Prioritisation of issues identified through the horizon scanning process

Strategic delivery:	☒ Setting standards	☐ Increasing and informing choice	☐ Demonstrating efficiency economy and value
Details:			
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)		
Agenda item	5		
Paper number	SCAAC(02/16)01		
Meeting date	03 February 2016		
Author	Anna Rajakumar, Scientific Policy Manager		
Output:			
For information or decision?	For decision		
Recommendation	• Members are asked to: • note the issues identified as high priority through the horizon scanning process, including the progress of research (since February 2015) • consider the high priority issues and work recommendations; and • consider whether advice from additional external advisors would help in achieving the work recommendations.		
Resource implications	Dependant on the number of issues that the Committee agrees to be high priority		
Implementation date	The Committee workplan for 2016		
Communication(s)	Work priorities (as defined by the Committee) will be communicated to the Head of Business Planning		
Organisational risk	☐Low	☒ Medium	☐ High
Annexes	Annex A: Prioritisation of issues identified through horizon scanning process Annex B: Issues identified through the horizon scanning process		

1. Background

1.1. The Authority established a horizon scanning function in 2004, the purpose of which is 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 licence applications and prepare, if necessary, a policy or position.

1.2. Issues are identified from journal articles, conferences and contact with experts such as members of the Authority's Horizon Scanning Panel. The Horizon Scanning Panel is an international panel of experts who meet annually and are contacted via email throughout the year.

1.3. The horizon scanning process is an annual cycle that feeds into the business planning for the Executive, the Scientific and Clinical Advances Advisory Committee (SCAAC) and the Authority's consideration of ethical issues and standards. The issues identified in this cycle of the horizon scanning process will be incorporated into the 2016/17 business plan and workplan for the Executive, SCAAC and the Authority.

2. Prioritisation process

2.1. A full list of all issues identified since February 2015 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 of high priority. New techniques which have been identified this year have been categorised as low, medium or high priority using the following criteria:

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

2.3. New techniques are considered to be high priority if they meet at least three of these criteria and medium if they meet at least two. Low priority issues are unlikely to impact on research or treatment in the near future.

2.4. High priority is also given to established techniques or issues which fall within the HFEA's remit and require ongoing monitoring.

3. High priority issues

3.1. The Executive considers the following topics to be of high priority and these are therefore recommended for consideration in 2016/17. Briefings about these issues, based on horizon scanning findings, can be found at Annex A unless otherwise stated.

- Genome editing
- In vitro derived gametes
- Use of ICSI
- Non-invasive methods for assessing embryo viability

3.2. Briefings have not been written for the remaining high priority areas, as listed below, as these topics are ongoing, have recently been considered by the Committee and are monitored annually.

- Embryo culture media
- Alternative methods for the creation of ES or ES-like cells
- Health outcomes of children conceived from ART
- New technologies in genetic testing (including embryo biopsy)

3.3. Following discussions on the briefings, and their priority status, the Executive asks Members to prioritise these issues to assist the business planning process. Members may think that some of the medium priority issues should be considered by SCAAC and therefore should be made high priority, or vice versa.

4. Recommendations

4.1. Members are asked to:

- note the issues identified as high priority through the horizon scanning process, including the progress of research (since February 2015)
- consider the high priority issues and work recommendations; and
- consider whether advice from additional external advisors would help in achieving the work recommendations.

5. Next Steps

5.1. Following discussions by the Committee, the prioritised issues, in addition to the other work areas, will be used to formulate the Committee work plan for 2016/17. Any areas of work which are likely to go beyond

the Committee's scope, and may impact on the work of other Authority committees, will be considered for inclusion in the business plan for 2016/17.

Annex A - Prioritisation of issues identified through the horizon scanning process

1. Genome editing

Background

1.1. Recent developments in genome editing technologies allow for the potential to insert, delete, or modify DNA with increased specificity and efficiency. This process was developed in human somatic gene transfer and has been discussed by this committee which has considered the potential for pluripotent stem cells that may be used to prevent disease and also provide potential therapeutic applications. More recent research has explored techniques that may be used for human germline modification.

