ionophore	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients • Embryo formation and early development for most fertility patients • Live birth rate for patients with failed fertilisation in previous ICSI treatments • Embryo formation and early development for patients with failed fertilisation in previous ICSI treatments GREY for all outcomes [Only 1 moderate/high quality study, no safety concerns]	2 RCTs 10 NRSIs
Assisted hatching	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients GREY [Only one moderate/high quality study, no safety concerns]	18 RCTs 4 NRSIs
Elective freeze all cycles	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients  or • OHSS outcomes for most fertility patients • OHSS outcomes for populations at increased risk of OHSS	13 RCTs 10 NRSIs

		 • Obstetric/neonatal outcomes for most fertility patients • Time to birth for most fertility patients • Time to birth for populations at increased risk of OHSS • Live birth for populations at increased risk of OHSS • Obstetric/neonatal outcomes for populations at increased risk of OHSS AMBER for live birth [Conflicting findings from 4 moderate/high quality studies]; GREEN for OHSS [On balance, consistent evidence]; GREY for obstetric/neonatal outcomes [Studies underpowered for these].	
Endometrial receptivity array (ERA)	 Live birth rate for most fertility patients	 • Live birth rate for most fertility patients RED (No moderate/high quality studies and safety concerns raised by Cozzolino 2022).	1 RCTs 3 NRSIs
Endometrial scratching	 Live birth rate for most fertility patients	 • Live birth rate for most fertility patients AMBER/GREEN [The more recent evidence reviewed above does not materially affect the previous review but the terminology of the grading has changed. The large, moderate-high quality studies do not consistently conclude benefit but do not conflict with each other. Meta-analysis is inconclusive at the standard 95% confidence level but “on balance” there is evidence for a small beneficial effect in terms of live birth. The Committee needs to balance this against cost, inconvenience and pain of the procedure].  • Live birth rate for patients with recurrent implantation failure	40 RCTs 5 NRSIs

		GREY [No moderate/high quality studies for the sub-population].	
Hyaluronate enriched medium (eg EmbryoGlue)	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients Recommendation: The number of moderate/high quality studies depends on whether the intervention by Kleijkers is considered eligible for this comparison. GREY (only two moderate/high quality studies) if not. GREEN (three moderate/high quality studies, consistent results) if so.	6 RCTs 7 NRSIs
Intracytoplasmic morphologic sperm injection (IMSI)	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients GREY [Only 1 moderate/high quality study, no safety concerns] • Live birth rate for male-factor infertility patients GREY [No moderate/high quality study, no safety concerns]	7 RCTs 5 NRSIs
Intrauterine culture	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients Since no further studies have been identified since the last review in October 2021, no summary has been provided by the independent reviewer for this meeting.	0 RCTs 1 NRSIs
Physiological intracytoplasmic sperm injection (PICSI)	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients • Live birth rate for male-factor infertility patients 	9 RCTs 5 NRSIs

		• Live birth rate for older women  or • Miscarriage rate for most fertility patients • Miscarriage rate for male-factor infertility patients • Miscarriage rate for older women GREY for all outcomes for most fertility patients [Only 1 moderate/high quality study, no safety concern] NB: The one study is large, of high quality, and may reasonably be considered definitive. The committee may conclude that there is sufficient evidence to grade as BLACK for live birth and GREEN for miscarriage. The magnitude of study required to confirm a plausible effect size makes unlikely the collection of further robust evidence: a randomised trial with 90% power to detect a difference in live birth rates between 25% and 27% would require in excess of 20,000 participants. Recommendation: GREY for all outcomes for male factor [Only 1 moderate/high quality study, no safety concern] N.B. Miller 2019 comprised 95% participants with male factor. The committee could consider grading as BLACK for live birth and GREEN for miscarriage. GREY for all outcomes for older women [Only 1 moderate/high quality study, no safety concern]. Given consistency with general population, the committee could consider grading GREEN for miscarriage.	
Pre-implantation genetic testing for aneuploidy (PGT-A)	Live birth rate for most fertility patients	 or • Live birth rate for most fertility patients  or • Time to birth for most fertility patients	7 RCTs 5 NRSIs

		 • Miscarriage rate for most fertility patients • Miscarriage rate for older women  or  • Live birth rate for older women  • Time to birth for older women Recommendation: GREEN for miscarriage for general population [several moderate/high quality studies, consistent]; RED/BLACK for live birth [Yan looks definitive but could argue either way]; RED/GREY for time to success [Yan looks definitive but just 1 study of this] GREEN for miscarriage for older women [1 study but consistent with general population]; BLACK/GREY for live birth [1 study but consistent with general population]; GREY for time to success [1 study, no safety concerns]	
Immunological test and treatments for infertility	 Live birth rate for most fertility patients	The independent review did not make an overarching recommendation for immunological tests and treatments	-
Intralipids	 Live birth rate for most fertility patients	 or  • Live birth rate for most fertility patients • Miscarriage rate for most fertility patients • Live birth rate for populations with immunological testing • Miscarriage rate for populations with immunological testing	6 RCTs 1 NRSIs

		GREY/RED for all outcomes [No moderate/high quality studies. Question over whether committee considers the safety concerns raised over congenital malformations justify the red rating].	
Intravenous immunoglobulin	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients • Miscarriage rate for most fertility patients AMBER for all outcomes for general population [3 RCTs providing moderate quality evidence. Not 'conflicting' but results too imprecise to determine effectiveness at this stage].  • Live birth rate for populations with immunological testing • Miscarriage rate for populations with immunological testing GREY for all outcomes for Populations with immunological testing [No moderate/high quality studies, no safety concerns]	4 RCTs 4 NRSIs
Steroids (glucocorticoids)	Live birth rate for most fertility patients	 • Live birth rate for most fertility patients • Miscarriage rate for most fertility patients • Live birth rate for populations with immunological testing • Miscarriage rate for populations with immunological testing GREY for all outcomes for general population. [Insufficient evidence from moderate/high quality studies, no safety concerns]. GREY for all outcomes for Populations with immunological testing. [No moderate/high quality studies, no safety concerns].	5 RCTs 6 NRSIs
Time-lapse imaging and incubation			13 RCTs 4 NRSIs

	Live birth rate for most fertility patients	• Live birth rate for most fertility patients BLACK [4 moderate/high quality studies with consistent results]

1. Artificial egg activation calcium ionophore

Artificial egg activation calcium ionophore was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

The panel recommended the review for this add-on to include outcomes relating to embryo formation and early development in addition to live birth rate. Outcomes for patients with failed fertilisation in previous ICSI treatments were requested in addition to outcomes for the general population.

2. Assisted hatching

Assisted hatching was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned a red traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

3. Elective freeze all cycles

Elective freeze all cycles was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

The panel recommended the review for this add-on to include ovarian hyperstimulation syndrome (OHSS) outcomes, obstetric/neonatal outcomes, and time to birth, in addition to live birth rate. Outcomes for patients at increased risk of OHSS were requested in addition to outcomes for the general population.

The committee is asked to consider whether OHSS outcomes for patients at increased risk of OHSS should be rated green or grey. There is only one high quality study available, but it is consistent with studies on the general population for which OHSS outcomes are rated green.

4. Endometrial receptivity array (ERA)

Endometrial receptivity array (ERA) was introduced to the HFEA's traffic light rated list of add-ons in June 2021 and was assigned a red traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

5. Endometrial scratching

Endometrial scratching was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

The panel recommended the review for this add-on to include outcomes for patients with recurrent implantation failure in addition to outcomes for the general population.

The Committee is asked to consider whether the rating for live birth rate in most fertility patients should be amber or green given the independent reviewer's comment that "the large, moderate-high quality studies do not consistently conclude benefit but do not conflict with each other. Meta-analysis is inconclusive at the standard 95% confidence level but "on balance" there is evidence for a small beneficial effect in terms of live birth."

6. Hyaluronate enriched medium (eg EmbryoGlue)

Hyaluronate enriched medium was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

7. Intracytoplasmic morphologic sperm injection (IMSI)

IMSI was introduced to the HFEA's traffic light rated list of add-ons in October 2018 and was assigned a red traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

The panel recommended the review for this add-on to include outcomes for patients with male-factor infertility in addition to outcomes for the general population.

8. Intrauterine culture

Intrauterine culture was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned a red traffic light rating by the Committee. Only one published study has been identified for this add-on and no safety concerns have been raised. This results in a grey rating as per the evidence decision tree as there is an insufficient number of publications.

Since no further studies have been identified since the last review in October 2021, no summary has been provided by the independent reviewer for this meeting.

9. Physiological intracytoplasmic sperm injection (PICSI)

PICSI was introduced to the HFEA's traffic light rated list of add-ons as in October 2018 and was assigned a red traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

The panel recommended the review for this add-on to include miscarriage rate, in addition to live birth rate. Outcomes for patients with male-factor infertility and older women were requested in addition to outcomes for the general population

The committee is asked to consider whether live birth rate for the general population and male-factor infertility patients should be rated as grey or black. This is in light of the summary provided by the independent reviewer that although there is only one high quality study available (Miller 2019), it is "large, of high quality and may reasonably be considered definitive".

The committee is asked to consider whether miscarriage rate for all populations should be rated as grey or green. As above, this due to the high-quality Miller 2019 study.

10. Pre-implantation genetic testing for aneuploidy (PGT-A)

PGT-A for day five embryos was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee, this rating was changed to a red traffic light by the Committee in October 2019.

The panel recommended the review for this add-on to include miscarriage rate and time to birth, in addition to live birth rate. Outcomes for older women were requested in addition to outcomes for the general population

The committee is asked to consider whether live birth rate and time to birth for the general population should be rated as red. This is in light of the summary provided by the independent reviewer that the Yan 2021 study looks definitive that the add-on reduces treatment effectiveness, but there is only one study of this so it could be argued another way.

The committee is asked to consider whether live birth rate for older women should be rated as grey or black. This is in light of the summary provided by the independent reviewer that although there is only one high quality study available, it is consistent with studies on the general population for which live birth rate is rated black or red (see point above).

The committee is asked to consider whether miscarriage rate for older women should be rated as grey or green. This is in light of the summary provided by the independent reviewer that although there is only one high quality study available, it is consistent with studies on the general population for which miscarriage rate is rated green.

11. Immunological test and treatments for infertility

Immunological test and treatments for infertility was introduced to the HFEA's traffic light rated list of add-ons as an umbrella term covering all immunological test and treatments for infertility treatments in February 2017 and was assigned a red traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

At the **October 2020** SCAAC meeting it was proposed that immunological test and treatments for infertility be broken down by treatment type and an individual traffic light rating be allocated to each type.

The independent reviewer did not make an overarching recommendation for immunological tests and treatments for infertility. The committee is asked to consider the independent reviewer's recommendations on intralipids, intravenous immunoglobulin and steroids (glucocorticoids), and recommend an overall rating for the group if appropriate.

The panel recommended the review for this add-on to include miscarriage rate in addition to live birth rate. Outcomes for patients undergoing immunological testing, such as natural killer cell blood tests, were requested in addition to outcomes for the general population

The committee is asked to consider whether outcomes for intralipids should be rated red or grey. This is in light of the summary provided by the independent reviewer around concerns over raised congenital malformations with use of this add-on.

12. Time-lapse imaging and incubation

Time-lapse incubation and imaging was introduced to the HFEA's traffic light rated list of add-ons in February 2017 and was assigned an amber traffic light rating by the Committee. No changes have been made to this traffic light rating since then.

The panel recommended the review for this add-on to report any differences in outcomes for the use of manual annotation of time lapse images by an embryologist vs automated annotation of time lapse images using a computer software or artificial intelligence.

There is little variation in method of annotation for the studies included in the literature search, with the majority using manual annotation. The committee is asked to consider the categories to report treatment outcomes using time-lapse with manual or automated annotation.

Annex C. References of reviewed studies

Bold indicates studies added for the 2023 update.

Adjunct	Study	DOI/reference
Artificial Egg Activation	Meerschaut 2012	10.1093/humrep/des097
	Ebner 2012	**10.1016/j.fertnstert.2012.07.1134**
	Montag 2012	**10.1016/j.rbmo.2012.02.002**
	Liu 2013	**10.1017/S0967199411000530**
	Aytac 2015	10.1016/j.fertnstert.2015.07.1163
	Caglar 2015	**10.1016/j.fertnstert.2015.07.1163**
	Darwish 2015	**10.1016/j.rbmo.2015.08.012**
	Ebner 2015	**10.1016/j.rbmo.2014.11.012**
	Aydinuraz 2016	10.1080/14647273.2016.1240374
	Fawzy 2018	10.1093/humrep/dey258
	Li 2019	**10.1016/j.rbmo.2019.03.216**
	Shebl 2021	**10.1007/s10815-021-02338-3**
	Yin 2022	**10.1007/s00404-021-06329-8**
Assisted Hatching: Stored	Balaban 2006	10.1093/humrep/del097
	Ge 2008froz	RBMO 2008;16(4):589-96.
	Valojerdi 2010	10.1016/j.rbmo.2009.11.002
	Fang 2010	10.1016/j.fertnstert.2009.08.014
	Figueria 2012	**10.1016/j.ejogrb.2012.05.022**
	Wan 2014	**10.1016/j.rbmo.2014.01.006**
	Wang 2016	10.3892/br.2016.716
	Knudtson 2016	F&S 2016;106(3) Suppl:e141
	Safari 2017	10.1016/j.repbio.2017.05.003
	Kirienko 2019	**10.1016/j.rbmo.2019.06.003**
Assisted Hatching: Fresh	Sagoskin 2007	10.1016/j.fertnstert.2006.07.1498
	Ge 2008fresh	RBMO 2008;16(4):589-96.
	Balakier 2009	10.1016/j.fertnstert.2008.07.1729
	Hagemann 2010	10.1016/j.fertnstert.2009.01.116
	Kutlu 2010young	10.1007/s10815-010-9431-6
	Kutlu 2010old	10.1007/s10815-010-9431-6
	Razi 2013	Iran J reprod Med 2013;11(12):1021-6.
	Shi 2016	10.1177/1933719116641764
	Chang 2016	F&S 2016;106(3) Suppl:e314
	Nada 2018	10.1007/s00404-017-4604-5
	Fawzy 2020	**10.1093/humrep/deaa160**
	Zhang 2022	**10.3389/fendo.2022.927834**
Embryo Glue	Morbeck 2007	NCT005882250
	Mahani 2007	EMHJ 2007;13(4):876-80.
	Friedler 2007	10.1093/humrep/dem220
	Korosec 2007	RBM0 2007;15(6):701-7.
	Hazlett 2008	10.1016/j.fertnstert.2007.05.063
	Urman 2008	10.1016/j.fertnstert.2007.07.1294

	Dittmann-Muller 2009	Hum Reprod 2009;24 Suppl 1:167.
	Fancsovits 2015	10.1007/s00404-014-3541-9
	Singh 2015	10.4103/0974-1208.170398
	Kleijkers 2016	**10.1093/humrep/dew156**
	Zbořilová 2018	https://europepmc.org/abstract/med/30764616
	Kandari 2019	**10.1016/j.fertnstert.2021.02.015**
	Yung 2021	**10.1016/j.fertnstert.2021.02.015**
Endometrial Receptivity	Simón 2020	10.1016/j.rbmo.2020.06.002
	Cohen 2020	**10.1080/19396368.2020.1824032**
	Cozzolino 2020	**10.1007/s10815-020-01948-7**
	Cozzolino 2022	**10.1016/j.fertnstert.2022.07.007**
Endometrial Scratching	Raziel 2007	10.1016/j.fertnstert.2006.05.062
	Karimzadeh 2009	10.1111/j.1479-828X.2009.01076
	Narvekar 2010	10.4103/0974-1208.63116
	Abdelhamid 2012	10.1007/s00404-013-2785-0
	Baum 2012	**10.3109/09513590.2011.650750**
	Nastri2013	**10.1002/uog.12539**
	Gibreel 2013	10.1111/j.1447-0756.2012.02016.x
	Parsanezhad 2013	IRCT:2012082510657NI
	Zarei 2014	IRCT:2012070810210NI
	Zhang 2014	**10.1007/s00404-014-3382-6**
	Zhang 2015	**10.1007/s11655-014-1843-1**
	Bord 2015	**10.1007/s00404-015-3954-0**
	Wadhwa 2015	J Hum Reprod Sci 2015;8(3):151-8.
	El Khayat 2015	10.1016/j/ejogrb.2015.08.025
	Mahey 2015	10.1016/j.fertnstert.2015.07.1163
	Maged 2016	10.1177/1933719115602776
	Bahaa Eldin 2016	**10.1177/1933719116638191**
	Siristatidis 2017	**10.1080/09513590.2016.1255325**
	Goel 2017	10.1007/s10815-017-0949-8
	Mak 2017	10.1016/j.rbmo.2017.04.004
	Aleyamma 2017	10.1016/j.ejogrb.2017.05.005
	Helmy 2017	10.1002/ijgo.12178
	Senocak 2017	10.1016/j.jogoh.2017.09.003
	Ashrafi 2017	10.1111/jog.13401
	Maged 2018	10.1002/ijgo.12355
	Frantz 2019	10.1093/humrep/dey334
	Lensen 2019	10.1056/NEJMoa1808737
	Olesen 2019	10.1016/j.fertnstert.2019.08.010
	Gürgan 2019	**10.1016/j.rbmo.2019.02.014**
	Tumanyan 2019	**10.1080/09513590.2019.1632085**
	Mackens 2020	**10.1093/humrep/deaa018**
	Berntsen 2020	10.1016/j.ejogrb.2020.06.034
	Ghuman 2020	10.1016/j.ejogrb.2020.08.010
	Rodriguez 2020	10.1007/s43032-020-00204-8

	van Hoogenhuijze 2021	10.1093/humrep/deaa268
	Metwally 2021	10.1093/humrep/deab041
	Yavangi 2021	10.18502/ijrm.v19i5.9255
	Aghajanpour 2021	**10.1016/j.jri.2021.103426**
	Glanville 2022	**10.1016/j.rbmo.2021.10.008**
	Izquierdo 2022	**10.1016/j.jogoh.2022.102335**
	Madhuri 2022	**10.1016/j.ejogrb.2021.10.028**
	Metwally 2022	**10.3310/JNZT9406**
	Wong 2022	**10.1016/j.fertnstert.2021.12.009**
Freeze All	Aflatoonian 2010	10.1007/s10815-010-9412-9
	Shapiro 2011a	10.1016/j.fertnstert.2011.05.050
	Shapiro 2011b	10.1016/j.fertnstert.2011.02.059
	Magdi 2017	10.1016/j.fertnstert.2017.04.020
	Shi 2018	10.1056/NEJMoa1705334
	Vuong 2018	10.1056/NEJMoa1703768
	Le 2018	**10.1093/humrep/dey253**
	Rahav Koren 2018	**10.1159/000479557**
	Ye 2018	**10.1186/s12958-018-0373-7**
	Deng 2019	**10.1007/s11596-019-2031-5**
	Shrem 2019	**10.1016/j.rbmo.2019.04.014**
	Wei 2019	10.1016/S0140-6736(18)32843-5
	Stormlund 2020	10.1136/bmj.m2519
	Santos-Ribeiro 2020	10.1093/humrep/deaa226
	Boynukalin 2020	**10.1371/journal.pone.0234481**
	Li 2021	**10.3389/fendo.2021.730059**
	Deepika 2021	**10.5935/1518-0557.20200028**
	Huang 2021	**10.1038/s41598-021-02227-w**
	Vuong 2021	**10.1007/s10815-021-02180-7**
	Wong 2021	10.1093/humrep/deaa305
	Maheshwari 2022	**10.1093/humrep/deab279**
	Maheshwari 2022a	**10.3310/AEFU1104**
IMSI	**Knez 2012**	**10.1016/j.rbmo.2012.03.011**
	De Vos 2013	10.1093/humrep/des435
	Leandri 2013	10.1111/j.2047-2927.2013.00104.x
	Setti 2013	10.1016/j.ejogrb.2013.09.006
	Marci 2013	10.1186/1742-4755-10-16
	Kim 2014	10.5653/cerm.2014.41.1.9
	Cassuto 2014	10.1016/j.rbmo.2013.08.013
	Setti 2014	**10.1016/j.ejogrb.2014.10.008**
	Sifer 2014	**10.1016/j.ejogrb.2014.07.017**
	La Sala 2015	10.1186/s12958-015-0096-y
	Mangoli 2019	10.1111/and.13340
	Mangoli 2020	**10.1007/s10815-020-01910-7**
Intralipids	**El-Khayat 2015**	**10.1016/j.fertnstert.2015.07.080**
	Meng 2016	**10.1007/s00404-015-3922-8**

