

## Scientific and Clinical Advances Advisory Committee Paper

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| <b>Paper title</b>              | <b>Prioritisation of issues identified through the horizon scanning process</b>   |
| <b>Paper number</b>             | SCAAC(02/15)05  |
| <b>Meeting date</b>             | 04 February 2015  |
| <b>Agenda item</b>              | <b>5</b>  |
| <b>Author</b>                   | Anjeli Kara (Senior Scientific Policy Officer)  |
| <b>Information/decision</b>     | Decision  |
| <b>Resource implications</b>    | Dependant on the number of issues that the Committee agree to be high priority  |
| <b>Implementation</b>           | The Committee workplan for 2015/16  |
| <b>Communication</b>            | Work priorities (as defined by the Committee) will be communicated to the Head of Business Planning   |
| <b>Organisational risk</b>      | Medium  |
| <b>Committee recommendation</b> | <p>Members are asked to:</p> <ul style="list-style-type: none"> <li>• note the issues identified as priority through the horizon scanning process, including the progress of research (since January 2014)</li> <li>• consider the high priority issues and work recommendations; and</li> <li>• consider whether advice from additional external advisors would help in achieving the work recommendations.</li> </ul> |
| <b>Evaluation</b>               | None  |
| <b>Annexes</b>                  | <p>Annex A: Issues identified through the horizon scanning process</p> <p>Annex B: Prioritisation of issues identified through the horizon scanning process</p>   |

## **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, conference attendance 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; 'the Committee') 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 2015/16 business plan and workplan for the Executive, SCAAC and the Authority.

## **2. Prioritisation process**

- 2.1 A full list of all issues identified since January 2014 can be found in Annex A 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 2015/16. Briefings about these issues, based on horizon scanning findings, can be found at Annex B of this document unless otherwise stated.
  - Freeze-all cycles

- New technologies in genetic testing
  - Preimplantation genetic screening [SCAAC(02/15)05]
  - Safety of embryo biopsy (ongoing)
- 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 (ongoing)
  - Alternative methods for the creation of ES or ES-like cells (ongoing)
- 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. Other work areas**
- 4.1. In addition to areas of work identified through the horizon scanning process, the Committee will need to advise on any other issues they feel should be prioritised and also be aware that a number of issues related to project work during the year may be areas identified by the Authority. For example:
- Update on blastocyst transfer
  - Data on multiple births rates
  - In vitro derived gametes
- 5. Recommendations**
- 5.1. Members are asked to:
- note the issues identified as high priority through the horizon scanning process, including the progress of research (since January 2014)
  - consider the high priority issues and work recommendations; and
  - consider whether advice from additional external advisors would help in achieving the work recommendations.
- 6. Next steps**
- 6.1. 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 2015/16. 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 2015/16.

## ANNEX B: Prioritisation of issues identified through the horizon scanning process

### 1. Freeze-all cycles

#### Background

- 1.1 In recent years, it has been suggested that cryopreservation techniques have improved to such an extent that it is a viable to freeze-all embryos from a stimulated cycle and subsequently transfer embryos in a natural cycle. This is due to reports that note the endometrium in stimulated cycles is not optimally prepared for implantation and that by transferring embryos in a natural cycle, the incidence of ovarian hyperstimulation syndrome (OHSS) is avoided.
- 1.2 Research in freeze-all cycles is fast evolving. In the last briefing note on this topic to the Committee<sup>1</sup>, it was highlighted that pregnancy rates are increased following frozen embryo transfers (FET) and that perinatal outcomes are less affected, and that consideration should be given to how freeze-all cycles are reported to the Authority and how this information is presented on the Authority's website. It has since been agreed by the Authority that cumulative live birth rates will be used as a headline figure which will accommodate freeze-all cycles.
- 1.3 This briefing note provides a summary of recent studies that explores the use of frozen versus fresh embryo transfer, the effects of FET on implantation, and the impact on live birth rates, and maternal and neonatal outcomes.

#### Summary of developments

##### *Frozen embryo transfers following prior implantation failure*

- 1.4 The sector's improved ability to store and thaw embryos for later use has reduced the general reliance on fresh embryo transfer cycles. Shapiro et al (2014) has continued to look into the effectiveness of frozen versus fresh embryo transfer, by comparing the outcomes in patients with prior implantation failure who elected to undergo another fresh or frozen cycle. Analysis indicated that freeze all was associated with a significantly greater live birth rate when compared with another fresh cycle. A subsequent analysis followed and showed a greater cumulative live birth rate when compared to a fresh cycle. These findings suggest that following implantation failure with fresh blastocysts, patients have a significantly greater chance of having a live birth with freeze all than fresh cycles.
- 1.5 A second study by the same group (Shapiro et al, 2014) compared outcomes of fresh and FET cycles in patients with a prior history of implantation failure with fresh embryos. Using a match-cohort approach to obtain comparable study groups (90 cycles per group), the study found that the fresh group had significantly more blastocysts, more transferred blastocysts, and comparable proportion of transfers of at least one top-grade blastocyst when compared to matched cycles in the FET group. The ongoing pregnancy rate per patient and the implantation rate were

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<sup>1</sup> The Scientific and Clinical Advances Advisory Committee: Prioritisation of issues identified through the horizon scanning process [SCAAC(02/14)02]. February 2014. Available at: [www.hfea.gov.uk/8733.html](http://www.hfea.gov.uk/8733.html)

significantly lower in the fresh group. The data demonstrates that patients with prior histories of fresh embryo implantation failure have significantly increased ongoing pregnancy and implantation rates, if they choose FET in their next cycle.