1.2. At the forefront of these technologies are techniques such as CRISPR-Cas9, which hold such promise due to their targeted approach, simplicity, efficiency, affordability and speed.

1.3. Genome editing of embryos for use in treatment is illegal. It has been permissible in research since 2009, as long as the research project meets the criteria in the legislation and it is done under an HFEA licence. The Authority recently received an application to use CRISPR-Cas9 in one of its licensed research projects, which is currently being considered. Despite this technology not being legal in clinical practice it is important to monitor progress of research in this area.

Summary of developments

1.4. At SCAAC's June 2015 meeting the Committee discussed the recent research conducted by a Chinese group using CRISPR-Cas9 (Liang et al.). The group demonstrated that CRISPR-Cas9 could effectively cleave the endogenous β-globin gene (HBB). However, the efficiency of homologous recombination directed repair (HDR) of HBB was low and the resulting edited embryos were mosaic. The research group highlighted that their work demonstrated a need to further improve the fidelity and specificity of this technique. The Committee agreed that this topic should be noted as high priority and developments in this area monitored.

1.5. Basic research into CRISPR-Cas9 efficacy is moving quickly. In 2015, Slaymaker et al. conducted research seeking to improve the specificity of Cas (the RNA-guided endonuclease) which is used as a genome editing tool. Cas9 creates double-strand breaks at targeted genomic loci

complementary to a short RNA guide. However, Cas9 can cleave off-target sites. The group used structure-guided protein engineering to improve the specificity of Streptococcus pyogenes Cas9 (SpCas9). They demonstrated that “enhanced specificity” SpCas9 (eSpCas9) variants reduce off-target effects and maintained robust on-target cleavage by utilising targeted deep sequencing and unbiased whole-genome off-target analysis, to analyse Cas9-mediated DNA cleavage in human cells. Therefore this study highlights that eSpCas9 could be useful for genome editing applications requiring a high level of specificity.

1.6. A further study (Yu et al. 2015) looked at the bacterial CRISPR-Cas9 system as a potential tool for sequence-specific gene knockout through non-homologous end joining (NHEJ).They developed a reporter-based screening approach for high-throughput identification of chemical compounds that can modulate precise genome editing through homology-directed repair (HDR). The group use small molecules that have been identified to enhance CRISPR-mediated HDR efficiency, 3-fold for large fragment insertions and 9-fold for point mutations. The group also found that a small molecule that inhibits HDR can enhance frame shift insertion and deletion (indel) mutations mediated by NHEJ. The identified small molecules were shown to function well in diverse cell types with minimal toxicity and may therefore provide a straightforward and effective strategy to improve genome engineering applications .

Impact

1.7. The benefits of new technologies such as CRISPR-Cas9 in gene editing mean that the potential to modify human germ cells to be disease free could exist. For the present, research focusses on improving the specificity of the gene editing tool and improving its efficiency.

Level of work recommendation

1.8. The Executive will keep abreast of the progress of research in this area to ensure that developments are monitored. The Committee is, therefore, asked to consider whether there are any further studies or developments in the area and identify particular concerns or issues that should be highlighted.

References

Slaymaker IM. et al. Rationally engineered Cas9 nucleases with improved specificity. *Science 1;351(6268):84-8*

Liang P. et al. (2015) CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein & Cell: 6 (5); 363-372*

Yu, C. et al. Small Molecules Enhance CRISPR Genome Editing in Pluripotent Stem Cells. *Cell Stem Cell, 2015; 16(2), pp.142–147.*

2. In vitro derived gametes

Background

2.1. Human germ cells (sperm and eggs) are derived from a type of stem cell called primordial germ cells. They are derived by the process of gametogenesis in the testes and ovaries of men and women. Researchers are investigating whether it is possible to carry out gametogenesis in the laboratory using primordial germ cells, embryonic stem cells or other human cells. Sperm and eggs derived from such cells in the laboratory are called in vitro derived gametes.