	Dakhly 2016	10.1016/j.ijgo.2016.06.026
	Gamaleldin 2018	**10.1002/central/CN-01911196/full**
	Singh 2019	10.1016/j.ejogrb.2019.06.007
	Al-Zebeidi 2019	10.1080/09513590.2019.1631280
	Rogenhofer 2021	**10.1111/aji.13506**
IV Immunoglobulin	Stephensen 2010	10.1093/humrep/deq179
	Christiansen 2014	10.1111/1471-0528.13192
	Cohen 2015	**PMID: 26380487**
	Yamada 2015	**10.1016/j.jri.2015.01.008**
	Christiansen 2015	**10.1111/1471-0528.13192**
	Lee 2016	**10.1111/aji.12442**
	Meng 2016	**10.1007/s00404-015-3922-8**
	Ahmadi 2017	**10.1016/j.imlet.2017.10.003**
	Jørgensen 2020	**10.1016/j.jri.2020.103128**
PGT-A	Yang 2012	Molec Cytogen 2012;5:24
	Forman 2013	10.1016/j.fertnstert.2013.02.056
	Scott 2013	10.1016/j.fertnstert.2013.04.035
	Ikuma 2015	**10.1371/journal.pone.0129958**
	Ubaldi 2017	**10.1016/j.fertnstert.2017.03.007**
	Verpoest 2018	**10.1093/humrep/dey262**
	Ozgur 2019	10.1007/s10815-018-01399-1
	Munné 2019	10.1016/j.fertnstert.2019.07.1346
	Cimadomo 2019	**10.1093/humrep/dez078**
	Yan 2021	**10.1056/NEJMoa2103613**
	De Munck 2022	**10.1371/journal.pone.0267241**
	Idárraga 2022	**10.5935/1518-0557.20210085**
PICSI	**Parmegiani 2012**	**10.1016/j.fertnstert.2012.05.043**
	Worrilow 2013	10.1093/humrep/des417
	Majumdar 2013	10.1007/s10815-013-0108-9
	Mokanszki 2014	10.3109/19396368.2014.948102
	Troya 2015	10.5935/1518-0557.20150015
	Lohinova 2017	PMID: 29099693
	Erberelli 2017	10.5935/1518-0557.20170002
	Korosi 2017	**PMID: 28724183**
	Avalos-Durán 2018	**10.5935/1518-0557.20180027**
	Miller 2019	10.1016/S0140-6736(18)32989-1
	Hasanen 2020	**10.1007/s10815-020-01913-4**
	Novoselsky 2021	**10.1111/andr.12982**
	Hozyen 2022	**10.1007/s43032-021-00642-y**
Steroids	Fawzy 2013	10.1007/s00404-013-3020-8
	Fan 2016	10.1111/aji.12559
	Taiyeb 2017	10.1007/s12020-017-1446-7
	Yeganeh 2017	10.1080/01443615.2017.1346593
	Kaye 2017	**10.1016/j.fertnstert.2017.04.003**
	Milardi 2017	**10.1111/andr.12300**
	Siristatidis 2018	**10.1080/09513590.2017.1380182**

	Liu 2018	10.1111/cen.13824
	Huang 2021	**10.1016/j.jri.2020.103245**
	Thalluri 2022	**10.1093/humrep/deac142**
	Zhou 2022	**10.1186/s12884-022-04532-2**
Time Lapse	Kirkegaard 2012	10.1007/s10815-012-9750-x
	Kahraman 2013	10.1177/205891581200300204
	Rubio 2014	10.1016/j.fertnstert.2014.07.738
	Park 2015	**10.1093/humrep/deu316**
	Wu 2016	**10.1186/s12958-016-0181-x**
	Goodman 2016	10.1016/j.fertnstert.2015.10.013
	Wang 2016	J Reprod Med 61(5):254-262
	Insua 2017	10.1016/j.fertnstert.2017.06.031
	Kaser 2017	**10.1093/humrep/dex231**
	Alhelou 2018	10.1016/j.repbio.2017.12.003
	Yang 2018	10.1093/humrep/dey047
	Kovacs 2019	10.1016/j.ejogrb.2018.12.011
	Chen 2020	10.1093/humrep/deaa268
	Ahlstrom 2022	**10.1093/humrep/deac020**
	Meng 2022	**10.1016/j.fertnstert.2022.02.015**
	Zhang 2022	**10.1016/j.rbmo.2022.06.017**

Traffic Light System for Treatment Add-ons

Andy Vail, January 2023

INTRODUCTION

The HFEA website provides patients with digestible information on treatment add-ons in the form of a rating system. The purpose of this report is to inform the Scientific and Clinical Advances Advisory Committee's deliberations on updating this information. In particular, this update extends the ratings system to five categories, supplements sparse evidence from randomised trials with additional data and considers outcomes other than live birth.

The aim of the work reported below is to critically appraise, interpret and summarise, for consideration by the HFEA, the reports of identified studies.

METHOD

Dina Halai, Scientific Policy Manager, provided references and hyperlinks to identified studies for consideration. All newly incorporated papers were published since 2012.

Critical review of studies included assessment of risk of bias from allocation method, blinding, selective reporting, unexplained attrition, unplanned interim analysis and other miscellaneous errors in the design, conduct or reporting of results. To classify a randomised trial as providing moderate/high quality evidence I have applied the default classification of the Cochrane Gynaecology and Fertility review group. Specifically, for a study to be considered in this category it must describe an adequately concealed randomisation process to prevent selection bias. It must also not be identified as at high risk of bias in other regards ('unclear' is acceptable) other than where blinding is unrealistic. Where HFEA specifically requested results for a sub-population of interest, I have presented first the studies addressing the general population and then studies addressing the specific sub-populations. The extent to which interpretation of sparse results for a sub-population should borrow from the broader information available is addressed on a case-by-case basis.

To calculate odds ratios, published results were re-calculated applying the intention to treat (ITT) principle and using two-sided confidence intervals. As these were being interpreted as indicative rather than inferential, no technical adjustments were applied for multiple testing, covariate adjustment or planned interim analyses. Odds ratios were calculated for the latest clinical outcome presented. That is, live birth rate was first choice, followed by ongoing, clinical, unspecified or biochemical pregnancy. An odds ratio greater than 1.0 for these outcomes implies benefit of the add-on under study. Additional outcomes, particularly those relating to safety such as OHSS incidence and miscarriage, are reported where these are a particular aim of the add-on or have been requested by HFEA. An odds ratio greater than 1.0 for adverse outcomes implies detriment of the add-on under study.

RESULTS

1. Artificial egg activation

The previous review in 2019 included four studies: two within-patient designs on sibling oocytes and two RCTs that each suggested promise but studied quite different populations and were dogged by

methodological issues. These are included below alongside nine additional studies, categorised as requested with the additional consideration of outcomes relating to embryo formation and early development.

1 (i) *General population*

Ebner 2012 prospectively recruited 66 couples undergoing ICSI with severe male factor and "sufficient" number of oocytes. All were treated with calcium ionophore immediately following ICSI. Unfortunately, there are several methodological issues with this study that preclude statistical interpretation. The presentation and analysis do not account for the multiple cycles per participant. Comparison is made with multiple historic cycles of the same participants. Comparison also fails to account for the inherent matching and is almost guaranteed to show 'benefit' given that regression to the mean, Hawthorne and placebo biases all favour the intervention. The authors reported higher blastocyst formation, implantation and clinical outcomes in the intervention arm, with 26 (39%) participants achieving live birth.

Liu 2013 conducted a non-clinical study with what appears to have been considered 'waste product'. From previous ICSI cycles they took oocytes that had failed to develop (germinal vesicle or metaphase I). These had been vitrified, thawed and then matured for 24-36 hours, with 204 oocytes maturing to be subject to ICSI using donor sperm. They then describe randomly assigning these to either standard cleavage medium or activation for 6 minutes in 7% ethanol prior to standard cleavage medium. There is no detail to assess the allocation but it appears to have been done regardless of sibling status. The number of women who provided the oocytes is not reported and there appears to have been no intention to transfer any resulting embryos. Reported fertilisation rates were similar between groups. Cleavage rate was higher in the activated arm and only this arm produced any high quality embryos (n=16), blastocysts (n=8) and high quality blastocysts (n=4). Caution is required with statistical interpretation as it is unclear whether these were independent observations or from just one or two donors, for example.

Aytac 2015, also identified as Caglar 2015, randomised 296 couples with diminished ovarian reserve but normal sperm parameters and no previous fertilisation failure. This appears to have been a well-designed trial for the clinical question. Similar numbers of oocytes progressed to cleavage stage and the distribution of embryo grade was also similar between arms. However, transfer was more common in the activation group (68% vs 56%) and there were more pregnancies per transfer, leading to a higher ongoing pregnancy rate: OR=1.9 (0.80 to 4.4).

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

Shebl 2021 presented a within-patient, sibling oocyte design in 78 couples undergoing ICSI with either a history of <50% fertilisation (n=47) or severe male factor (n=31). Activation was by ionophore (calcimycin) for 15 minutes within 10 minutes of ICSI. All embryos were then cultured in a

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

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

Current rating amber.
Recommendation: GREY for all outcomes [Only 1 moderate/high quality study, no safety concerns]

1 (ii) *Failed fertilisation in previous ICSI cycle*

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

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

Darwish 2015 undertook a similar but far smaller 'preliminary' study. They prospectively recruited four couples whose previous ICSI cycle was incomplete due to 2PN arrest. The same statistical issues apply to interpretation of the data. All four participants progressed to embryo transfer with a total

of eleven embryos transferred. Only one had a positive pregnancy test and this resulted in a healthy twin delivery at term from three transferred embryos.

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

Aydinuraz 2016 presented a within-patient, sibling oocyte design in 21 couples with terato-zoospermia and a low fertilisation rate in the previous cycle. Unfortunately, their presentation and all analyses ignored the matching of the design, precluding statistical interpretation of their data. However, it is clear that only 13 of the 21 couples produced at least one top quality embryo from artificially activated oocyte, whereas 20 achieved this from conventionally cultured oocytes.

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

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

2. Assisted hatching

The previous review included 14 RCTs and three other designs considering a range of techniques for assistance (laser thinning or creation of hole by laser or chemically) in various settings (fresh, frozen-thaw and vitrified; oocytes, embryos, blastocysts). Results were conflicting but no study was deemed of moderate/high quality. Five additional studies are considered below.

Figueira 2012 reported results from a trial of 60 participants receiving vitrified donor oocytes. Assisted hatching was enabled by laser drilling of a 30µm hole. The allocation process was not reported in sufficient detail to assess risk of bias and the average of more than two embryos transferred at a time may have implications for generalisability to the UK. Clinical pregnancy rate was slightly higher in the intervention arm: OR= 1.5 (0.54 to 4.4).

Wan 2014 randomised 203 highly selected participants. Low grade, cleavage stage embryos were allowed to develop to blastocysts and then vitrified if high or fair quality. These were then offered to patients who had exhausted, through fresh and vitrified cycles, all cleavage stage embryos that

had been assessed as high or fair grade. At this stage participants were enrolled and apparently randomised for use of assisted hatching. Unfortunately, there was no information on which to assess the risk of allocation bias. Assisted hatching was enabled by use of a laser to open 25% of the zona pellucida. Reported results for live birth slightly favoured the intervention arm: OR= 1.6 (0.88 to 2.9).

Kirienko 2019 randomised 419 participants. Assisted hatching was by mechanical removal of the zona pellucida from vitrified-warmed blastocysts assessed as high grade at the time of vitrification. Unfortunately, there was no information on which to assess the risk of allocation bias. The ongoing pregnancy rate was similar between groups: OR= 0.94 (0.63 to 1.4).

Fawzy 2020 randomised 966 participants who were undergoing a first or second cycle of ICSI. Assistance entailed a laser pulse to open the zona pelucida of all metaphase II oocytes to facilitate ICSI. This appears to have been a methodologically strong study. Clinical results for ongoing pregnancy favoured the control arm: OR= 0.79 (0.61 to 1.0).

Zhang 2022 conducted an early-phase sibling-embryo study in participants undergoing their first IVF cycle who had more than two highly fragmented day-3 embryos. Sibling embryos were randomised between laser thinning and laser opening of the zona pellucida on day 4, with vitrification of all viable and good quality blastocysts on day 5 or 6. No detail was given to assess risk of bias in the allocation process but analysis correctly accounted for sibling status. No marked differences were identified in blastocyst assessments.

Current rating red.
Recommendation: GREY [Only one moderate/high quality study, no safety concerns]

3. Embryo glue

The previous review in 2021 covered eleven studies including nine RCTs with a total of over 3000 participants. Most were of poor quality with high risk of bias. However, the largest and methodologically strongest study, Urman 2008, found significantly increased live birth rate when using embryo glue in fresh embryo transfers at day 3 or day 5: OR = 1.5 (1.2 to 1.9). Three additional studies were identified.

Kleijkers 2016 randomised 836 participants who were undergoing either a first IVF/ICSI cycle or their first following previous success. Rather than Embryo Glue as such, allocation was for culture throughout in G5, a medium containing hyaluronan, or HTF, a medium without this component. This was a well-designed and well-reported study comparing cumulative outcome to 1 year of follow-up. Live birth was higher with the G5 medium: OR=1.3 (0.98 to 1.7).

Kandari 2019 randomised 321 participants with PCOS on the day of elective fresh single embryo transfer following time-lapse incubation. The transfer medium allocated was Embryo Glue or CSCM (Irvine, CA, USA). This study was only available as a conference abstract and details to assess risk of bias were not available.The authors reported fewer miscarriages using Embryo Glue and a large benefit for live birth: OR=2.7 (1.6 to 4.5).

Yung 2021, randomised 550 couples who had had an unsuccessful or cancelled fresh cycle to use of embryo glue in the subsequent frozen transfer. Like Urman, this study was of moderate/high quality. They reported similar live birth rates in the two groups: OR=0.98 (0.67 to 1.4). They also

reported very similar pregnancy losses, twin rates and obstetric outcomes. A clear difference from Urman was the use of frozen rather than fresh transfers. Other differences are likely to have occurred in standard care over the intervening period.

Current rating amber.
Recommendation: The number of moderate/high quality studies depends on whether the intervention by Kleijkers is considered eligible for this comparison. GREY (only two moderate/high quality studies) if not. GREEN (three moderate/high quality studies, consistent results) if so.

4. Endometrial receptivity analysis

The previous review considered only Simón 2020. This was a single, 3-arm randomised trial comparing 'personalised embryo transfer' based on ERA with two different control groups: elective frozen embryo transfer and fresh embryo transfer. Participants had not suffered previous recurrent implantation failure or miscarriages. The study suffered from a number of methodological issues, in particular from poor protocol adherence with more than 40% of participants not receiving the allocated intervention.

The current review identified three further papers.

Cohen 2020 reported results in a cohort of 97 patients with a history of implantation failure. All underwent ERA assessment. Those assessed to be 'receptive' underwent embryo transfer on the corresponding day of the subsequent cycle. Those assessed to be 'not receptive' were offered a choice on the recommended day of the subsequent cycle between embryo transfer or repeated ERA. Four participants did not progress to personalised embryo transfer, two because the biopsy was considered insufficient. One of the 14 who opted for repeat ERA was assessed to be 'not receptive' for a second time. Denominators presented for clinical outcomes differ without adequate explanation, but six miscarriages and three live births were observed among the 93 women undergoing a first personalised embryo transfer. Also reported by the authors was very low concordance between assessment of receptivity using ERA versus conventional histological dating in 35 women undergoing both: kappa -0.18 (-0.5 to 0.14).

Cozzolino 2020 reported a retrospective cohort analysis of 2110 patients with history of recurrent implantation failure in at least three consecutive cycles during which neither ERA not PGT-A had been used. Patients with abnormal karyotype and various known potential aetiologies were excluded from consideration. This was a very poorly reported study. It is not clear what criteria were used to decide on use of ERA, PGT-A, both or neither. It is also unclear how patients with multiple cycles using different methods were classified into just one of these four categories. There were 3000 analysed cycles of treatment. It also appears that an 'improper' cohort approach may have been used, in which patients were eligible for consideration only if their treatment cycle resulted in a transfer. This may not be a major source of bias for assessment of ERA as it would not be anticipated that the result of the ERA intervention would prevent transfer. However, PGT-A may do so, so any correlation between selection for the two approaches may have indirectly led to bias. Ongoing pregnancy rates were very similar between the 126 patients categorised as receiving ERA and those not: OR= 0.99 (0.69 to 1.4).

Cozzolino 2022 similarly reported a retrospective analysis of 5372 patients with a previous failed embryo transfer, excluding any who had taken part in Simón 2020 (above). This appears again to have only included patients who progressed to receive a transfer. Two of the authors are noted as

"inventors of the endometrial receptivity array patent". All results were presented with participants divided according to receipt or not of donated oocytes, use of PGT and whether standard (non-ERA) cycles used fresh or frozen embryo transfer. Live birth rates were considerably lower with ERA: OR=0.51 (0.41 to 0.62). Cumulative live birth rates were also lower

Current rating red.
Recommendation: RED (No moderate/high quality studies and safety concerns raised by Cozzolino 2022).

5. Endometrial scratching

The previous review considered 27 studies reporting outcomes for a total of more than 6000 participants. Results for natural/IUI cycles were consistently positive but tended to be from early, small studies at questionable risk of bias. More recently, several large and well-designed studies had reported results for IVF/ICSI cycles with odds ratios for live birth or ongoing pregnancy consistently between 1.0 and 1.4, suggesting possible benefit of a few percentage points but not reaching statistical significance.

The current review identifies fifteen further papers, including eight specifically in participants with recurrent implantation failure (RIF) and four new studies in the general population published since the last review.

5 (i) *General population*

Nastri 2013 allocated 158 participants to a single procedure 7-14 days preceding controlled ovarian stimulation. The study appears to be biased to an unpredictable extent by planned repeated analyses conducted without consideration of cumulative error. It stopped after the fourth such analysis on what appeared at face value to be a significant finding in favour of the scratch procedure: live birth OR=2.4 (1.2 to 4.8).

Bahaa Eldin 2016 allocated 349 participants undergoing IUI for unexplained or mild male factor infertility to receive either a scratch procedure on day 5-7 of the controlled ovarian hyperstimulation cycle with prophylactic antibiotic or just the antibiotic. Timing and process of the randomisation procedure was unclear. Follow-up only extended to diagnosis of clinical pregnancy. This outcome clearly favoured the scratch procedure: OR=2.8 (1.4 to 5.6).

Mackens 2020 allocated 200 participants to a scratch procedure on day 6-8 of the ovarian stimulation cycle for fresh ART transfer. This was a well-designed study that stopped after the second planned interim analysis due to safety concerns regarding miscarriage. Results show higher numbers of clinical pregnancies in the intervention arm with more miscarriages leading to slightly lower live birth rate: OR=0.84 (0.47 to 1.5).

Glanville 2022 allocated 117 participants with polycystic ovary syndrome to a scratch procedure on day 1-12 of the cycle preceding three consecutive cycles of planned ovarian induction. This was a well-designed study but struggled to recruit. The authors acknowledge the resulting imprecision. Live birth was higher after the first cycle but cumulatively lower after the third: OR=0.72 (0.30 to 1.8).

Izquierdo 2022 published a follow-up of the previously reviewed trial, Rodriguez 2020. They report detailed follow-up information on up to four subsequent treatment cycles over the 12 months following the planned randomised comparison. Subsequent attempts, and whether or not each involved a preceding endometrial scratch procedure, were at the discretion of treating clinicians and the participants. They report a total of 120 live births in the initially allocated scratch participants and 114 in the control arm but it is not clear even how this intention to treat perspective should be interpreted.

Madhuri 2022 reported 168 participants with previously failed IUI cycles. They randomised to scratch on day 9 preceding the first of up to three planned cycles of ovarian stimulation for IUI. This was a well-designed study but too small to give a precise result. Live birth was higher after each cycle with ultimate OR=2.2 (0.90 to 5.6). There were just two miscarriages, both in the active arm, and no multiple pregnancies.

Metwally 2022 is the detailed HTA Monograph describing the study previous reviewed as Metwally 2020.

Wong 2022 allocated 220 participants with unexplained infertility planning up to three natural cycles. They randomised to scratch on day 1-12 of the first cycle. As with Glanville 2022 above (same study team) this was a well-designed study but fell short of its initial recruitment target. Live birth was higher after each cycle with ultimate OR=1.4 (0.51 to 3.8).

Current rating amber.
Recommendation: AMBER/GREEN [The more recent evidence reviewed above does not materially affect the previous review but the terminology of the grading has changed. The large, moderate-high quality studies do not consistently conclude benefit but do not conflict with each other. Meta-analysis is inconclusive at the standard 95% confidence level but "on balance" there is evidence for a small beneficial effect in terms of live birth. The Committee needs to balance this against cost, inconvenience and pain of the procedure].

5 (ii) *Recurrent Implantation Failure (RIF)*
Baum 2012 randomised 36 participants with recurrent implantation failure to scratch procedures on days 9-12 and 21-24 of the cycle preceding a planned fresh transfer, IVF cycle. The randomisation process was not clearly described. All four live births and five of the six pregnancies occurred in the control group, who underwent a sham procedure.

Zhang 2014 reported a retrospective study that included 55 participants who had received either endometrial scratch or intracavitary physiotherapy. Unfortunately these were 'improper' cohorts, defined by having gone on to receive embryo transfer in the following cycle rather than by receipt of the intervention itself, rendering the results uninterpretable. On face value those who had undergone the scratch procedure had marginally higher clinical and ongoing pregnancy rates.

Zhang 2015 reported a randomised comparison that included 55 participants who had received "hysteroscopic examination and mechanical stimulation" and 57 receiving conventional transfers. Eligible participants had recurrent implantation failure and adequate quality frozen-thawed embryos for transfer. Much concerning the design is unclear, including the timing and process of allocation, making assessment of the results challenging. Clinical results reported for the hysteroscopy group were substantially better than those for control participants. A third group undergoing Chinese medicine prior to embryo transfer had results similar to those of the hysteroscopy group.

Bord 2015 reported a retrospective analysis of 854 cycles in patients with recurrent implantation failure. Unfortunately these cycles were in 183 (or 184) patients and the presented analyses are invalid as they reverse the risk factors and clinical outcome. It is not possible from the paper to determine either the number of patients undergoing the scratch procedure or the success rates.

Siristatidis 2017 initiated a randomised trial in patients with recurrent implantation failure defined as at least two failed transfers each of at least two good quality embryos. Unfortunately, they found randomisation to be impractical "early after the initiation" of the study. It is not clear exactly why this was the case nor whether and, if so, how recruitment continued after this point. The final data suggested a strong benefit of the scratch procedure in terms of live birth, with low miscarriage and multiple pregnancy rates in both arms.

Gürgan 2019 randomised 305 participants with recurrent implantation failure to receive scratch on day 10-12 of the cycle preceding scheduled IVF treatment. The study is at unclear risk of bias given a lack of information on the timing and process of randomisation. Presented analyses excluded more than 20% of randomised participants. However, assuming unsuccessful outcome in excluded participants allows calculation of an 'intention to treat' effect of live birth as OR=2.1 (1.1 to 4.2).

Tumanyan 2019 reported a comparison of 62 patients with recurrent implantation failure scheduled for IVF. It is unclear whether the study was retrospective or prospective, with inclusion criteria including a stipulation that patients had to undergo consecutive fresh and frozen/thaw cycles to be eligible. Results purported to strongly favour those undergoing a scratch procedure on day 20-22 of the preceding cycle.

Aghajanpour 2021 randomised just 20 participants to scratch procedure on day 9-11 of the cycle preceding IVF treatment. Their focus was on molecular changes and therefore all participants underwent biopsy on day 19-21 of the same cycle. Clinical pregnancy, miscarriage and live birth rates were all unsurprisingly similar given the small numbers and intervention in each arm.

Recommendation: GREY [No moderate/high quality studies for the sub-population].

6. Freeze all

The previous review considered 11 studies including several moderate/high quality RCTs. These are included below alongside additional studies, categorised as requested with the additional consideration of outcomes including ovarian hyperstimulation syndrome (OHSS), time to birth and obstetric outcomes.

6 (i) *General population*

Aflatoonian 2010 described good trial methods but was retracted following "results of an investigation" due to "serious methodological flaws". Clearly results cannot be relied upon.

Shapiro 2011a and Shapiro 2011b compared freezing of all oocytes followed by blastocyst transfer with fresh blastocyst transfer, selecting the best one or two for transfer in each case. The difference was in eligibility criteria, reporting 'normal responders' (8 to 15 antral follicles) in 2011a and 'high responders' (>15 antral follicles) in 2011b. Each used an insecure method of allocation concealment and blinding would not have been possible. Both stopped early on planned interim analyses: the first for efficacy and the second due to unacceptably high multiple conception rate. They did not

report OHSS explicitly but one fresh cycle in 2011a and two in 2011b were “cancelled for medical reasons”. None was cancelled in the corresponding intervention arms. Both reported statistically non-significant higher rates of 10-week pregnancy with the freeze-all policy: OR=1.9 (0.95 to 3.7) and 1.5 (0.74 to 3.2) respectively. Neither reported later outcomes.

Magdi 2018 studied 171 couples undergoing ICSI following unexplained, recurrent implantation failure in at least three previous ICSI cycles with fresh embryo transfer. Unfortunately, allocation was by alternation rather than randomisation, leaving high risk of selection bias. It should also be noted that the high number of embryos transferred in each cycle (>2 in each trial arm) may also limit applicability to the UK setting. They did not report OHSS explicitly and it is possible that cancelled cycles were omitted from the report, which would explain an imbalance in reported group size despite having allocated by alternation. Results for ongoing pregnancy were promising even after adjustment of the report for an intention to treat approach: OR = 2.2 (1.1 to 4.2). Later outcomes were not reported.

Shi 2018 randomised over 2000 good prognosis couples to a fresh or freeze-all strategy for day 2 or day 3 embryos. This was a well-designed and reported study. OHSS was lower in the intervention arm: OR=0.31 (0.13 to 0.74). Live birth was quite similar between arms: OR=0.94 (0.80,1.1). There were no statistically significant differences in reported obstetric outcomes (gestational diabetes and hypertension, pre-eclampsia, preterm delivery) or in neonatal outcomes (birthweight, congenital anomalies, neonatal death).

Vuong 2018 randomised nearly 800 good prognosis couples to a fresh or freeze-all strategy for day 3 embryos. This was a well-designed and reported study but it is worth noting that the standard policy was for double embryo transfer. OHSS was only a little lower in the intervention arm: OR=0.75 (0.17 to 3.4). Live birth was quite similar between arms: OR= 1.1 (0.82 to 1.5). Median time to pregnancy was delayed by 1.4 months in the intervention arm. Most obstetric outcomes were similar but the authors noted a lower proportion being small for gestational age and correspondingly higher mean birthweight in the intervention arm.

Le 2018 present a cost effectiveness analysis based on the comparison and data presented by Vuong 2018 (immediately above). They obtained costs for 704 couples. Assuming that those lost to follow-up or declining to provide data were not atypical, the authors estimated that costs were higher on average in the intervention arm. Higher direct medical costs were driven by the additional freezing and thawing entailed. Direct non-medical and indirect costs were similar between arms. Given the similar chance of success observed, it follows that it is unlikely that the freeze-all strategy could be cost effective for this population.

Wei 2019 randomised 1650 good prognosis couples to a fresh or freeze-all strategy for blastocysts. This was a well-designed and reported study using single blastocyst transfer. OHSS was lower in the intervention arm: OR=0.44 (0.14 to 1.4). Live birth was higher: OR= 1.6 (1.3 to 2.0). Time to pregnancy or live birth was not reported but with only one transfer cycle per participant must have been later by design. Most obstetric outcomes were similar but the authors noted a higher proportion with pre-eclampsia and higher proportion being large for gestational age in the intervention arm.

Stormlund 2020 randomised 460 good prognosis couples to a fresh or freeze-all strategy for blastocysts. They randomised early to incorporate the opportunity to reduce risk of OHSS by using a gonadotrophin releasing hormone agonist to trigger final oocyte maturation. This was a pragmatic comparison using a conventional trigger for fresh transfer but allowing those at high risk of OHSS to delay until a frozen cycle. This was a well-designed and reported study using single blastocyst

transfer. There was only one case of OHSS. This occurred in the control arm and required hospital admission. Live birth was quite similar between arms: OR= 0.90 (0.60 to 1.4). Most obstetric outcomes were similar but the authors noted a lower proportion with pre-term delivery and higher mean birthweight in the intervention arm. Further outcomes are promised but not reported.

Simón 2020 was intended as a study of ERA (see 4 above) but the two 'control' groups provide a comparison of elective freeze-all with fresh transfer in 310 low risk women scheduled for blastocyst transfer. This was a poorly designed study in that early randomisation allowed substantial protocol non-adherence, with 40% of participants not receiving their allocated intervention. Under 'per protocol' analysis, OHSS occurred in just one participant who was in the control arm. Success rates were lower in the frozen transfer group: live birth OR (95% CI) = 0.71 (0.45 to 1.1); and cumulative birth: OR=0.95 (0.61 to 1.5). Under per protocol analysis, obstetric complications were rare and similar between arms. There was one neonatal death in the intervention arm and slightly higher mean birthweight in both singletons and twins.

Boynukalin 2020 reported retrospective analysis of all patients undergoing a single blastocyst transfer after elective freeze-all versus all those undergoing a similar transfer after rejecting the offer of elective freeze-all. As well as being subject to clear selection bias through the patient preference design, this study makes the mistake of defining the cohort by those reaching a later stage (single blastocyst transfer) that could have been affected by the preceding decision. They report much lower rates of moderate/severe OHSS in the elective freeze-all arm: OR= 0.04 (0.01 to 0.12). Live birth rates were higher in the freeze-all arm: OR=1.5 (1.3 to 1.8) for first transfer and for cumulative live birth. Obstetric complications were similar between groups. Birthweights were higher in the freeze-all arm.

Li 2021 randomised 360 couples who were about to undergo endometrial preparation for their first frozen transfer in a freeze-all cycle. Their comparison was between preparation methods: down-regulation ovulation-induction using HMG versus a modified natural cycle approach. The study was at risk of bias due to insecure concealment of the allocation process. Cycles were cancelled for five participants in the down-regulation arm and none in the conventional arm to prevent OHSS. Despite this, higher average number of embryos per transfer and higher average quality of embryos in the conventional arm, the ongoing pregnancy rate was higher in the down-regulation arm: OR=2.2 (1.5 to 3.4). The paper did not report obstetric and neonatal outcomes outcomes.

Wong 2021 randomised 204 couples with any indication, regardless of available numbers of follicles or embryos, undergoing their first treatment cycle. They randomised before the start of down-regulation and compared a policy of cryopreservation of all embryos on day 6 with a strategy of fresh single blastocyst transfer on day 5 followed by cryopreservation of all surplus embryos on day 6. This was a well-designed and reported study. There were three cases of OHSS requiring hospitalisation, all in the control arm: OR= 0 (0 to 2.4). Success rates were much lower in the freeze-all arm: live birth OR (95% CI) = 0.27 (0.11 to 0.66); and cumulative birth to 12 months OR=0.54 (0.28 to 1.1). They did not report detailed obstetric outcomes. Time to ongoing pregnancy was reported with a statistically significant log-rank test (p=0.02) favouring the control arm. The authors report no evidence of a difference in birthweights or other neonatal outcomes, and confirm that there were no congenital abnormalities in either arm.

Maheshwari 2022 randomised 619 couples between freeze-all and fresh transfer strategies if they were undergoing a first, second or third cycle of IVF treatment with their own gametes and had no clinical indication for elective freeze-all. Fuller details are presented in Maheshwari 2022a and used here. Unfortunately, this study suffered from poor recruitment and from very high non-adherence with the freeze-all strategy: 31% received a fresh transfer despite their allocation. Moderate/severe

OHSS was lower in the freeze-all arm: OR=0.27 (0.10 to 0.73). Live birth was also lower: OR= 0.76 (0.54 to 1.1). This conclusion was similar under re-analyses using different strategies (per protocol, as treated and compliance-adjusted). There was no evidence of differences in obstetric outcomes such as gestational diabetes or hypertension. Birthweights and rate of congenital anomalies were also similar between trial arms.

Current rating amber.

Recommendation: AMBER for live birth [Conflicting findings from 4 moderate/high quality studies]
GREEN for OHSS [On balance, consistent evidence]
GREY for obstetric/neonatal outcomes [Studies underpowered for these]

6 (ii) *Populations at increased risk of OHSS*

Santos-Ribeiro 2020 randomised 209 couples at high risk of OHSS defined by their high response to ovarian stimulation. They were allocated to a fresh or freeze-all strategy for either day 3 or day 5 transfer using the same pre-defined criteria in each arm. This was a well-designed and reported study using single or double embryo transfer. There were nine cases of moderate/severe OHSS, all in the control arm: OR= 0 (0 to 0.49). Live birth rate was very similar between arms: OR= 1.1 (0.61 to 1.8). Cumulative live birth to 24 months was also similar. Time to pregnancy was slightly reduced in the control arm: HR= 0.92 (0.68,1.2) but with very similar trajectories after the second month. That is, similar patterns with a one-cycle lag with the freeze-all strategy. They did not report detailed obstetric or neonatal outcomes.

Rahav Koren 2018 reports a retrospective analysis of their clinic strategy. They replaced their standard ovulation trigger with a GnRH agonist. Those with more than 20 oocytes retrieved were considered high risk and given freeze-all. Others were given a 'rescue' hCG bolus and fresh transfer. I have only been able to access the abstract for this study but it does not inform a comparison of strategies as the interventions are uncontrolled. It does add evidence for safety. No OHSS occurred in either group. The only other outcome reported was clinical pregnancy rates: 25% in high-risk freeze-all versus 32% in the low-risk, fresh transfer arm. The associated p-value implies a total sample size of less than 100 participants.

Ye 2018 reported a retrospective comparison of 110 patients receiving each of two freeze-all strategies for women at high risk of OHSS defined by PCOS. One group received progestin-primed ovarian stimulation using a lower dose of hMG with 50mg clomiphene citrate. The other received standard stimulation. There was one case of moderate/severe OHSS in each arm. Cumulative live birth was higher in the standard stimulation arm: OR= 0.59 (0.33 to 1.1). They did not report obstetric outcomes. Birthweights were similar between arms.