2.2. In vitro derived gametes can be used for research purposes, eg, research into germ cell development and cell differentiation. In vitro derived gametes could potentially also be used in treatment. For people who are unable to produce their own eggs or sperm, in vitro derived gametes are a potential method by which they could have children that are genetically related to them.

2.3. The legislation in the UK (the Human Fertilisation and Embryology Act 1990, as amended) prohibits the use of in vitro derived gametes in treatment. Section 1(4) of the Act defines a gamete as "*live human eggs, including cells of the female germ line at any stage of maturity…"* or *"live human sperm, including cells of the male germ cell line at any stage of maturity"*. Comment 27 of the explanatory note to the Act states that the term *"gametes"* in the Act *"has been amended to expressly encompass not only mature eggs and sperm, but also immature gametogenic cells such as primary oocytes, and spermatocytes."* Section 3ZA requires that sperm or eggs permitted for treatment are *"produced by or extracted from the ovaries of a woman/testes of a man".*

2.4. An HFEA research licence would be required by researchers in the UK if they wished to investigate whether human sperm and eggs derived in vitro could undergo fertilisation and the early stages of embryo development. It is therefore important that the HFEA is aware of progress into research on in vitro derived gametes.

2.5. The Committee reviewed research on in vitro derived human gametes in September 2009 and more recently in 2011. It was the Committee's view that one of the main hindrances to in vitro derived gametes was incorrect imprinting. It was suggested that transplanting gamete precursor cells to their normal environment for the later stages of gamete maturation could help resolve this. However, the transplantation of human gamete precursor cells (derived in vitro) was not at the time a viable, safe approach. Despite progress, no research published at the time convincingly showed that human embryonic stem cells could be differentiated in vitro into mature human sperm.

Summary of developments

2.6. A recent review by Azim et al (2015) highlights that research recently unpicked the mechanism of human primordial germ cell (hPGC) specification and ability to reset the epigenome for totipotency. The article discusses that regulators of hPGC specification also initiate resetting of the epigenome, resulting in a comprehensive erasure of DNA methylation, erasure of imprints and X reactivation in early hPGCs in vivo. The review states that within the extreme hypomethylated environment of the early human germline are loci that are resistant to DNA demethylation, with subsequent predominant expression in neural cells. The article concludes that these loci provide a model for studies on the mechanism of epigenetic inheritance, and their response to environmental factors. The article highlights that these studies reveal differences with the mouse model, which are probably due to differences in the regulation of human pluripotency, and in postimplantation development at gastrulation, thus emphasising the need to produce studies in the human model.

2.7. A study by Tang et al. (2015) showed that the transcriptional program for hPGCs differs from mouse models, with co-expression of somatic specifiers and naive pluripotency genes TFCP2L1 and KLF4. The articles states that this unique gene regulatory network, , drives comprehensive germline DNA demethylation. Further analysis revealed that in week 5-7 in vivo hPGCs progressive DNA demethylation to basal levels. At the same time, hPGCs undergo chromatin reorganization, X reactivation, and imprint erasure. The study highlights that some loci associated with metabolic and neurological disorders are also resistant to DNA demethylation. This could have implications for transgenerational epigenetic inheritance. Tang et al. (2015) provide useful insight into early human germline transcriptional network and epigenetic reprogramming that may have relevance tohuman development and disease.

2.8. An article by Irie et al. (2015) demonstrates specification of hPGC-like cells (hPGCLCs) from germline competent pluripotent stem cells. In this study the characteristics of hPGCLCs were shown to be consistent with the embryonic hPGCs and a germline seminoma that share a CD38 cell-surface marker. The study highlights that put together this may define the potential progression of the early human germline. The article states thatSOX17 is the key regulator of hPGC-like fate, whereas BLIMP1 represses endodermal and other somatic genes during specification of hPGCLCs. Differences between mouse and human PGC specification could be attributed to their divergent embryonic development and pluripotent states, which might affect other early cell-fate decisions. Irie et al. (2015) have established a foundation for future studies on resetting

of the epigenome in hPGCLCs and hPGCs for totipotency and the transmission of genetic and epigenetic information.