Deng 2019 reported a retrospective cohort of 21 patients at high risk of OHSS defined by having at least 30 follicles of at least 11mm diameter or pre-trigger peak oestradiol of >10,000pg/mL. All patients were undergoing ovarian stimulation with a GnRH antagonist protocol and all received a second dose of GnRHa 12 hours after the first and again at 0.25mg/day for three days following oocyte retrieval. There were 15 (71%) cases of mild OHSS but none progressed to moderate/severe. No comparison was made and no outcomes were reported regarding subsequent transfers.

Shrem 2019 reported a retrospective cohort of 480 patients at high risk of OHSS defined by having PCOS, antral follicle count>8, or 18 follicles >10mm diameter. All patients underwent a GnRH antagonist protocol, GnRH agonist trigger and a freeze-all strategy. In addition to those receiving the standard trigger, one group received 0.5mg/day oral cabergoline for 7 days and one received this plus 5 days of GnRH antagonist from the day of oocyte retrieval. As with Deng 2019 (immediately

above), this was more a study of how to prevent OHSS and contained no comparison of the freeze-all strategy. There were no cases of severe OHSS. Mild/Moderate OHSS was reported for 80 (38%), 48 (29%) and 19 (18%) of patients in each of the three groups. No outcomes were reported regarding subsequent transfers.

Deepika 2021 presented follow-up data from an earlier randomised trial of 210 participants undergoing a first treatment cycle scheduled for a freeze-all strategy. Randomisation was between trigger using GnRH agonist versus conventional hCG. Sixteen (8%) participants had ceased follow-up prior to this follow-on report. Moderate/Severe OHSS was the primary outcome in the original. This occurred in none of the GnRH agonist arm and 38 of the hCG arm: OR=0 (0 to 0.07). Using the GnRH agonist trigger, live birth was higher in the first cycle: OR=1.5 (0.81 to 3.0). This was also the case for cumulative live birth measured up to three transfers: OR=2.2 (1.2 to 3.8). The paper did not report obstetric and neonatal outcomes.

Huang 2021 presented a retrospective analysis of 333 couples with PCOS undergoing their first IVF cycle using a freeze-all strategy. They reported results for 160 couples using GnRH antagonist to prevent premature LH surge prior to a change in their routine practice and 173 couples after switching from GnRH antagonist to dydrogesterone for this purpose. They observed no cases of OHSS in either group. Live birth rate was similar in the two groups: OR=1.0 (0.68 to 1.6). The paper did not report obstetric and neonatal outcomes.

Vuong 2021 randomised 40 couples undergoing in vitro maturation at high risk of OHSS due to high antral follicle count, including those with PCOS. Randomisation was to fresh embryo transfer or a freeze-all strategy for day 3 embryos, with all but one couple receiving two embryos at transfer. This was a well-designed and reported study. There were no cases of OHSS and no cases of either gestational diabetes or hypertension. Live birth was higher in the freeze-all arm: OR=6 (1.5 to 25) but it should be noted that this was based on small numbers. Time to live birth for those delivering after the first cycle was unsurprisingly a median of 43 days less in the fresh transfer arm. Birthweights were similar but too sparse for meaningful comparison and there were no congenital abnormalities.

Current rating amber.

Recommendation: GREY for live birth [Only 1 moderate/high quality study]
GREY/GREEN for OHSS [1 study but consistent with general population]
GREY for obstetric/neonatal outcomes [No evidence]

7. IMSI

The previous review considered eight studies including just one randomised trial that provided moderate/high quality evidence, Setti 2013. They studied IMSI in couples consisting of a woman aged over 37 years and a fertile man, with the hypothesis that older eggs may be less able to repair DNA damage, and found improved ongoing pregnancy rate: OR= 4.1 (1.2 to 15). Further studies are considered below categorised as requested.

7 (i) *General population*

Setti 2014 undertook a review of comparative studies, without regard to study design. They identified a few studies as randomised controlled trials between 2008 and 2011 that have not been reviewed here. From the summary information presented these studies appear to favour IMSI over

ICSI for the outcome of 'pregnancy rate'. However, the risk of bias inherent in these studies is not clear and nor can it be assumed that the trial authors and reviewers have correctly analysed by randomised participants rather than by numbers of treatment cycles.

Current rating red.
Recommendation: GREY [Only 1 moderate/high quality study, no safety concerns]

7 (ii) *Male factor*

Knez 2012 randomised 122 couples with male factor (isolated teratozoospermia) and at least six mature oocytes to receive IMSI or conventional ICSI with a policy of transferring up to two blastocysts. The methods of this study were poorly reported making it hard to assess risk of the most common biases. In particular there was no information on the method or timing of randomisation and no explanation for the imbalance in sample size between arms. The only reported clinical outcome was clinical pregnancy rate, which was higher in the IMSI arm: OR=3.2 (1.5 to 7.0).

Sifer 2014 studied 91 couples with no more than 2 previous failed ICSI cycles where the man had severe teratozoospemia. All underwent IMSI using fresh sperm with the strategy of transferring up to two embryos at day 2 or 3. Their study groups were defined by the availability of sperm using the Vanderzwalmen criteria: Grade I & II available or having to use Grade III or IV. Both clinical pregnancy and live birth were marginally higher in the second group. However, interpretation is unclear given the potential confounding inherent in this design. It may be that the grading is not relevant to viability or that, for example, those with higher grade sperm may have been in couples with poorer female prognosis.

Mangoli 2020 randomised 95 couples with male factor, primary infertility where the woman was considered healthy and had at least six mature oocytes. They compared IMSI with ICSI under a policy of transferring two day-3 embryos. There was no description of the randomisation process to allow assessment of risk of bias. Note that this study was concurrent in the same centre as Mangoli 2019 (see previous review). The difference here is that these participants had at least 3 years of primary infertility, which was listed as an exclusion criterion in the earlier paper. Forty couples in each arm underwent transfer and live birth was more frequent in the IMSI arm: OR=1.5 (0.56 to 3.9).

Current rating red.
Recommendation: GREY [No moderate/high quality study, no safety concerns]

8. Intralipids

The previous review considered three RCTs that were each at high risk of bias but consistently supported the use of intralipids. These are included below alongside additional studies, categorised as requested with the additional consideration of miscarriage rates.

8 (i) *General population*

El Khayat 2015 is reported only as a conference abstract. They report randomisation of 203 participants with recurrent implantation failure undergoing ICSI. Intervention was to receive IV infusion from days 4 to 9 of the ovarian stimulation followed by a further dose within one week of a positive pregnancy test. Control was no treatment and therefore unblinded. Details for risk of bias

assessment are otherwise scant. The authors report a markedly higher live birth rate with intervention: OR=3.3 (1.2 to 6.8). By subtraction from reported clinical pregnancy rates, the miscarriage rate was similar with just two per arm.

Gamaleldin 2018 is also reported only as an abstract. They report randomisation to IV intralipid or saline of 97 women with unexplained recurrent implantation failure undergoing IVF. Use of saline implies double-blinding but otherwise details for risk of bias assessment are scant. The authors report slightly higher live birth rate with intervention: OR=1.8 (0.70 to 4.8). Miscarriage rates appear similar: OR=1.3 (0.34 to 5.2). The authors raise a concern regarding two fetal malformations (external ear anomalies) arising in the intralipid group.

Singh 2019 studied about 100 women with recurrent implantation failure undergoing IVF. Infusions were given immediately following oocyte retrieval and again one hour after embryo transfer. This too was a poorly reported study at risk of bias from both allocation concealment and blinding. It was also conducted with a policy of transferring two or three embryos when available. The reported result was a marked increase in live birth rate with intervention: OR (95% CI) = 3.3 (1.2 to 8.8). Just two participants, both in the intervention arm, failed to progress from clinical pregnancy to live birth.

Al-Zebeidi 2019 studied nearly 150 women with unexplained recurrent implantation failure undergoing ICSI. Infusions in this study were given at the time of embryo transfer and again at the time of pregnancy testing. This too was a poor study at risk of bias from allocation concealment and with no attempt at blinding. A double embryo transfer policy was used with three embryos allowed for older women. Again, the reported live birth result favoured intervention but this time without reaching statistical significance: OR (95% CI) = 1.4 (0.57 to 3.4). This despite more reported miscarriages: OR = 1.4 (0.57 to 3.4).

Current rating [?].
Recommendation: GREY/RED for all outcomes [No moderate/high quality studies. Question over whether committee considers the safety concerns raised over congenital malformations justify the red rating].

8 (ii) *Populations with immunological testing*

Dakhly 2016 randomised nearly 300 participants with secondary recurrent miscarriage and elevated levels of natural killer cells (>12%), who were undergoing IVF, to either IV infusion on the day of oocyte retrieval or matching placebo. Unfortunately this was a poorly reported study with scope for serious bias in the allocation and blinding processes. It was conducted with a policy of transferring two or three embryos. The reported result was a marked increase in live birth rate with intervention: OR=2.1 (1.3 to 3.5). There were also fewer miscarriages in the intervention arm: OR=0.66 (0.35 to 1.2).

Meng 2016 recruited 192 participants with recurrent miscarriage and $CD56^{+}CD16^{+}$>20%. Participants were randomised between IV intralipid and IV immunoglobulin. Each started monthly and continued through to week 12 of gestation in the event of pregnancy. Injections were continued monthly for three months then stopped for three months, with the pattern repeated for up to 24 months. There is no suggestion that interventions were blinded and too little information to judge risk of bias in the allocation process. Substantial loss to follow-up occurred after intervention was completed at 12 weeks gestation. Ongoing pregnancy rate to this point was quite similar: OR=1.2 (0.62 to 2.2). Miscarriage within this timeframe was lower in the intralipid arm: OR=0.58 (0.23 to 1.5).

Rogenhofer 2021 described a patient preference study of 12 participants with recurrent miscarriage that was unexplained other than being positive for anti-trophoblast antibodies (ATAb) activity. Ten chose to accept off-label IV infusions of intralipids from their positive pregnancy test every three weeks up to the 33rd week of gestation. The remaining two agreed to repeated monitoring. These two both miscarried a euploid fetus within the first trimester. There was one miscarriage of a fetus with trisomy 16 in the active arm. All other pregnancies continued to live birth with no neonatal malformations. The study is not of a suitable design or scale to draw statistical conclusions regarding these clinical outcomes. The focus was on ATAb activity which was noted to decrease progressively throughout pregnancy with intralipid treatment.

Recommendation: GREY/RED for all outcomes [No moderate/high quality studies, no safety concerns specific to this sub-population but may need to consider safety concern raised above]

9. IV immunoglobulin

The previous review considered two well designed but small RCTs in participants with unexplained secondary recurrent miscarriage. These are included below alongside additional studies, categorised as requested with the additional consideration of miscarriage rates.

9 (i) *General population*

Stephenson 2010 randomised 77 participants with idiopathic secondary recurrent miscarriage in a double-blind, placebo controlled trial. IVIG was delivered at a dose of 500mg/kg two to three weeks before the next anticipated menstrual period and then every four weeks for up to 6 cycles or until reaching 18 to 20 weeks gestation. This was a well-designed study but small. The size of study ruled out very little. The live birth odds ratio was 1.2 (0.47 to 2.9), consistent with the intervention more than doubling or halving the odds of success. Miscarriage was lower in the intervention group but, again, with wide confidence intervals: OR=0.38 (0.07 to 2.1).

Christiansen 2014 conducted a study of similar size in a similar patient population. The main difference was that IVIG was first given on confirmation of pregnancy by repeated biochemical testing. A total of eight infusions was given up to week 15 of gestation at a dose of approximately 25g for those up to 75kg of weight and 35g for heavier women. This was a well-designed study but small. Live birth was similar in the two arms: OR=1.2 (0.51 to 2.9). Miscarriage rates were also very similar: OR=1.1 (0.40 to 2.8).

Jørgensen 2020 reported further blood analyses from a trial by Christiansen 2002. They found that participants in the IVIG arm had markedly boosted production and release of smaller extracellular vesicles. The initial study randomised 58 women with recurrent miscarriage to IVIG or placebo from the time of positive pregnancy test. It was a methodologically strong study but too small to give a precise estimate of effectiveness. Infusions of 0.8g/kg bodyweight were given weekly from week 5 to week 10 of gestation then fortnightly through to week 20. From then to week 26 the fortnightly dose increased to 1.0g/kg. Live births and, conversely, miscarriages were identical between the two groups: OR=1.0 (0.36 to 2.8).

Current rating [?].
Recommendation: AMBER for all outcomes [3 RCTs providing moderate quality evidence. Not 'conflicting' but results too imprecise to determine effectiveness at this stage].

9 (ii) *Populations with immunological testing*

Cohen 2015 undertook a retrospective analysis of 90 women with recurrent miscarriage and elevated natural killer cells who received IV immunoglobulin treatment. This is only available as an abstract and no further details are available on definitions. They report that 78 became pregnant and 14 of these pregnancies ended in first trimester miscarriages. No comparative data are presented.

Yamada 2015 conducted a prospective, single group study of 14 women with unexplained recurrent miscarriage (13 primary, one secondary) and previous failure of low dose aspirin and heparin treatment. 20g IV immunoglobulin was given on each of three days immediately following confirmation of a gestational sac. Natural killer cell status was not an eligibility criterion. Four of the 14 pregnancies resulted in healthy live birth. Eight ended in first trimester miscarriage and two in 'stillbirths' at 17 and 21 weeks gestation. Natural killer cell activity was reduced in all but three of the participants, each of whose pregnancy resulted in miscarriage. No comparative data are presented.

Lee 2016 conducted a retrospective analysis of 189 women with at least two previous miscarriages who had undergone full assessment and had known follow-up data. The latter feature could bias comparisons but the extent of this concern would depend on the number of potential participants excluded from the cohort, which is not reported. Women were categorised according to known or unknown aetiology and presence or absence of a cellular immune abnormality, defined by natural killer cells or T helper cells. Those with immune abnormality were treated with IVIG 400mg/kg at week 4-6 of gestation, repeated every three weeks up to 30 weeks gestation. All patients were given standard care according to any known aetiology. In total, 111 women received IVIG with 94 live births and 17 miscarriages. This was compared with the 70 live births and 8 miscarriages observed in 78 women who were not diagnosed with immune abnormalities and therefore did not receive IVIG.

Meng 2016 recruited 192 participants with recurrent miscarriage and $CD56^{+}CD16^{+}$>20%. Participants were randomised between IV intralipid and IV immunoglobulin under the hypothesis that intralipid would achieve similar therapeutic aims with reduced adverse effect profile. The study is described more fully above under 8 (ii). In brief, treatments were started prior to pregnancy and continued through to week 12 of gestation in the event of pregnancy. The study was poorly reported and at risk of bias. Ongoing pregnancy to 12 weeks gestation was quite similar between groups: OR=1.2 (0.62 to 2.2). Miscarriage within this timeframe was lower in the intralipid arm: OR=0.58 (0.23 to 1.5).

Ahmadi 2017 reported a prospective patient preference study in 94 participants with recurrent miscarriage and abnormal flow cytometry for either natural killer or T helper cells. Participants who volunteered were given 400mg/kg IVIG after a positive pregnancy test and every 4 weeks up to 32 weeks gestation. Their outcomes were compared with a concurrent control group of those who chose not to receive IVIG. The study is subject to bias both from the allocation process and the lack of blinding. Results were strongly in favour of intervention: live birth OR=8.7 (3.1 to 24) and, conversely, miscarriage OR=0.11 (0.04 to 0.32).

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

10. PGT-A

The previous review considered five randomised trials that made subtly different comparisons in a range of settings. Previously reviewed studies are included below alongside seven additional publications, categorised as requested with the additional consideration of miscarriage rates and time to birth.

10 (i) General population

Yang 2012 randomised 112 couples undertaking a first cycle of ICSI and scheduled for elective single embryo transfer. The study was restricted to women under 35 years old. They employed assisted hatching on day 3 in both groups to facilitate PGT-A of the blastocyst and compared outcomes in the fresh transfer cycle. Yang 2012 did not report allocation concealment but attempted to blind patients to their intervention. There were only three miscarriages reported: OR= 0.49 (0.04 to 5.6). Ongoing pregnancy (beyond 20 weeks) was much higher in the PGT-A arm: OR=3.8 (1.7 to 8.3). The single cycle comparison precluded consideration of time to success.

Forman 2013 randomised 175 couples undertaking a first or second cycle of IVF who had produced at least two good quality blastocysts. They employed assisted hatching on day 3 in both groups to facilitate PGT-A of the blastocyst and compared outcomes in the first transfer cycle. They compared single transfer in the PGT-A arm with double transfer (DET) in the controls. They reported a secure randomisation process but did not attempt blinding. Miscarriage was lower in the PGT-A arm: OR=0.44 (0.17 to 1.1). Higher clinical pregnancy in the control arm meant that ongoing pregnancy rate was similar: OR= 0.83 (0.45 to1.5). The single cycle comparison precluded consideration of time to success.

Scott 2013 was from the same research team as Forman 2013 with apparently overlapping recruitment periods. They randomised 155 couples undertaking a first or second cycle of IVF who had produced at least two good quality blastocysts. They employed assisted hatching on day 3 in both groups to facilitate PGT-A of the blastocyst and compared outcomes in the first transfer cycle. They reported a secure randomisation process but did not attempt blinding. Miscarriage was lower in the PGT-A arm: OR=0.41 (0.15 to 1.1). Despite slightly higher clinical pregnancy in the control arm, live birth rate was higher as a result: OR= 6.5 (2.3 to 18). The single cycle comparison precluded consideration of time to success.

Ikuma 2015 undertook retrospective analysis of couples with recurrent (at least two) pregnancy loss with reciprocal or Robertsonian translocations. Participants had chosen between PGD and natural conception. PGD was performed by FISH analysis of day-3 embryos “at about the 8-cell stage”. Unfortunately, there were substantial exclusions from each study group, including all women over 35 years of age who underwent PGD. Even if an eligible intervention for this comparison, the methods presented make it hard to interpret the results, which were reported to favour natural conception in terms of live birth but PGD in terms of cumulative miscarriage.

Ozgur 2019, randomised 220 couples undertaking ICSI under a freeze-all policy who had produced at least two good quality blastocysts. The study was restricted to women no more than 35 years old and compared only the first transfer cycle. Ozgur 2019 did not report allocation concealment but attempted to blind clinicians but not patients to the intervention. Miscarriage was lower in the PGT-A arm: OR=0.44 (0.15 to 1.3). This did not compensate for lower clinical pregnancy rate so live birth

rate was also lower: 0.75 (0.44 to 1.3). The single cycle comparison precluded consideration of time to success.

Munné 2019 randomised 661 couples undertaking ICSI under a freeze-all policy with similar characteristics to those of Ozgur 2019. They allowed women up to 40 years old with zero to two previous failed attempts and compared only the first transfer cycle. They reported a secure randomisation process with blinding of clinical staff and patients. Miscarriage was similar in the two arms: OR=0.87 (0.51 to 1.5). Live birth results were also similar: OR (95% CI) = 0.93 (0.69 to 1.3). The single cycle comparison precluded consideration of time to success.

Cimadomo 2019 reported a retrospective study of transfers using poor quality blastocysts. Their clinic approach included PGT-A of all blastocysts regardless of morphological grade. Following ICSI, blastocysts were routinely biopsied and then vitrified for subsequent use in single transfers. Unfortunately, the study is reported entirely in terms of cycles and blastocysts so it is unclear how many couples contributed data and consequently not possible to draw any clinical conclusions. There were 2757 retrievals, 2217 of which resulted in at least one blastocyst and 724 of which culminated with live birth. The paper does demonstrate that, although prognosis is poorer, it remains possible to achieve live birth using euploid poor quality embryos with 21 such deliveries. Their analysis found no evidence of safety concerns in terms of obstetric or neonatal outcomes.

Yan 2021 randomised 1212 couples with good prognosis undertaking their first cycle. Participants with at least three good quality blastocysts were assigned to selection based on PGT-A using next-generation sequencing or conventional morphological criteria. All blastocysts were then cryopreserved before use in successive single transfers for up to 1 year. This was a high quality study using concealed randomisation but not attempting to blind clinicians or participants. After the first transfer cycle there were fewer miscarriages in the PGT-A arm: OR=0.69 (0.45 to 1.1). A similar pattern occurred in each subsequent transfer but it is not possible to discern from the reporting how many different couples experienced miscarriage. Although live birth was higher from the first transfer, cumulative live birth was lower in the PGT-A arm: OR=0.75 (0.57 to 1.0). Time to conception resulting in live birth was also significantly longer (p=0.01 from Kaplan-Meier presented in supplement).

De Munck 2022 presented secondary analysis of a previously published sibling oocyte study. They studied 30 couples who had each produced at least ten cumulus oocyte complexes and were scheduled for PGT-A selection of blastocysts using next-generation sequencing. Oocytes were randomised to conventional IVF or ICSI but there was no description of the randomisation process in either report. The original paper concluded that euploid blastocysts were as likely using conventional IVF but the design does not allow for clinical comparisons. De Munck 2022 adds comparison between conventional IVF and ICSI for morphokinetic parameters from time-lapse imaging but nothing regarding the potential clinical benefit of using PGT-A for blastocyst selection.

Idárraga 2022 presented retrospective analyses of 54 couples who had undergone PGT-A either of day 3 embryos or of blastocysts. Their focus was on reporting results of the testing and there is no clinical comparison presented. In all, 32 couples had progressed to transfer at the time of the report and 13 of these had progressed to live birth. No first trimester miscarriages were reported.

Current rating red.

Recommendation: GREEN for miscarriage [several moderate/high quality studies, consistent]
RED/BLACK for live birth [Yan looks definitive but could argue either way]
RED/GREY for time to success [Yan looks definitive but just 1 study of this]

10 (ii) Older women

Ubaldi 2017 reported a retrospective analysis of 137 couples with women aged 44, 45 or 46 years. All participants underwent ICSI using a policy of blastocyst selection based on PGT-A with elective freeze-all. (See Cimadomo 2019 above). With 13 couples undergoing a second cycle, the cumulative live birth was 12 (9%) from 13 clinical pregnancies and just one miscarriage before 20 weeks.

Verpoest 2018 randomised 396 couples with women aged 36 to 40 years in a multi-national trial. Couples were eligible if they had no history of poor ovarian response in previous cycles, no more than two previous cycle failures and no more than two previous miscarriages of a clinical pregnancy. Participants received the standard ICSI protocol for their centre. PGT-A was by polar body biopsy six to nine hours after ICSI. Participants were then followed up to record live births within 12 months. This was a well-designed, clearly reported trial. It should be noted however that outcomes of spontaneous pregnancies were not reported and that the definition of 'live birth within 12 months' could favour premature over term deliveries. Miscarriage was lower in the PGT-A arm: OR=0.45 (0.23 to 0.88). Live birth following the first transfer was similar between arms and cumulative live birth showed a similar pattern: OR=1.0 (0.66 to 1.7) for the latter. Time to live birth was presented graphically showing similar patterns between groups: log-rank test p=0.82.

Current rating red.

Recommendation: GREEN for miscarriage [1 study but consistent with general population]
BLACK/GREY for live birth [1 study but consistent with general population]
GREY for time to success [1 study, no safety concerns]

11. PICSI

The previous review considered seven studies. It was dominated by the well-designed and reported trial of Miller 2019 that ruled out any major effect of PICSI in their population of couples using own gametes and scheduled for fresh transfer on days 3 to 5. Previously reviewed studies are included below alongside six additional publications, categorised as requested with the additional consideration of miscarriage rates.

11 (i) *General population*

Parmegiani 2012 randomised 100 couples to PICSI or to 'Sperm Slow' selection. Women were aged up to 41 years and sperm counts were at least one million with 5% motility. The randomisation process was not well described so at unclear risk of bias, although baseline characteristics of the two groups were very similar. There was one less miscarriage in the PICSI arm, OR= 0.78 (0.20 to 3.1), and two more live births: OR=1.2 (0.52 to 2.8).

Majumdar 2013 studied couples undergoing their first cycle of IVF-ICSI for unexplained infertility (normal semen parameters) and excluded women over 38 years old. The study was at unclear risk of bias with missing detail on allocation method and blinding, as well as post-randomisation exclusions (no embryo transfer) whose group assignment was unreported. There were fewer miscarriages in the PICSI arm: OR=0.46 (0.11 to 1.9). Odds of clinical pregnancy were equal in the two groups, with a slight and non-significant benefit for live birth: OR=1.3 (0.62 to 2.6). If the participants not reaching embryo transfer were assigned to the intervention group, which would still remain smaller, the OR for live birth reduces to 1.1.

Troya 2015 studied unselected couples (normal semen parameters) undergoing ICSI. There was high risk of bias with no information on allocation method or blinding and no explanation for imbalanced group sizes, suggesting the possibility of unreported, post-randomisation exclusions. There were only three miscarriages between clinical and 'ongoing' pregnancy: OR=0.58 (0.05 to 6.6). No evidence was found for clinical benefit of PICSI over conventional ICSI. In 102 reported couples, pregnancy ongoing at 20 weeks gave OR=2.0 (0.85 to 4.7).

Miller 2019 was a pragmatically designed, well-conducted and well-reported trial of more than 2700 participants across 16 sites. ICSI had been recommended on the basis of semen assessment in over 95% of participants. Miscarriage rates were lower in the PICSI arm: OR=0.60 (0.43 to 0.83). The primary analysis ruled out major differences in the outcome of live birth: OR (95% CI) = 1.1 (0.95 to 1.3). Further secondary analyses considered stratification by factors identified in the earlier trials including, for example, hyaluronan sperm binding score, none of which showed evidence of differential effects.

Novoselsky Persky 2021 conducted an unusual retrospective analysis of 45 couples undergoing ICSI. All had accepted half their oocytes being "randomly assigned" to PICSI as part of routine clinical practice for staff to gain experience of the new method. There is no detail on the process of allocation and little on eligibility beyond "mainly couples with previous failure". Nearly two thirds of retrospectively identified couples had male factor infertility and women were aged from 27 to 34 years. The best embryo(s) selected for fresh transfer was deemed to come from the PICSI arm on 22 occasions, from ICSI on 13, and from a mix on 9. The remaining couple had no embryos of sufficient quality from either method.

Current rating: red.
Recommendation: GREY for all outcomes [Only 1 moderate/high quality study, no safety concern]

NB: **The one study is large, of high quality, and may reasonably be considered definitive. The committee may conclude that there is sufficient evidence to grade as BLACK for live birth and GREEN for miscarriage. The magnitude of study required to confirm a plausible effect size makes unlikely the collection of further robust evidence: a randomised trial with 90% power to detect a difference in live birth rates between 25% and 27% would require in excess of 20,000 participants.**

11 (ii) *Male factor infertility*

Worrilow 2013 studied infertile men and excluded women over 40 years old. The design effectively comprised two separate trials: the first in couples for whom hyaluronic acid (HA) binding was greater than 65% in unprocessed semen, and the second for whom binding was less than 65%. In an otherwise well-conducted study at apparently low risk of bias, the presentation of results was confused by stratifying results by non-design features (post-processing parameters). There was sufficient information from combining text and figures to recalculate the results of the randomised comparison. Miscarriage of clinical pregnancy before classification as 'ongoing' was below 5% in all arms bar that of the routine selection arm of the low binding stratum: OR=1.4 (0.23 to 8.7) for high binding; OR=0.18 (0.4 to 0.84) for low binding. Contrary to the article title, the clinical outcomes were slightly worse in the PICSI group with no evidence of a difference. The ongoing pregnancy rate figures gave OR= 0.87 (0.52 to 1.4) for those with high binding and OR= 0.99 (0.63 to 1.6) for those with low binding.

Mokanszki 2014 presented a study of infertile men in which the proportion of HA binding determined treatment selection for the most part (PICSI if HA binding ≤60%), supplemented with

cases undergoing ICSI because PICSI was contra-indicated and eight cases who were selected to undergo PICSI. It was not possible to determine numerators or denominators from reported percentages, which did not appear to be based on numbers of either women or transfers. Even if numbers were available, it is unclear how useful these results would be for the comparison of interest here.

Lohinova 2017 presented a small controlled trial of PICSI methods - 'SpermSlow' versus 'PICSI cup' - for infertile men with previous IVF failure. There was a high risk of bias with no claim of randomisation and no information regarding blinding. It was not possible to derive numerators or denominators from presented graphs of clinical outcome. Results appeared very similar with the two methods.

Erberelli 2017 reported outcomes of PICSI and ICSI for couples with 'moderate to severe' male factor. There was high risk of bias with no suggestion of randomisation or blinding. It was also unclear whether the 56 cycles reported were for 56 couples or included repeat cycles. Cycles using PICSI had twice the average number of oocytes (12 vs 6) and higher clinical pregnancy rate in this small (n=56) study. Later clinical outcomes, including miscarriage and live birth, were not reported.

Korosi 2017 reported a comparison of pre-treatment with oral supplement for subfertile men scheduled for PICSI. The pre-treated participants also had their semen incubated for 2 hours in Myo-Inositol immediately prior to selection. All participants received PICSI under the protocol. The methods state that data were excluded from analyses for men non-adherent with study medication, which clearly breaches the intention-to-treat principle. It is not explained why the active arm remained substantially larger than the control arm. They reported no clinical pregnancies in the 13 control couples and 11 (50%) in the active arm. Of these, two miscarried, four were ongoing at the time of report and five had led to live births.

Avalos-Duran 2018 undertook a systematic review and meta-analysis of trials comparing PICSI with ICSI for infertile men in terms of live birth, miscarriage and other outcomes. They identified two small trials (Parmegiani 2010 and Castillo-Baso 2012). Neither is reviewed in this exercise. Both were of unclear risk of bias regarding allocation process and at high risk of bias for other aspects. The reviewers found no evidence or suggestion of effect for either miscarriage or live birth rates.

Hasanen 2020 randomised 413 couples on the day of autologous ICSI to selection using either PICSI or MACS. All couples had sperm DNA fragmentation, at least one million progressive motile sperm, at least five mature oocytes and women aged 18 to 35 years. Unfortunately, 17 (6%) participants were excluded post-randomisation for not having met eligibility criteria and a further 59 (14%) were omitted from presented analyses having vitrified all available embryos. The mean number of embryos per transfer was 2.3 in each arm of the trial. Ongoing pregnancy from fresh transfer was similar between arms: OR=1.1 (0.72 to 1.6). I was unable to calculate either the number of clinical pregnancies or number of miscarriages from the presented data.

Hozyen 2022 also recruited couples with sperm DNA fragmentation, at least one million progressive motile sperm, at least five COCs and women aged <37 years from the same clinic as Hasanen 2022 during the same period of time. They additionally specified the requirement to have "at least one mature oocyte developed to a blastocyst with fresh embryo transfer", although it is unclear how this could be known at the time of randomisation. They reported a four-group randomised trial comparing sperm preparation methods including PICSI alongside density gradient centrifugation (DGC), testicular sperm and MACS. PICSI had the highest clinical pregnancy and ongoing pregnancy rates. There was no description of an adequate concealment for the randomisation process. The

comparison of PICSI with DGC suggested higher ongoing pregnancy rate, OR=2.0 (1.0 to 3.8) and similar miscarriage rate, OR=1.2 (0.3 to 4.5).

Current rating: red.
Recommendation: GREY for all outcomes [Only 1 moderate/high quality study, no safety concern] N.B. Miller 2019 comprised 95% participants with male factor. The committee could consider grading as BLACK for live birth and GREEN for miscarriage.

11 (iii) *Older women*

Miller 2019 (see 11 i) presented pre-planned subgroup analysis of their primary outcomes by maternal age, including a cohort of 331 women aged at least 35 years. These data give OR=1.3 (0.97 to 1.7) for term live birth and OR=0.48 (0.30 to 0.75) for miscarriage.

Current rating: red.
Recommendation: GREY for all outcomes [Only 1 moderate/high quality study, no safety concern]. Given consistency with general population, the committee could consider grading GREEN for miscarriage.

12. Steroids (glucocorticoids)

The previous review considered four RCTs and a further controlled trial that were each at risk of bias but consistently supported the use of steroids. These are included below alongside additional studies, categorised as requested with the additional consideration of miscarriage rates.

12 (i) *General population*

Fawzy 2013 studied over 300 women with previous unexplained implantation failures. The intervention consisted of oral prednisolone 20 mg/day from the day of stimulation with 1mg/kg/day subcutaneous low molecular weight heparin (LMWH) from the day after oocyte retrieval until the day of pregnancy test (if negative) or week 8 of pregnancy. The authors reported a large increase in ongoing pregnancy but this study was unblinded and, more importantly, used entirely predictable alternation rather than randomisation to allocate participants. Results are therefore unreliable. A large benefit in terms of clinical and ongoing pregnancy rates of intervention was claimed with similar miscarriage rates.

Taiyeb 2017 studied 240 men with anti-sperm antibodies. Treatment consisted of following a course of tapering prednisolone repeated in each of three menstrual cycles prior to IVF/ICSI. There was risk of bias from both unclear allocation concealment and blinding processes and methodological issues with post-randomisation exclusions. Reconstruction of an intention to treat comparison suggested a small and non-statistically significant advantage of treatment on clinical pregnancy rate. Miscarriage rates were not reported.

Yeganeh 2017 studied over 200 women with PCOS with the aim of reducing the risk of OHSS. Intervention consisted of methylprednisolone: 1g intravenous on the days of oocyte retrieval and embryo transfer plus 16mg oral daily from the first day of stimulation through to pregnancy testing. This was another unblinded study at high risk of bias regarding allocation concealment but reported

very similar clinical pregnancy rate in each group: OR= 1.2 (0.53 to 2.9). Miscarriage rates were not reported.

Kaye 2017 retrospectively analysed 876 embryo transfer procedures before and after a change in their routine practice. The earlier cohort had received prophylactic antibiotic and steroid for four days preceding the transfer. No medication was received by the later cohort. Patients from the earlier cohort were more likely to receive fresh transfer, less likely to be at blastocyst stage and, on average, received more embryos per transfer. Note that these are also 'improper' cohorts as they are defined from undergoing transfer rather than from initiation of treatment. Live birth rates were similar: OR= 0.95 (0.73 to 1.2). Miscarriage rates were lower in the treated cohort: OR= 0.68 (0.44 to 1.0).

Milardi 2017 undertook a study of 90 men with oligozoospermia and evidence of abacterial prostate-vesiculo-epididymitis. They randomised participants to one of three doses of daily prednisone given for 1 month: 5; 12.5; 25mg. No clinical outcomes were reported with the focus on sperm parameters. These improved to some extent in the anticipated direction in all three groups. Unfortunately, the analyses were within-group rather than comparative but there was some evidence of a dose-response relationship.

Siristatidis 2018 initiated a randomised trial in patients with recurrent implantation failure defined as at least two failed transfers each of at least two good quality embryos. Unfortunately, they found randomisation to be impractical "early after the initiation" of the study. It is not clear exactly why this was the case nor whether and, if so, how recruitment continued after this point. The final data suggested higher live birth with almost identical miscarriage rates: OR=1.0 (0.14 to 7.5). It is worth noting the similar recruitment period, eligibility criteria and design difficulties to the study by the same first author reviewed under 'endometrial scratch' above.

Liu 2018 undertook an unblinded trial of 450 women undergoing their first IVF cycle with no history of recurrent miscarriage who experienced raised progesterone levels on the third or fourth day of gonadotrophin stimulation. They compared 0.75mg daily oral dexamethasone with no treatment in another unblinded study. They reported very similar live birth rates in the fresh transfer cycle: OR=1.1 (0.72 to 1.5). Miscarriage rates were also similar: OR=0.85 (0.40 to 1.8). Follow-up for two years of all frozen transfers suggested a possible advantage of intervention for the outcome of cumulative live birth: OR= 1.5 (1.0 to 2.2).

Thalluri 2022 reported a retrospective study of live births resulting from IVF/ICSI cycles. They identified 618 mothers who had received oral corticosteroids (prednisolone or dexamethasone) either during the cycle or within the first trimester. Typical indications for such treatment were recurrent implantation failure or recurrent miscarriage of presumed immune aetiology. This design does not allow for consideration of implantation or pregnancy outcomes such as miscarriage. The focus was on congenital anomalies some of which were reported to be higher in the treated group. However, the authors acknowledge that it was not possible to control for the characteristics that led to the clinical decision to treat with corticosteroids. It is therefore valuable to note the numbers of specific anomalies but not possible to distinguish to what extent these may have been a result of modifiable clinical factors such as steroid treatment.

Current rating [?].
Recommendation: GREY for all outcomes. [Insufficient evidence from moderate/high quality studies, no safety concerns].

12 (ii) *Populations with immunological testing*

Fan 2016 studied 130 women undergoing IVF with antinuclear antibody who had experienced a previous implantation failure. Treatment consisted of prednisolone 10mg daily plus aspirin 100mg daily from 3 months before ovulation induction until clinical pregnancy. The trial was unblinded and unclear regarding allocation concealment. Results are therefore not reliable. A large benefit in terms of ongoing pregnancy was reported: OR=3.9 (1.8 to 8.5). A large benefit in terms of miscarriage was also reported: OR=0.43 (0.11 to 1.7).