Impact

2.9. For people who are unable to produce their own eggs or sperm, in vitro derived gametes are a potential method by which they could have children that are genetically related to them. This technology could therefore have an impact on a variety of patient groups. As with genome editing, the clinical application of this technology is not legal, however, it is important to be aware of how research is progressing and the potential implications.

Level of work recommendation

2.10. The Executive needs to keep abreast of the progress of research in this area to ensure that developments are monitored. The Committee is, therefore, asked to consider whether there are any further studies or developments in the area and identify particular concerns or issues that should be highlighted.

References

Azim SM. Human Germline: A New Research Frontier. *Stem Cell Reports. 2015; 4(6): 955–960.*

Irie N. et al. i SOX17 Is a Critical Specifier of Human Primordial Germ Cell Fate. *Cell. 2015; 160(1-2): 253–268*.

Tang WWC. et al. A unique gene regulatory network resets the human germline epigenome for development *Cell 2015; 161*.

3. Use of ICSI

Background

3.1. Intra cytoplasmic sperm injection (ICSI) is the process of injecting a single sperm into an egg. ICSI techniques currently account for around two thirds of ART treatments in Europe. In recent years experts have been debating whether ICSI is being used appropriately.

3.2. In 2009 SCAAC considered the use of ICSI and the potential risks. The HFEA issued guidance to licensed fertility centres regarding information which they should provide to patients about the risks involved with ICSI[1] eg, risks of eggs being damaged in the procedure, risk of miscarriage, risk of embryos/children having genetic abnormalities, imprinting disorders (such as Angleman's syndrome) and male infertility being passed onto the next generation[1]. Research exploring the impacts of ICSI has continued to grow and SCAAC have monitored developments through their horizon scanning functions looking at health outcomes in ART children. A summary of recent discussion in this area is provided below.

3.3. In 2014 SCAAC raised the use of ICSI as a high priority issue and it was agreed that the Committee would reconsider this topic on publication of the most recent and relevant, professional body guidance.

Summary of developments

3.4. In 2010, at the Advancing Science Serving Society (ASSS) Conference, Professor van Steirteghem highlighted the possibility that ICSI might enable fertilisation with genetically-defective sperm, raising the prospect that problems like diabetes, heart disease and obesity could be passed on, suggesting that ICSI should only be used if conventional IVF does not work.

3.5. In 2012, Prof. Michael Davies presented his recent work to SCAAC, exploring the extent to which birth defects in children born from fertility treatment may be explained by underlying parental factors. The study (Davies et al 2012) showed that an increased risk of birth defects associated with IVF was no longer significant after adjustment for parental factors. The risk of birth defects associated with ICSI remained increased after multivariate adjustment, although the possibility of residual confounding factors could not be excluded. The Committee discussed the study and emphasised the need to consider the risk of birth defects in IVF and particularly ICSI as an important area of research. Members felt that while the study highlighted a correlation

[1] http://www.hfea.gov.uk/docs/2009-05-12_SCAAC_paper_-_ICSI_-_Annex_A.pdf and http://www.hfea.gov.uk/docs/2009-05-12_SCAAC_paper_-_ICSI.pdf

between ICSI practices and increased risk of birth defects, it was not clear whether some confounding influences, such as underlying infertility issues, were a significant influence. The Committee raised concerns about the extrapolation of the study's findings, suggesting that the study was confined to two regionally specific sites and a small sample size. The Committee agreed that this was an important area of research and suggested that larger long term follow-up and observational studies were required to more comprehensively explore any possible links between birth defects and IVF/ICSI.

3.6. In the same year, a study by Hodez-wertz et al (2012) determined whether the use of ICSI in couples who previously underwent ICSI cycles elsewhere could be decreased without compromising the pregnancy rate. The group retrospectively analysed the records of 149 fresh, in vitro fertilisation-embryo transfer cycles in patients who underwent ICSI elsewhere and subsequent fertilisation by insemination only (all insemination group) or half insemination and half ICSI. They compared fertilisation, implantation, and clinical pregnancy and live birth rates[2]. The group found no statistically significant difference in the live birth rate between the two groups. This study therefore suggests that stringent criteria for ICSI may not compromise the clinical outcome and fertilisation can be achieved whether or not ICSI is used.