Huang 2021 studied 19 women with recurrent implantation failure. They were all given prednisolone 10mg daily in the month preceding an intended natural cycle frozen embryo transfer. Treatment continued to the day of a negative pregnancy test or through to 12 weeks gestation. Four live births and one miscarriage were observed. The focus was on biomarkers of immune balance. Although not selected on the basis of immunological testing, these markers were shown to be worse at baseline than in a control group of fertile mothers and some markers improved by follow-up.

Zhou 2022 studied 346 women who underwent a first cycle of IVF/ICSI who were euthyroid but had tested positive for anti-thyroperoxidase or thyroglobulin antibodies. This was a retrospective study of those who had or had not received combined prednisone and aspirin treatment from the day of transfer until confirmation of pregnancy according to clinician inclination. Clinical pregnancy was slightly higher in the treated arm, but livebirth was lower: OR=0.91 (0.59 to 1.4). This was a result of higher miscarriage in the intervention arm: OR=2.0 (1.0 to 3.8). These figures refer to unadjusted effect measures but this was not a randomised study. In multifactorial analyses of clinical pregnancy and miscarriage, stratified by fresh/frozen transfer status, adjusted effect estimates were very similar.

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

13. Time lapse

Time lapse incubation involves two distinct processes both hypothesised to deliver clinical benefits. First, the ability to leave the embryo undisturbed during repeated assessment may be beneficial to the development process. Independently, the additional information available through time-lapse imaging may bring benefits for embryo selection. The previous review in 2021 identified studies in three broad categories evaluating effects of:

i) the environment for embryo development (one safety study and one ongoing RCT);

ii) the embryo selection process (two low quality studies reported non-significant benefits); and

iii) the combined effect of the two (4 studies at high risk of bias with contrasting results).

13 (i). *Studies of the environment*

The previous review contained just a single safety study of this question that contributed no clinical outcomes. The current review includes two new RCTs.

Park 2015 randomised over 350 couples in a 2:1 ratio. Their focus was on embryo quality but they also reported clinical outcomes with more than 95% single embryo transfers. This was a well-designed study. They reported lower ongoing pregnancy rate with the stable environment of the time-lapse incubator [OR=0.64 (0.38 to 1.1). They also reported similar clinical pregnancy and higher

miscarriage rates. The authors note that their use of day 2 transfer may have led to atypical results but the study appeared reasonable from a methodological perspective.

Wu 2016 reported both a small pilot RCT of couples (n=49) and an even smaller study (n=7) in which oocytes/embryos were alternately assigned to the time-lapse or standard incubator. Neither was methodologically strong and the pilot RCT in particular suffered from substantial post-randomisation loss to follow-up. Neither study supported the use of the time lapse system.

13 (ii). Studies of the selection process

The previous review contained two small RCTs that randomised couples to use a selection algorithm based on time lapse data or conventional morphology. Each suggested promise of the intervention but was subject to high risk of bias. This review incorporates two additional RCTs of question (ii) above.

Kaser 2017 reported a 3-way comparison of single embryo transfer based on Eeva classification on either day 3 or day 5 versus conventional morphology on Day 5. Highest clinical pregnancy, lowest miscarriage and highest ongoing pregnancy rates were observed in the conventional arm. This was a pilot study (n=163) that the sponsor stopped prematurely due to "funding priorities" but appeared methodologically sound in other key regards. The estimated effect for ongoing pregnancy in the combined Eeva groups versus conventional morphology was OR=0.69 (0.36 to 1.4).

Ahlstrom 2022 also studied elective single embryo transfer. 676 patients with at least two good blastocysts on day 5 were randomised between selection based on KIDScore or conventional morphology (Gardner/Schoolcraft). The study stopped earlier than intended as a result of the global pandemic but appears otherwise strong methodologically. Clinical pregnancy rate was a little lower in the time lapse group [OR=0.95 (0.72 to 1.3)] with higher early pregnancy loss: OR=1.2 (0.75 to 1.8).

13 (iii). Trials of environment and selection

The previous review contained four studies of the combined question, none of which was at low risk of bias. Results of the two largest studies were starkly contrasting, with claims of both significant detriment and significant benefit. This review incorporates two additional RCTs of question (iii) above.

Meng 2022 compared time lapse incubation with day 3 KIDScore versus conventional incubation and morphology in 139 couples. This appears to have been a well-designed study but stopped early, seemingly at a planned interim review, due to the magnitude of difference observed. Live birth was markedly lower in the time lapse arm: OR=0.38 (0.19 to 0.76). Reported miscarriage was very low in both groups (n=6 total).

Zhang 2022 compared time lapse incubation with 'Geri assess' versus conventional incubation and morphology (Alpha consensus) in over 1200 couples. The study design and conduct appears methodologically strong. Usually two embryos were transferred, which may affect generalisability to UK practice. Live birth rate was similar but slightly higher in the time lapse arm: OR=1.1 (0.85 to 1.4) and cumulative live birth even more similar. Patients were generally good prognosis (e.g. aged <35yrs, first cycle)

Current rating amber.
Recommendation: BLACK [4 moderate/high quality studies with consistent results]

DISCUSSION

Caution is required as the assessments above are made from a methodological perspective without expertise in the clinical or scientific context.

The recommendations for rating are only intended as a starting point for committee discussion.

Some comparisons contain a range of interventions (e.g. steroids taken by the male or female partner). Many post-hoc but biologically plausible rationales could be put forward to 'lump' or further 'split' categories presented above.

REFERENCES: Reviewed studies (Bold indicates full references added for 2023 update)

Adjunct	Study	DOI/reference
Artificial Egg Activation	Meerschaut 2012	10.1093/humrep/des097
	Ebner 2012	**10.1016/j.fertnstert.2012.07.1134**
	Montag 2012	**10.1016/j.rbmo.2012.02.002**
	Liu 2013	**10.1017/S0967199411000530**
	Aytac 2015	10.1016/j.fertnstert.2015.07.1163
	Caglar 2015	**10.1016/j.fertnstert.2015.07.1163**
	Darwish 2015	**10.1016/j.rbmo.2015.08.012**
	Ebner 2015	**10.1016/j.rbmo.2014.11.012**
	Aydinuraz 2016	10.1080/14647273.2016.1240374
	Fawzy 2018	10.1093/humrep/dey258
	Li 2019	**10.1016/j.rbmo.2019.03.216**
	Shebl 2021	**10.1007/s10815-021-02338-3**
	Yin 2022	**10.1007/s00404-021-06329-8**
Assisted Hatching: Stored	Balaban 2006	10.1093/humrep/del097
	Ge 2008froz	RBMO 2008;16(4):589-96.
	Valojerdi 2010	10.1016/j.rbmo.2009.11.002
	Fang 2010	10.1016/j.fertnstert.2009.08.014
	Figueria 2012	**10.1016/j.ejogrb.2012.05.022**
	Wan 2014	**10.1016/j.rbmo.2014.01.006**
	Wang 2016	10.3892/br.2016.716
	Knudtson 2016	F&S 2016;106(3) Suppl:e141
	Safari 2017	10.1016/j.repbio.2017.05.003
	Kirienko 2019	**10.1016/j.rbmo.2019.06.003**
Assisted Hatching: Fresh	Sagoskin 2007	10.1016/j.fertnstert.2006.07.1498
	Ge 2008fresh	RBMO 2008;16(4):589-96.
	Balakier 2009	10.1016/j.fertnstert.2008.07.1729
	Hagemann 2010	10.1016/j.fertnstert.2009.01.116
	Kutlu 2010young	10.1007/s10815-010-9431-6
	Kutlu 2010old	10.1007/s10815-010-9431-6
	Razi 2013	Iran J reprod Med 2013;11(12):1021-6.
	Shi 2016	10.1177/1933719116641764
	Chang 2016	F&S 2016;106(3) Suppl:e314
	Nada 2018	10.1007/s00404-017-4604-5
	Fawzy 2020	**10.1093/humrep/deaa160**
	Zhang 2022	**10.3389/fendo.2022.927834**
Embryo Glue	Morbeck 2007	NCT005882250
	Mahani 2007	EMHJ 2007;13(4):876-80.
	Friedler 2007	10.1093/humrep/dem220
	Korosec 2007	RBM0 2007;15(6):701-7.
	Hazlett 2008	10.1016/j.fertnstert.2007.05.063
	Urman 2008	10.1016/j.fertnstert.2007.07.1294
	Dittmann-Muller 2009	Hum Reprod 2009;24 Suppl 1:167.
	Fancsovits 2015	10.1007/s00404-014-3541-9
	Singh 2015	10.4103/0974-1208.170398
	Kleijkers 2016	**10.1093/humrep/dew156**
	Zbořilová 2018	https://europepmc.org/abstract/med/30764616
	Kandari 2019	**10.1016/j.fertnstert.2021.02.015**
	Yung 2021	**10.1016/j.fertnstert.2021.02.015**
Endometrial Receptivity	Simón 2020	10.1016/j.rbmo.2020.06.002
	Cohen 2020	**10.1080/19396368.2020.1824032**
	Cozzolino 2020	**10.1007/s10815-020-01948-7**
	Cozzolino 2022	**10.1016/j.fertnstert.2022.07.007**
Endometrial Scratching	Raziel 2007	10.1016/j.fertnstert.2006.05.062

	Karimzadeh 2009	10.1111/j.1479-828X.2009.01076
	Narvekar 2010	10.4103/0974-1208.63116
	Abdelhamid 2012	10.1007/s00404-013-2785-0
	Baum 2012	**10.3109/09513590.2011.650750**
	Nastri2013	**10.1002/uog.12539**
	Gibreel 2013	10.1111/j.1447-0756.2012.02016.x
	Parsanezhad 2013	IRCT:2012082510657NI
	Zarei 2014	IRCT:2012070810210NI
	Zhang 2014	**10.1007/s00404-014-3382-6**
	Zhang 2015	**10.1007/s11655-014-1843-1**
	Bord 2015	**10.1007/s00404-015-3954-0**
	Wadhwa 2015	J Hum Reprod Sci 2015;8(3):151-8.
	El Khayat 2015	10.1016/j/ejogrb.2015.08.025
	Mahey 2015	10.1016/j.fertnstert.2015.07.1163
	Maged 2016	10.1177/1933719115602776
	Bahaa Eldin 2016	**10.1177/1933719116638191**
	Siristatidis 2017	**10.1080/09513590.2016.1255325**
	Goel 2017	10.1007/s10815-017-0949-8
	Mak 2017	10.1016/j.rbmo.2017.04.004
	Aleyamma 2017	10.1016/j.ejogrb.2017.05.005
	Helmy 2017	10.1002/ijgo.12178
	Senocak 2017	10.1016/j.jogoh.2017.09.003
	Ashrafi 2017	10.1111/jog.13401
	Maged 2018	10.1002/ijgo.12355
	Frantz 2019	10.1093/humrep/dey334
	Lensen 2019	10.1056/NEJMoa1808737
	Olesen 2019	10.1016/j.fertnstert.2019.08.010
	Gürgan 2019	**10.1016/j.rbmo.2019.02.014**
	Tumanyan 2019	**10.1080/09513590.2019.1632085**
	Mackens 2020	**10.1093/humrep/deaa018**
	Berntsen 2020	10.1016/j.ejogrb.2020.06.034
	Ghuman 2020	10.1016/j.ejogrb.2020.08.010
	Rodriguez 2020	10.1007/s43032-020-00204-8
	van Hoogenhuijze 2021	10.1093/humrep/deaa268
	Metwally 2021	10.1093/humrep/deab041
	Yavangi 2021	10.18502/ijrm.v19i5.9255
	Aghajanpour 2021	**10.1016/j.jri.2021.103426**
	Glanville 2022	**10.1016/j.rbmo.2021.10.008**
	Izquierdo 2022	**10.1016/j.jogoh.2022.102335**
	Madhuri 2022	**10.1016/j.ejogrb.2021.10.028**
	Metwally 2022	**10.3310/JNZT9406**
	Wong 2022	**10.1016/j.fertnstert.2021.12.009**
Freeze All	Aflatoonian 2010	10.1007/s10815-010-9412-9
	Shapiro 2011a	10.1016/j.fertnstert.2011.05.050
	Shapiro 2011b	10.1016/j.fertnstert.2011.02.059
	Magdi 2017	10.1016/j.fertnstert.2017.04.020
	Shi 2018	10.1056/NEJMoa1705334
	Vuong 2018	10.1056/NEJMoa1703768
	Le 2018	**10.1093/humrep/dey253**
	Rahav Koren 2018	**10.1159/000479557**
	Ye 2018	**10.1186/s12958-018-0373-7**
	Deng 2019	**10.1007/s11596-019-2031-5**
	Shrem 2019	**10.1016/j.rbmo.2019.04.014**
	Wei 2019	10.1016/S0140-6736(18)32843-5
	Stormlund 2020	10.1136/bmj.m2519
	Santos-Ribeiro 2020	10.1093/humrep/deaa226
	Boynukalin 2020	**10.1371/journal.pone.0234481**

	Li 2021	**10.3389/fendo.2021.730059**
	Deepika 2021	**10.5935/1518-0557.20200028**
	Huang 2021	**10.1038/s41598-021-02227-w**
	Vuong 2021	**10.1007/s10815-021-02180-7**
	Wong 2021	10.1093/humrep/deaa305
	Maheshwari 2022	**10.1093/humrep/deab279**
	Maheshwari 2022a	**10.3310/AEFU1104**
IMSI	**Knez 2012**	**10.1016/j.rbmo.2012.03.011**
	De Vos 2013	10.1093/humrep/des435
	Leandri 2013	10.1111/j.2047-2927.2013.00104.x
	Setti 2013	10.1016/j.ejogrb.2013.09.006
	Marci 2013	10.1186/1742-4755-10-16
	Kim 2014	10.5653/cerm.2014.41.1.9
	Cassuto 2014	10.1016/j.rbmo.2013.08.013
	Setti 2014	**10.1016/j.ejogrb.2014.10.008**
	Sifer 2014	**10.1016/j.ejogrb.2014.07.017**
	La Sala 2015	10.1186/s12958-015-0096-y
	Mangoli 2019	10.1111/and.13340
	Mangoli 2020	**10.1007/s10815-020-01910-7**
Intralipids	**El-Khayat 2015**	**10.1016/j.fertnstert.2015.07.080**
	Meng 2016	**10.1007/s00404-015-3922-8**
	Dakhly 2016	10.1016/j.ijgo.2016.06.026
	Gamaleldin 2018	**10.1002/central/CN-01911196/full**
	Singh 2019	10.1016/j.ejogrb.2019.06.007
	Al-Zebeidi 2019	10.1080/09513590.2019.1631280
	Rogenhofer 2021	**10.1111/aji.13506**
IV Immunoglobulin	Stephensen 2010	10.1093/humrep/deq179
	Christiansen 2014	10.1111/1471-0528.13192
	Cohen 2015	**PMID: 26380487**
	Yamada 2015	**10.1016/j.jri.2015.01.008**
	Christiansen 2015	**10.1111/1471-0528.13192**
	Lee 2016	**10.1111/aji.12442**
	Meng 2016	**10.1007/s00404-015-3922-8**
	Ahmadi 2017	**10.1016/j.imlet.2017.10.003**
	Jørgensen 2020	**10.1016/j.jri.2020.103128**
PGT-A	Yang 2012	Molec Cytogen 2012;5:24
	Forman 2013	10.1016/j.fertnstert.2013.02.056
	Scott 2013	10.1016/j.fertnstert.2013.04.035
	Ikuma 2015	**10.1371/journal.pone.0129958**
	Ubaldi 2017	**10.1016/j.fertnstert.2017.03.007**
	Verpoest 2018	**10.1093/humrep/dey262**
	Ozgur 2019	10.1007/s10815-018-01399-1
	Munné 2019	10.1016/j.fertnstert.2019.07.1346
	Cimadomo 2019	**10.1093/humrep/dez078**
	Yan 2021	**10.1056/NEJMoa2103613**
	De Munck 2022	**10.1371/journal.pone.0267241**
	Idárraga 2022	**10.5935/1518-0557.20210085**
PICSI	**Parmegiani 2012**	**10.1016/j.fertnstert.2012.05.043**
	Worrilow 2013	10.1093/humrep/des417
	Majumdar 2013	10.1007/s10815-013-0108-9
	Mokanszki 2014	10.3109/19396368.2014.948102
	Troya 2015	10.5935/1518-0557.20150015
	Lohinova 2017	PMID: 29099693
	Erberelli 2017	10.5935/1518-0557.20170002
	Korosi 2017	**PMID: 28724183**
	Avalos-Durán 2018	**10.5935/1518-0557.20180027**
	Miller 2019	10.1016/S0140-6736(18)32989-1

	Hasanen 2020	**10.1007/s10815-020-01913-4**
	Novoselsky 2021	**10.1111/andr.12982**
	Hozyen 2022	**10.1007/s43032-021-00642-y**
Steroids	Fawzy 2013	10.1007/s00404-013-3020-8
	Fan 2016	10.1111/aji.12559
	Taiyeb 2017	10.1007/s12020-017-1446-7
	Yeganeh 2017	10.1080/01443615.2017.1346593
	Kaye 2017	**10.1016/j.fertnstert.2017.04.003**
	Milardi 2017	**10.1111/andr.12300**
	Siristatidis 2018	**10.1080/09513590.2017.1380182**
	Liu 2018	10.1111/cen.13824
	Huang 2021	**10.1016/j.jri.2020.103245**
	Thalluri 2022	**10.1093/humrep/deac142**
	Zhou 2022	**10.1186/s12884-022-04532-2**
Time Lapse	Kirkegaard 2012	10.1007/s10815-012-9750-x
	Kahraman 2013	10.1177/205891581200300204
	Rubio 2014	10.1016/j.fertnstert.2014.07.738
	Park 2015	**10.1093/humrep/deu316**
	Wu 2016	**10.1186/s12958-016-0181-x**
	Goodman 2016	10.1016/j.fertnstert.2015.10.013
	Wang 2016	J Reprod Med 61(5):254-262
	Insua 2017	10.1016/j.fertnstert.2017.06.031
	Kaser 2017	**10.1093/humrep/dex231**
	Alhelou 2018	10.1016/j.repbio.2017.12.003
	Yang 2018	10.1093/humrep/dey047
	Kovacs 2019	10.1016/j.ejogrb.2018.12.011
	Chen 2020	10.1093/humrep/deaa268
	Ahlstrom 2022	**10.1093/humrep/deac020**
	Meng 2022	**10.1016/j.fertnstert.2022.02.015**
	Zhang 2022	**10.1016/j.rbmo.2022.06.017**

Synthetic embryonic-like entities – literature review

Details about this paper

Area(s) of strategy this paper relates to:	Shaping the future
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Annexes	None

Output from this paper

For information or decision?	For decision
Recommendation	Members are asked to: • consider the progress of research (since June 2016) into alternative methods to derive embryonic or embryonic-like stem cells; • advise the Executive if they are aware of any other recent developments; • review whether any outputs from the HFEA are required
Resource implications	Recommendations will be considered by the HFEA for implementation in due course
Implementation date	Recommendations will be considered by the HFEA for implementation in due course
Communication(s)	Recommendations will be considered by the HFEA for implementation in due course
Organisational risk	☒ Low ☐ Medium ☐ High

1. Introduction

1.1. Pharmaceuticals intended for use in patients with childbearing potential must be tested for teratogenicity to ensure that the agent does not disrupt embryo or fetal development. Restrictions on embryo use and the 3R principles (replace, reduce, and refine) have prompted the use of in vitro models. However, in vitro models currently available lack the spatiotemporal and morphological characteristics of a developing embryo, thus the development of synthetic embryo-like models. Additionally, the use of embryo models such as synthetic embryo-like entities (ELEs) allows for better understanding of early embryonic development from the blastocyst to the gastrulation stage in an accessible and scalable format in vitro.

1.2. The HFE Act's 2008 amendments do not explicitly ban research in this field, but the application of synthetic embryos in treatment is not allowed.

1.3. The HFEA are responsible for the regulation of human embryo research; given the potential of these models to be used in future human research, the SCAAC continue to monitor follow-up studies through its horizon scanning processes.

1.4. The Authority first introduced this topic in February 2018, where it was raised as a high-priority issue (previously known as synthetic human entities with embryo-like features) for the horizon scanning process. Subsequently, in June 2018, a paper was brought to the SCAAC meeting on synthetic ELEs where the different structures and methods of deriving synthetic ELEs were discussed. In addition to how closely they resemble the standard human embryo. The June 2018 meeting concluded that given the complex developmental potential of some of these structures, synthetic embryo-like entities should continue to be part of the annual horizon scanning process, for the Authority to continue to monitor any developments. The SCAAC last considered research in this area as part of its horizon scanning process in January 2022.

2. Summary of developments

Assembloids

2.1. Models of human post-attached embryos are challenging to replicate in vitro. In June 2022, Simunovic, Siggia & Brivanlou, proposed propose a strategy of modelling the post-attachment human embryo using extra-embryonic (xEM) cells and a pre-formed polarised epithelial epiblast (2022). Their embryo assembloids allows cells to self-organise into a structure similar to that of the dish-attached human embryo. Further, the assembloids mimic *in vitro* attachment and anteroposterior symmetry breaking and result in cells transcriptionally akin to human gastrula

Axioloids

2.2. In December 2022, Yamanaka et al., introduced a pluripotent stem cell (PSC)-derived mesoderm-based 3D model of human segmentation and somitogenesis, that they termed 'axioloid', that captures accurately the oscillatory dynamics of the segmentation clock and the morphological and molecular characteristics of sequential somite formation in vitro (2022). Axioloids show proper rostrocaudal patterning of forming segments and robust anterior-posterior FGF/WNT signalling gradients and retinoic acid (RA) signalling components. Authors identified an unexpected critical role of RA signalling in the stabilisation of forming segments, indicating distinct, but also synergistic effects of RA and extracellular matrix (ECM) on the formation and epithelialization of somites. Importantly, comparative analysis demonstrated striking similarities of

axioloids to the human embryo, further validated by the presence of a HOX code in axioloids. Lastly, the authors demonstrate the utility of axioloids to study the pathogenesis of human congenital spine diseases, by using patient-like iPSCs with mutations in HES7 and MESP2. These results suggest that axioloids represent a promising novel platform to study axial development and disease in humans.

Blastoids

2.3. In January 2022, Kagawa et al., published their model of the human blastocyst, that is able to mimic aspects of implantation was derived from naïve human pluripotent stem cells (2022). This model has the ability to generate and spatially pattern cellular analogues of the human blastocyst stage including similar developmental pace (4 days) and sequence and a greater that 70% efficiency. This human blastoid model has potential to be used to investigate human embryonic development and implantation, and in the future inform the identification of therapeutic targets for preclinical modelling.

2.4. In July 2022, Seong et al., defined an optimal set of molecules secreted by the epiblast (inducers) that captures in vitro stable, highly self-renewing mouse trophectoderm stem cells (TESCs) resembling the blastocyst stage. When exposed to suboptimal inducers, these stem cells fluctuate to form interconvertible subpopulations with reduced self-renewal and facilitated differentiation, resembling peri-implantation cells, known as TR stem cells (TSCs). TESCs have enhanced capacity to form blastoids that implant more efficiently in utero due to inducers maintaining not only local TR proliferation and self-renewal, but also WNT6/7B secretion that stimulates uterine decidualization. Overall, the epiblast maintains sustained growth and decidualization potential of abutting TR cells, while, as known, distancing imposed by the blastocyst cavity differentiates TR cells for uterus adhesion, thus patterning the essential functions of implantation. Authors conclude that their study provides a framework to explain how the conceptus leverages inductions and TR state fluctuation to maintain progenitors, facilitate differentiation, or allocate and balance the functions necessary for implantation to occur.

2.5. In August 2022, Kagawa et al., published their protocol for human blastoids that model blastocyst development and implantation (2022b). Their blastoids formed by triple inhibition of Hippo, TGF-β, and ERK pathways possess the features of highly efficient morphogenesis, correct sequence of lineage specification, high purity of blastocyst-like cells at the transcriptome level, and capacity to model peri-implantation development. Authors conclude these features of blastoids can facilitate building hypotheses on blastocyst development and implantation, however, they do not recapitulate earlier stages of embryonic development.

2.6. In October 2022, Vrij et al., created a partial mouse embryo model to elucidate the principles of epiblast (Epi) and extra-embryonic endoderm co-development (XEn). They triggered naive mouse embryonic stem cells to form a blastocyst-stage niche of Epi-like cells and XEn-like cells (3D, hydrogel free and serum free). Once established, these two lineages autonomously progressed in minimal medium to form an inner pro-amniotic-like cavity surrounded by polarised Epi-like cells covered with visceral endoderm (VE)-like cells. Progression occurred through reciprocal inductions by which the Epi supports the primitive endoderm (PrE) to produce a basal lamina that subsequently regulates Epi polarisation and/or cavitation, which, in return, channels the transcriptomic progression to VE. This VE then contributes to Epi bifurcation into anterior- and posterior-like states. Similarly, boosting the formation of PrE-like cells within blastoids supports

developmental progression. Authors conclude that self-organisation can arise from lineage bifurcation followed by a pendulum of induction that propagates over time.

2.7. In November 2022, Zhang et al., generated blastoids by using the optimised trophectoderm (TE)-like cells and the undifferentiated human extended pluripotent stem cell (EPSCs) through three-dimensional culture system. With the addition epidermal growth factor (EGF) they aimed to improve the quality of the reconstructing blastoids. By assessing TE-like cells derived from multiple human pluripotent stem cells (hPSCs), they demonstrated that TE-like cells derived from human EPSCs represented the TE of preimplantation embryos and optimised TE-like cell induction by adding EGF. The application of optimised TE-like cells effectively improved the efficiency of reconstructing blastoids and provided a robust method to generate PE cells. Authors concluded that the addition of EGF enhanced TE lineage differentiation and human blastoids reconstruction and the optimised blastoids could be used as a blastocyst model for simulating early embryonic development.

Embryoids

2.8. In December 2021, Langkabel et al., published data on a new ELE that progressed from rosette formation to lumenogenesis were derived from induced trophoblast stem cells (iTSCs) and termed Rosette-to-Lumen-embryoids (RtL- embryoids). RtL-embryoids are synthetic, integrated, cell-based embryo models for exploration of spatio-temporal regulation of gene expression and cell communication during early murine embryogenesis. Reprogramming stem cells towards iTSC and induced extra-embryonic endoderm (iXEN) cell fate in 3D co-culture led to the induction of the respective cell lineage and compartmented embryo like structures. The study provided evidence that ELEs can be generated via a 3D co-culture system made up of transcription factor mediated reprogramming of embryonic stem cells via embryonic stem cells (ESCs) as the only starting population. Authors hypothesise that in using this method there is crosstalk between cells undergoing cell-fate conversion leading to the emergence of complex embryonic, multicellular, and extra-embryonic tissues.

2.9. In March 2022, studies by Mazid et al., demonstrated the generation of eight-cell stage-like cells (8CLCs) from primed naïve human pluripotent stem cells, using a transgene-free, rapid and controllable method for producing (8CLCs) (2022). These 8CLCs transcriptionally and epigenetically resembled the human eight-cell embryo. Their methods aimed to overcome the scarcity of embryos and the relevant ethical considerations to advancements. Additionally, they demonstrated that the 8CLCs were able to produce embryonic and extraembryonic lineages in vitro or in vivo in the form of blastoids and complex teratomas. Authors highlight the potential in using cultured cells that more closely resemble the early human embryo (8CLCs) to model the dynamics of developmental processes and facilitate the generation of optimal human blastoids and other embryonic structures.

2.10. In September 2022, Bao et al., show that a stem cell-specific cadherin code drives synthetic embryogenesis (2022). The extraembryonic endoderm (XEN) cell cadherin code enables XEN cell sorting into a layer below embryonic stem (ES) cells, recapitulating the sorting of epiblast and primitive endoderm before implantation. By optimising cadherin code expression in different stem cell lines, they tripled the frequency of correctly formed synthetic embryos Authors concluded that exploiting cadherin codes from different stages of development, lineage-specific stem cells bypass the preimplantation structure to directly assemble a post-implantation embryo.

2.11. In September 2022, studies by Tarazi et al., demonstrated that naïve mouse embryonic stem cells can self-organise into an embryo beyond gastrulation stages of development *ex utero* (2022). Development includes post-gastrulation derived organ specific progenitors and complex extra embryonic compartments. The mouse post-gastrulation synthetic whole embryo models (sEmbryos) provide a model for the mammalian embryo post-gastrulation and demonstrate the potential of naïve pluripotent cells to self-organise.

2.12. In October 2022, Lau et al., developed a mouse embryonic stem cell (ESC)-based in vitro model that reconstitutes the pluripotent ESC lineage and the two extraembryonic lineages of the post-implantation embryo by transcription-factor-mediated induction (2022). Their embryoid recapitulates developmental events from embryonic day 5.5 to 8.5, including gastrulation; formation of the anterior-posterior axis, brain, and a beating heart structure; and the development of extraembryonic tissues, including yolk sac and chorion. Comparing single-cell RNA sequencing from individual structures with time-matched natural embryos identified remarkably similar transcriptional programs across lineages but also showed when and where the model diverges from the natural program. Authors conclude that their findings demonstrate an extraordinary plasticity of ESCs to self-organise and generate a whole-embryo-like structure.

2.13. In October 2022, research by Amadei et al., assembled stem cell-derived embryos in vitro from mouse ES cells, TS cells and iXEN cells and showed that they recapitulate the development of whole natural mouse embryo in utero up to day 8.5 post-fertilisation (2022). Their embryoids, termed ETiX embryoids, display headfolds with defined forebrain and midbrain regions and develops a beating heart-like structure, a trunk comprising a neural tube and somites, a tail bud containing neuromesodermal progenitors, a gut tube, and primordial germ cells. The embryo model develops within an extraembryonic yolk sac that initiates blood island development. Moreover, authors demonstrated that the neurulating embryo model assembled from Pax6-knockout ES cells aggregated with wild-type TS cells and iXEN cells recapitulates the ventral domain expansion of the neural tube that occurs in natural, ubiquitous Pax6-knockout embryos. Their results demonstrate the self-organisation ability of ES cells and two types of extraembryonic stem cells to reconstitute mammalian development through and beyond gastrulation to neurulation and early organogenesis.

2.14. In October 2022, Rodriguez-Fraticelli, summarised two recent articles showing that synthetic mouse embryos derived from embryonic stem cells (ESCs) can be grown *ex vivo* and complete gastrulation up to the organogenesis stage (2022). Both studies used transcription factors to reprogram extraembryonic cells, which they combined with naive ESCs. Further culture of these aggregates using gas-exchange bioreactors allowed these aggregates to proceed through gastrulation and organogenesis, resembling E8.5 stage mouse embryos. These advanced synthetic embryos will allow the modelling of challenging stages of mammalian development. Authors concluded that translation of these findings to human pluripotent systems may allow the production of rare cell types for engineering and therapy.

2.15. In November 2022, Viukov et al., reported that, in the absence of WNT stimulation, transforming growth factor β (TGF-β) pathway inhibition leads to direct and robust conversion of primed human pluripotent stem cells (PSCs) into trophoblast stem cells (TSCs) (2022). The resulting primed PSC-derived TSC lines exhibit self-renewal, can differentiate into the main trophoblast lineages, and present RNA and epigenetic profiles that are indistinguishable from recently established TSC lines derived from human placenta, blastocysts, or isogenic human naïve PSCs expanded under human enhanced naïve stem cell medium (HENSM) conditions. Activation of nuclear Yes-

associated protein (YAP) signalling is sufficient for this conversion and necessary for human TSC maintenance. The author's findings underscore a residual plasticity in primed human PSCs that allows their in vitro conversion into extra-embryonic trophoblast lineages.

Extraembryonic mesoderm cells

2.16. In September 2022, Pham et al., discovered that naive human pluripotent stem cell (hPSC) cultures can specify the extraembryonic mesoderm cell (EXMC) fate, which provides a model to characterise extraembryonic mesoderm (EXM) specification in vitro molecularly and functionally (2022). In humans, EXM specification takes place after implantation and starts before gastrulation and is therefore inaccessible for experimentation. Authors conclude the induction and maintenance of EXMCs from multiple naive hPSC lines will enable the study of EXM in culture and molecular, genetic, and epigenetic manipulations. EXMCs may also allow the development of improved integrated ELE models in combination with trophoblast, epiblast, and PrE-lineage-derived cell types.

Gastruloids

2.17. In August 2022, Rossi et al., extended the culture conditions of gastruloids to capture features of embryonic blood development through a combination of immunophenotyping, detailed transcriptomics analysis, and identification of blood stem/progenitor cell potency (2022). They uncovered the emergence of blood progenitor and erythroid-like cell populations in late gastruloids and showed the multipotent clonogenic capacity of these cells, both in vitro and after transplantation into irradiated mice. They also identified the spatial localisation near a vessel-like plexus in the anterior portion of gastruloids with similarities to the emergence of blood stem cells in the mouse embryo. Authors conclude their results highlight the potential and applicability of gastruloids to the in vitro study of complex processes in embryonic blood development with spatiotemporal fidelity.

2.18. In November 2022, Cermola et al., showed that budesonide, a glucocorticoid drug widely used to treat asthma, prevents embryonic stem cell (ESC) aggregates to break symmetry (2022). Mechanistically, the effect of budesonide is glucocorticoid receptor independent. RNA sequencing and lineage fate analysis reveal that budesonide counteracts exit from pluripotency and modifies the expression of a large set of genes associated with cell migration, A-P axis formation, and WNT signaling. This correlates with reduced phenotypic and molecular cell heterogeneity, persistence of E-CADHERIN at the cell-cell interface, and cell aggregate compaction. The author's findings reveal that cell-cell adhesion properties control symmetry breaking and cell fate transition in 3D gastruloids and suggest a potential adverse effect of budesonide on embryo development.

2.19. In November 2022, Wehmeyer et al., presented approaches to expand the experimental potency of murine 3D gastruloids by using functional genetics in mouse embryonic stem cells (mESCs) to generate chimeric gastruloids (2022). In chimeric gastruloids, fluorescently labelled cells of different genotypes harbouring inducible gene expression or loss-of-function alleles are combined with wild-type cells. They showcased this experimental approach in chimeric gastruloids of mESCs carrying homozygous deletions of the Tbx transcription factor brachyury or inducible expression of Eomes. Resulting chimeric gastruloids recapitulate reported Eomes and brachyury functions, such as instructing cardiac fate and promoting posterior axial extension, respectively. Additionally, chimeric gastruloids revealed previously unrecognised phenotypes, such as the tissue sorting preference of brachyury deficient cells to endoderm and the cell non-autonomous

effects of brachyury deficiency on Wnt3a patterning along the embryonic axis, demonstrating some of the advantages of chimeric gastruloids as an efficient tool for studies of mammalian gastrulation.

Organoids

2.20. In December 2022, Pryzhkova, Boers & Jordan, developed a simple, bioreactor-based organoid system for modelling early human gonad development (2022). Male hPSC-derived organoids follow the embryonic gonad developmental trajectory and differentiate into multipotent progenitors, which further specialize into testicular supporting and interstitial cells. Authors demonstrated functional activity of the generated cell types by analysing the expression of cell type-specific markers. Furthermore, the specification of gonadal progenitors in organoid culture was accompanied by the characteristic architectural tissue organisation. They conclude that the model organoid system opens the opportunity for detailed studies of human gonad and germ cell development that can advance our understanding of sex development disorders.

Review papers

2.21. Veenvliet et al., produced a review on how unlocking distinct levels of embryo-like architecture through controlled modulations of the cellular environment enables the identification of minimal sets of mechanical and biochemical inputs necessary to pattern and shape the mammalian embryo (2021). Authors detail how this can be complemented with precise measurements and manipulations of tissue biochemistry, mechanics, and geometry across spatial and temporal scales to provide insights into the mechanochemical feedback loops governing embryo morphogenesis. Additionally, they discuss how, even in the absence of active manipulations, ELEs display intrinsic phenotypic variability that can be leveraged to define the constraints that ensure reproducible morphogenesis in vivo.

2.22. In January 2022, Zhai et al., published a review paper on advances in stem cell-based models included those that aim to better understand the human embryogenesis process from peri-implantation to gastrulation (2022). They reviewed ELEs such as the 3D blastoids, 3D Amnion-like structure, 3D gastruloid, 2D neuraloid, 3D foregut-midgut boundary organoid. Authors highlight optimising in vitro culture systems is required to make embryo models that are reflective of in vivo processes, and this could be done using strategies such as supplemented media, culture systems using microfluidics, 3D culturing systems, and the addition of different cell types. They state given research restrictions of ‘14-day rule’ the potential for in vitro studies of non-human primate embryos, that can be cultured through various developmental stages, is one of the most reliable and integrated models used to optimise culture conditions and shed light on human embryo development.