3.7. A study by Nangia et al (2011) evaluated the outcomes of treatment cycles for male factor infertility, and method of sperm collection. They used cycles from the Society for Assisted Reproductive Technology Clinic Outcomes Reporting System database, which were limited to three groups. These were, ICSI and ICSI cycles for tubal ligation only; non-ICSI and ICSI cycles for male factor infertility only; and all cycles (regardless of infertility diagnosis) using ICSI only. Their results showed that models for male factor infertility only versus tubal ligation only ICSI cycles had lower clinical intrauterine gestation (CIG) but not for live birth (LB). No difference was seen for non-ICSI cycles. Within male factor infertility only cycles, ICSI had a worse outcome than non-ICSI for CIG but not for LB. For all ICSI cycles with no male factor infertility and ejaculated sperm as the reference group, models showed better rates of CIG with male factor infertility ejaculated sperm and with male factor infertility aspirated sperm. The LB rate was higher with male factor infertility ejaculated sperm only. This study therefore concludes that ICSI and sperm source influence both, CIG and LB rates in male factor infertility cases.

[2] The fertilisation rate was 74% and 73% for the all insemination and the half ICSI groups, respectively. In the latter group 69% of inseminated and 78% of ICSI eggs were fertilised. No cycle showed complete fertilisation failure.

3.8. Most recently, Boulet et al (2015) analysed the largest dataset to date using data (from 1996-2012) from the US National Assisted Reproductive Technology Surveillance System to explore the trends in the use of ICSI. The researchers identified 1,395,634 fresh IVF cycles - fertilised eggs that had been transferred to the uterus without being frozen first. Of these, 908,767 (65.1%) cycles used ICSI and 486,867 (34.9%) used conventional IVF. Male factor infertility was identified in 499,135 (35.8%) of fresh IVF cycles. Cycles without male factor infertility - patients with unexplained infertility, two or more prior ART cycles with prior live birth, low oocyte (immature egg) yield, use of preimplantation genetic testing and female patients aged 38 years or older - accounted for the remaining 896,499 (64.2%) fresh IVF cycles. The team found that in the presence of male factor infertility, reproductive outcomes of fresh IVF cycles using ICSI were similar to outcomes of cycles using conventional IVF. In cycles using ICSI without male factor infertility, the team identified *"small but significant"* reductions in implantation, pregnancy, live birth and multiple live birth, compared with cycles using conventional IVF without male factor infertility.

Impact

3.9. It is important to understand what the implications are for using this method as an alternative to conventional IVF. If ICSI is currently being used unnecessarily this may be unnecessarily increasing the risk to those born as a result, as highlighted in current patient information[3]. However, it is important to note that ICSI may be appropriate in other circumstances, outside of male subfertility (for example to reduce the risk of viral transmission in virus positive and sero-discordant patients).

Level of work recommendation

3.10. The Committee is asked if they would like a wider literature review of the studies exploring the risks of ICSI specifically and whether the technique is currently being used appropriately, or if they feel this has been considered as part of the paper discussed in 2015 on health outcomes in children conceived by ART. Should this issue be considered further, the Executive will draft information for patients, informed by professional body guidance and with input from the Committee.

References

Boulet SL. et al. (2015) Trends in Use of and Reproductive Outcomes Associated with Intracytoplasmic Sperm Injection. *JAMA. 2015;313 (3):255-263.*

Brooke Hodes-Wertz et al. (2015) Is Intracytoplasmic Sperm Injection Overused. *Journal of Urology 187(2): 602–606*

[3] http://www.hfea.gov.uk/icsi-risks.html

Davies et al. (2012) Reproductive Technologies and the Risk of Birth Defects. *N Engl J Med. 10;366(19):1803-13.*
Nangia AK et al. (2011) National study of factors influencing assisted reproductive technology outcomes with male factor infertility. *Andrology 3:143-146*

4. Non-invasive methods for assessing embryo viability

Background

4.1. To date the Committee has considered in detail advancements in preimplantation genetic screening and preimplantation genetic diagnosis, exploring the clinical use of new techniques such as karyomapping and Next Generation Sequencing. With more non-invasive techniques such as time-lapse imaging becoming more common place in the lab, researchers continue to move towards non-invasive methods to diagnose embryos. Below we summarise some of the latest advancements towards non-invasive methods for PGD and PGS.