2.23. In January 2022, Ankeny, Munsie & Leach, provided a review on the creation of iBlastoids, derived via self-organisation of reprogrammed adult skin cells (2022). These iBlastoids resemble early human embryos prior to implantation. Authors investigated the ethical, philosophical, social, and regulatory issues related to this research. They conclude the need for reflexive, anticipatory, and deliberative ethical and conceptual work by researchers working in emerging and contentious research domains, in collaboration with interdisciplinary scholars, as well as regulators, funders, and publics.

2.24. In May 2022, Shao & Fu, explored the conceptual and technological frameworks used for developing high-fidelity embryoids and organoids that display tissue- and organ-level phenotypes

and functions, needed for decoding developmental programs and improving translational applications (2022). Authors reviewed recent progress in reconstructing multiscale structural orders in embryoids and organoids. In addition to, the bioengineering tools useful for multiscale, multimodal structural engineering of tissue- and organ-level cellular organisation and microenvironment that present integrative, bioengineering-directed approaches to achieve next-generation, high-fidelity embryoids and organoids.

2.25. In July 2022, Ai et al., reviewed recent research progress in the understanding of human peri-implantation embryogenesis based on extended in vitro cultured embryos and stem cell-based embryoids (2022). Findings lay a foundation for understanding early life, promoting research into human stem cells and their application, and preventing and treating infertility. Authors propose key scientific issues regarding peri-implantation embryogenesis and provide an outlook on future study directions. The paper highlights China's contribution to the field and future opportunities.

2.26. In July 2022, Zhang, Reis & Simunovic, reviewed recently developed strategies of making 3D embryoids. Authors focus on models aimed at reconstituting the 3D epithelial characteristics of the early human embryo, namely the intra/extraembryonic signalling crosstalk, tissue polarity, and embryonic cavities (2022). They identify distinct classes of embryoids based on whether they explicitly include extraembryonic tissues and argue for the merit of compromising on certain aspects of embryo mimicry in balancing the experimental feasibility with ethical considerations. They conclude that human embryoids open gates toward a new field of synthetic human embryology, allowing to study the long inaccessible stages of early human development at unprecedented detail.

2.27. In August 2022, Li et al., published a review paper of 3D culture models of human endometrium for the study of trophoblast endometrium interactions during implantation (2022). Authors concluded that in vitro 2D to 3D models of endometrium are good tools for understanding the molecular mechanism behind embryo implantation and early pregnancy in humans and introducing the newly established organoid concept including the endometrial glandular organoids, endometrial assembloids, trophoblast organoids and blastoid model. Moreover, in vitro 3D culture models can better recapitulate the trophoblast-endometrium interaction for investigation of the pathophysiology of implantation failure or pregnancy complications such as recurrent pregnancy loss and pre-eclampsia.

2.28. In August 2022, Arias, Marikawa & Moris, discussed the organisation and development of gastruloids in the context of the embryonic stages that they represent, pointing out similarities and differences between the two (2022). Authors point out gastruloids potential as a reproducible, scalable, and searchable experimental system and highlight some questions posed by the current menagerie of the ELE. They conclude that gastruloids are a useful model for understanding human mammalian development and a suitable for substrate for genetic, phenotypic, and drug screens. They highlight that gastruloids are not a substitute for embryo work, but a useful complementary tool. They suggest in the future that gastruloids could act as a vehicle for the development of new organoid systems.

2.29. In October 2022, Bao, Cornwall-Scoones & Zernicka-Goetz, published a review summarising recent research advancements in both human and mouse stem cell-based embryo models (embryoids), provided understanding of the foundational molecular and transcriptional processes of embryonic development (2022). Authors concluded that it remains uncertain as to how similar human embryoids mimic morphogenesis in vivo.

2.30. In September 2022, Tahmasbpour Marzouni et al., produced a review on the regenerative mechanisms, applications, and advantages of different types of stem cells for restoring gametogenesis in infertile patients, as well as major challenges that must be overcome before clinical application (2022). They explored the importance and limitations of *in vitro* generation of gametes from patient-specific human-induced pluripotent stem cells (hiPSCs) in the context of human reproduction. Moreover, discuss the potential role of organ-on-a-chip models that can direct differentiation of hiPSC-derived primordial germ cell-like cells to gametes and other reproductive organoids is also explored. They conclude these ELE technologies provide prospects for those who experience reproductive failure.

2.31. In November 2022, Sozen, Conkar & Veenvliet, summarise emergent ELEs that recapitulate aspects of human development in vitro (2022). Authors show how ELEs can provide insights into the molecular, cellular, and morphogenetic processes that fuel the formation of a fully formed fetus and discuss the potential of these platforms to revolutionise our understanding of human development in health and disease. They caution against over-interpretation the extent to which these in vitro platforms model the natural embryo, and discuss how fate, form, and function - a tightly coupled trinity in vivo, can be disconnected in vitro. They propose how careful benchmarking of existing models, in combination with rational protocol design based on an increased understanding of in vivo developmental dynamics and insights from mouse in vitro models of embryo development, will help guide the establishment of better models of human embryo development.

2.32. In October 2022, Niethammer et al., summarised the advantages and shortcomings of both in vivo and in vitro developmental toxicity testing, as well as the possibility of integrated testing strategies as a viable option in the near future (2022). They discuss the opportunity and challenges for in vitro models of human development, such as blastoids, gastruloids and human organoids, to be used to advance developmental toxicity testing to be reliable without compromising the protection of either humans or non-human animals. Authors conclude that to achieve this, protocols need to be improved and validated to support a potential application in a regulatory context.

2.33. In December 2022, Oldak, Aguilera-Castrejon & Hanna, discuss the most recent *ex utero* embryo-culture systems established to date for rodents, non-human primates, and humans (2022). They emphasise their technical aspects and developmental time frame and provide insights into the new opportunities that these methods will contribute to the study of natural and synthetic mammalian embryogenesis and the stem-cell field. Authors conclude that the intersection of synthetic embryoid models and advanced *ex utero* culture systems will pave the way for the study of different developmental stages as a continuum, possibly opening a new window into human natural and synthetic peri-implantation- and post-implantation-stage modelling.

2.34. In October 2022, Terhune et al., examine the wide variety of stem cell-based embryo models that have been developed to recapitulate and study embryonic events, from pre-implantation development through to early organogenesis (2022). They discuss the applications of these models, key considerations regarding their importance within the field, and how such models are expected to grow and evolve to achieve exciting new milestones in the future.

2.35. In January 2023, Amel, Rossouw & Goolam, explained that most of the work studying mammalian implantation stage development has focused on the use of gastruloids to model embryogenesis (2023). However, due to gastruloid's tractable nature and suitability for high-throughput scaling,

there is an unprecedented opportunity to investigate both developmental and environmental aberrations to the embryo as they occur in vitro. Authors summarised the recent developments in the use of gastruloids to model congenital anomalies, their usage in teratogenicity testing, and the current limitations of this emerging field.

3. Level of recommendations

3.1. Members are asked to:

- Consider the progress of research into synthetic embryo-like entities.
- Advise the Executive if they are aware of any other recent developments.
- Review whether any outputs from the HFEA are required.

4. References

Ai, Z., Yin, Y., Niu, B. & Li, T. (2022) Deconstructing human peri-implantation embryogenesis based on embryos and embryoids†. *Biology of reproduction*. 107 (1), 212–225. doi:10.1093/BIOLRE/IOAC096.

Amadei, G., Handford, C.E., Qiu, C., de Jonghe, J., Greenfeld, H., Tran, M., Martin, B.K., Chen, D.Y., Aguilera-Castrejon, A., Hanna, J.H., Elowitz, M.B., Hollfelder, F., Shendure, J., Glover, D.M. & Zernicka-Goetz, M. (2022) Embryo model completes gastrulation to neurulation and organogenesis. *Nature*. 610 (7930), 143–153. doi:10.1038/S41586-022-05246-3.

Amel, A., Rossouw, S. & Goolam, M. (2022) Gastruloids: A Novel System for Disease Modelling and Drug Testing. *Stem cell reviews and reports*. doi:10.1007/S12015-022-10462-5.

Ankeny, R.A., Munsie, M.J. & Leach, J. (2022) Developing a Reflexive, Anticipatory, and Deliberative Approach to Unanticipated Discoveries: Ethical Lessons from iBlastoids. *The American journal of bioethics : AJOB*. 22 (1), 36–45. doi:10.1080/15265161.2021.1974976.

Arias, A.M., Marikawa, Y. & Moris, N. (2022) Gastruloids: Pluripotent stem cell models of mammalian gastrulation and embryo engineering. *Developmental biology*. 488, 35–46. doi:10.1016/J.YDBIO.2022.05.002.

Bao, M., Cornwall-Scoones, J., Sanchez-Vasquez, E., Chen, D.Y., de Jonghe, J., Shadkhoo, S., Hollfelder, F., Thomson, M., Glover, D.M. & Zernicka-Goetz, M. (2022) Stem cell-derived synthetic embryos self-assemble by exploiting cadherin codes and cortical tension. *Nature cell biology*. 24 (9), 1341–1349. doi:10.1038/S41556-022-00984-Y.

Bao, M., Cornwall-Scoones, J. & Zernicka-Goetz, M. (2022) Stem-cell-based human and mouse embryo models. *Current opinion in genetics & development*. 76, 101970. doi:10.1016/J.GDE.2022.101970.

Cermola, F., Amoroso, F., Saracino, F., Ibello, E., de Cesare, D., Fico, A., Cobellis, G., Scalera, E., Casiraghi, C., D'Aniello, C., Patriarca, E.J. & Minchiotti, G. (2022) Stabilization of cell-cell adhesions prevents symmetry breaking and locks in pluripotency in 3D gastruloids. *Stem cell reports*. 17 (11), 2548–2564. doi:10.1016/J.STEMCR.2022.09.013.

Kagawa, H., Javali, A., Khoei, H.H., Sommer, T.M., Sestini, G., Novatchkova, M., Scholte op Reimer, Y., Castel, G., Bruneau, A., Maenhoudt, N., Lammers, J., Loubersac, S., Freour, T., Vankelecom, H., David, L. & Rivron, N. (2022a) Human blastoids model blastocyst development and implantation. *Nature*. 601 (7894), 600–605. doi:10.1038/S41586-021-04267-8.

Kagawa, H., Javali, A., Khoei, H.H., Sommer, T.M., Sestini, G., Novatchkova, M., Scholte Op Reimer, Y. & Rivron, N. (2022b) Protocol for Human Blastoids Modeling Blastocyst Development and Implantation. *Journal of visualized experiments : JoVE*. 2022 (186). doi:10.3791/63388.

Langkabel, J., Horne, A., Bonaguro, L., Holsten, L., Hesse, T., et al. (2021) Induction of Rosette-to-Lumen stage embryoids using reprogramming paradigms in ESCs. *Nature Communications 2021 12:1*. 12 (1), 1–18. doi:10.1038/s41467-021-27586-w.

Lau, K.Y.C., Rubinstein, H., Gantner, C.W., Hadas, R., Amadei, G., Stelzer, Y. & Zernicka-Goetz, M. (2022) Mouse embryo model derived exclusively from embryonic stem cells undergoes neurulation and heart development. *Cell stem cell*. 29 (10), 1445-1458.e8. doi:10.1016/J.STEM.2022.08.013.

Li, X., Kodithuwakku, S.P., Chan, R.W.S., Yeung, W.S.B., Yao, Y., Ng, E.H.Y., Chiu, P.C.N. & Lee, C.L. (2022) Three-dimensional culture models of human endometrium for studying trophoblast-endometrium interaction during implantation. *Reproductive biology and endocrinology : RB&E*. 20 (1). doi:10.1186/S12958-022-00973-8.

Mazid, M.A., Ward, C., Luo, Z., Liu, C., Li, Y., et al. (2022) Rolling back human pluripotent stem cells to an eight-cell embryo-like stage. *Nature*. 605 (7909), 315–324. doi:10.1038/S41586-022-04625-0.

Niethammer, M., Burgdorf, T., Wistorf, E., Schönfelder, G. & Kleinsorge, M. (2022) In vitro models of human development and their potential application in developmental toxicity testing. *Development (Cambridge, England)*. 149 (20). doi:10.1242/DEV.200933.

Oldak, B., Aguilera-Castrejon, A. & Hanna, J.H. (2022) Recent insights into mammalian natural and synthetic ex utero embryogenesis. *Current opinion in genetics & development*. 77. doi:10.1016/J.GDE.2022.101988.

Pham, T.X.A., Panda, A., Kagawa, H., To, S.K., Ertekin, C., et al. (2022) Modeling human extraembryonic mesoderm cells using naive pluripotent stem cells. *Cell stem cell*. 29 (9), 1346-1365.e10. doi:10.1016/J.STEM.2022.08.001.

Pryzhkova, M. v., Boers, R. & Jordan, P.W. (2022) Modeling Human Gonad Development in Organoids. *Tissue engineering and regenerative medicine*. 19 (6). doi:10.1007/S13770-022-00492-Y.

Rodriguez-Fraticelli, A.E. (2022) Synthetic Embryos Can Complete Gastrulation and Initiate Organogenesis Ex Utero. *Cellular reprogramming*. 24 (5), 223–224. doi:10.1089/CELL.2022.0111.

Rossi, G., Giger, S., Hübscher, T. & Lutolf, M.P. (2022) Gastruloids as in vitro models of embryonic blood development with spatial and temporal resolution. *Scientific reports*. 12 (1). doi:10.1038/S41598-022-17265-1.

Seong, J., Frias-Aldeguer, J., Holzmann, V., Kagawa, H., Sestini, G., et al. (2022) Epiblast inducers capture mouse trophectoderm stem cells in vitro and pattern blastoids for implantation in utero. *Cell stem cell*. 29 (7), 1102-1118.e8. doi:10.1016/J.STEM.2022.06.002.

Shao, Y. & Fu, J. (2022) Engineering multiscale structural orders for high-fidelity embryoids and organoids. *Cell stem cell*. 29 (5), 722–743. doi:10.1016/J.STEM.2022.04.003.

Simunovic, M., Siggia, E.D. & Brivanlou, A.H. (2022) In vitro attachment and symmetry breaking of a human embryo model assembled from primed embryonic stem cells. *Cell stem cell*. 29 (6), 962-972.e4. doi:10.1016/J.STEM.2022.05.001.

Sozen, B., Conkar, D. & Veenvliet, J. v. (2022) Carnegie in 4D? Stem-cell-based models of human embryo development. *Seminars in cell & developmental biology*. 131, 44–57. doi:10.1016/J.SEMCDB.2022.05.023.

Tahmasbpour Marzouni, E., Stern, C., Henrik Sinclair, A. & Tucker, E.J. (2022) Stem Cells and Organs-on-chips: New Promising Technologies for Human Infertility Treatment. *Endocrine reviews*. 43 (5), 878–906. doi:10.1210/ENDREV/BNAB047.

Tarazi, S., Aguilera-Castrejon, A., Joubran, C., Ghanem, N., Ashouokhi, S., et al. (2022) Post-gastrulation synthetic embryos generated ex utero from mouse naive ESCs. *Cell*. 185 (18), 3290-3306.e25. doi:10.1016/J.CELL.2022.07.028.

Terhune, A.H., Bok, J., Sun, S. & Fu, J. (2022) Stem cell-based models of early mammalian development. *Development (Cambridge, England)*. 149 (20). doi:10.1242/DEV.201015.

Veenvliet, J. v., Lenne, P.F., Turner, D.A., Nachman, I. & Trivedi, V. (2021) Sculpting with stem cells: how models of embryo development take shape. *Development (Cambridge, England)*. 148 (24). doi:10.1242/DEV.192914.

Viukov, S., Shani, T., Bayerl, J., Aguilera-Castrejon, A., Oldak, B., Sheban, D., Tarazi, S., Stelzer, Y., Hanna, J.H. & Novershtern, N. (2022) Human primed and naïve PSCs are both able to differentiate into trophoblast stem cells. *Stem cell reports*. 17 (11), 2484–2500. doi:10.1016/J.STEMCR.2022.09.008.

Vrij, E.J., Scholte Op Reimer, Y.S., Fuentes, L.R., Guerreiro, I.M., Holzmann, V., Aldeguer, J.F., Sestini, G., Koo, B.K., Kind, J., van Blitterswijk, C.A. & Rivron, N.C. (2022) A pendulum of induction between the epiblast and extra-embryonic endoderm supports post-implantation progression. *Development (Cambridge, England)*. 149 (20). doi:10.1242/DEV.192310.

Wehmeyer, A.E., Schüle, K.M., Conrad, A., Schröder, C.M., Probst, S. & Arnold, S.J. (2022) Chimeric 3D-gastruloids – a versatile tool for studies of mammalian peri-gastrulation development. *Development*. 149 (22). doi:10.1242/DEV.200812/280536/AM/CHIMERIC-3D-GASTRULOIDS-A-VERSATILE-TOOL-FOR.

Yamanaka, Y., Hamidi, S., Yoshioka-Kobayashi, K., Munira, S., Sunadome, K., Zhang, Y., Kurokawa, Y., Ericsson, R., Mieda, A., Thompson, J.L., Kerwin, J., Lisgo, S., Yamamoto, T., Moris, N., Martinez-Arias, A., Tsujimura, T. & Alev, C. (2022) Reconstituting human somitogenesis in vitro. *Nature*. 1–3. doi:10.1038/s41586-022-05649-2.

Zhai, J., Xiao, Z., Wang, Y. & Wang, H. (2022) Human embryonic development: from peri-implantation to gastrulation. *Trends in cell biology*. 32 (1), 18–29. doi:10.1016/J.TCB.2021.07.008.

Zhang, M., Reis, A.H. & Simunovic, M. (2022) Human embryoids: A new strategy of recreating the first steps of embryonic development in vitro. *Seminars in cell & developmental biology*. doi:10.1016/J.SEMCDB.2022.07.003.

Zhang, Y., An, C., Yu, Y., Lin, J., Jin, L., Li, C., Tan, T., Yu, Y. & Fan, Y. (2022) Epidermal growth factor induces a trophectoderm lineage transcriptome resembling that of human embryos during reconstruction of blastoids from extended pluripotent stem cells. *Cell proliferation*. 55 (11). doi:10.1111/CPR.13317.