Summary of developments

4.2. In June 2015, Pallini et al. published a review presenting some of the recent developments in the field of PGD and PGS, discussing their benefits and limitations, along with biopsy techniques and the use of new high-throughput technologies. This review also discusses the possibility of a non-invasive diagnosis using blastocoele fluid (the blastocoel is the enclosed fluid-filled cavity that forms within 5-day-old human embryos) and culture media, but highlights that their application is still theoretical and has not been used clinically.

4.3. In 2015, Galluzzi et al. published work evaluating the embryo extracellular matrices (spent medium and blastocoele fluid) as a source of DNA for embryo genotyping. The group first evaluated the amplifiability and the amount of genomic DNA in spent embryo culture media from day 3 and day 5/6. They also evaluated the possibility to genotype the MTHFR polymorphism C677T from media at day 5/6 and blastocoele fluids by direct sequencing. The C677T polymorphism detection rate was 62.5 and 44.4% in medium and fluid, respectively. This research adds to the body of work exploring non-invasive approaches for embryo genotyping, but highlights limitations due to low detection rate and potential allele dropout.

4.4. A study by Montsko et al. (2015) sought to find new candidate molecules to assess embryo viability in a non-invasive way. The group found a novel polypeptide marker that might be helpful to differentiate between potentially viable and nonviable embryos. This molecule was identified with tandem mass spectrometry as the α-1 fragment of human haptoglobin. Significant correlation was found in the amount of the peptide fragment and the outcome of pregnancy. In the culture media of embryos that were assigned in the biochemical assay as non-viable, there were no pregnancies detected; this assay revealed a 100%

successful selection of the non-viable embryos. In the group assigned as viable, the pregnancy rate was 54.7%. Viability of the embryo during the IVF process is assessed by microscopic inspection, resulting in a pregnancy rate of 25%–30%. The article concludes that detection and quantitation of the α-1 haptoglobin fragment of the culture medium proved to be a useful additional method for identifying non-viable embryos, increasing the success rate to 50%.

4.5. In 2015 Poli et al. investigated how the application of proteomic measurements could improve success rates in clinical embryology. They explored a procedure that allows the identification and quantification of proteins of embryonic origin, present in attomole concentrations in the blastocoele. By using targeted proteomics, this group demonstrates the feasibility of quantifying multiple proteins in samples derived from single blastocoeles and they suggest that these measurements correlate with indicators of embryo viability, such as chromosomal (ploidy) status. This study highlights the scope for high-sensitivity proteomics to measure clinically relevant biomarkers in minute samples and that important aspects of embryo competence could be measured using such a proteomic-based strategy.

Impact

4.6. Implementation of non-invasive techniques for PGD or PGS would limit the amount of intervention required on an embryo, the embryo would not need to undergo the trauma of embryo biopsy, eliminating any possible associated risks.

Level of work recommendation

4.7. The Committee is asked to consider whether a more comprehensive literature review in this area is required and if expert advisors should be invited to speak on this topic in order to further understand the implications and scope of this technology.

References

Galluzzi L. et al (2015) Extracellular embryo genomic DNA and its potential for genotyping *Future Science OA (1): 4*

Montsko G. et al (2015) Noninvasive embryo viability assessment by quantitation of human haptoglobin alpha-1 fragment in the in vitro fertilization culture medium: an additional tool to increase success rate. *Fertility and Sterility 103 (3):687–69*

Palinni S. et al (2015) Pre-implantation geneticdiagnosis and screening: now and the future *Gynecological Endocrinology 31:10*

Poli M. et al (2015) Characterization and quantification of proteins secreted by single human embryos priorto implantation *EMBO Mol Med. 2015 Nov; 7(11): 1465–1479.*