- 1.6 This correlation that FET cycles are more successful following an unsuccessful fresh cycle is supported by Doherty et al (2014), who questioned whether the outcomes of fresh blastocyst transfer cycles are predictive of the chances for pregnancy and live birth in subsequent frozen blastocyst transfer cycles, using sibling embryos from the same retrieval. Overall, clinical pregnancy rates and live birth rates were similar in fresh and frozen cycles; however, the clinical pregnancy and live birth rates in frozen cycles were significantly higher in patients who were unsuccessful following a fresh embryo transfer. Therefore, where fresh cycles might be unsuccessful, the remaining frozen blastocysts of the same cohort have the same chance of resulting in a clinical pregnancy than the fresh cycle. The group also suggested that frozen cycles following successful fresh cycles have significantly lower clinical pregnancy rate and live birth rate.

#### *Frozen embryo transfer and implantation*

- 1.7 A strong argument for the FET strategy is the prevention of OHSS<sup>2</sup>. Currently, the stimulation protocol that is best thought to eliminate the risk of both early and late OHSS is a GnRH antagonist protocol with a GnRH agonist trigger, followed by cryopreservation of all embryos. Van de Vijver et al (2014) retrospectively investigated the use of GnRH agonist downregulation in artificial endometrium priming cycles for cryopreserved embryo transfer, to establish whether this led to an increase in live birth rates. The study concluded that live birth rates did not increase when a GnRH agonist was administered after FET.
- 1.8 In order to determine whether endometrial hCG infusion at the time of human blastocyst transfer impacts upon implantation rates in fresh or frozen cycles, Hong et al (2014) conducted a randomised double-blinded placebo-controlled trial. It was determined, however, that routine inclusion of hCG infusion before blastocyst-stage embryo transfer, whether fresh or frozen, is not beneficial.

#### *Frozen embryo transfers and ectopic pregnancies*

- 1.9 It has been reported that the ectopic pregnancy rate is significantly reduced after the replacement of frozen embryos. To investigate this, Decler et al (2014) sought to analyse the incidence of ectopic pregnancies in fresh and frozen cycles, in a retrospective cohort study. The incidence of ectopic pregnancy per established clinical pregnancy was similar; as such, no significant difference could be demonstrated in a large cohort of patients.
- 1.10 In contrast Huang et al (2014), with the same aim, found that fresh embryo transfers had the greatest risk of ectopic pregnancy, followed by day-3 FET, and blastocyst FET which had the lowest risk. It was therefore concluded that FET are associated

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<sup>2</sup> The human chorionic gonadotropin (hCG) used to initiate egg maturation is thought to play an integral part in causing OHSS, as is trophoblast-derived hCG which can prolong the symptoms of severe OHSS.

with a statistically significantly lower risk of ectopic pregnancy when compared against fresh cycles; and are consistent with the notion that ovarian stimulation is associated with an increased risk of ectopic pregnancy.

*Maternal and neonatal outcomes following frozen embryo transfer*

- 1.11 There have been multiple studies to date comparing pregnancy outcomes from frozen blastocysts with fresh transfers. Roy et al (2014) completed a retrospective study to compare pregnancy and neonatal outcomes after fresh and frozen blastocyst transfers. From a set of approximately 1,200 infertile patients, similar clinical outcomes were achieved for fresh and frozen grade I and grade II blastocyst transfers. The study concluded that FET yield equivalent live birth rates and improved neonatal outcomes.
- 1.12 Using a sample size of around 270,000 patients, Ishihara et al (2014) also aimed to evaluate the relationship between frozen blastocyst transfer and neonatal and maternal outcomes of pregnancy. This retrospective single-centre analysis found that FET was associated with a significantly reduced occurrence of preterm birth, low birth weight, small for gestational age, yet increased large for gestational age neonates. FET was also linked with significantly increased maternal risk of placenta accreta<sup>3</sup> and pregnancy-induced hypertension. No significant association was identified between blastocyst transfer and maternal complications.
- 1.13 Pinborg et al (2014) questioned whether singletons born after FET are at increased risk of being born large for gestational age and if so, is this caused by intrinsic maternal factors or related to the freezing/thawing procedures. The national register-based controlled cohort study involved two populations of FET singletons: the first population consisted of all FET singletons compared with singletons born after fresh embryo transfer and also with that born after natural conception in Denmark from 1997 to 2006; and the second population included all sibling pairs, where one singleton was born after FET and the consecutive sibling born after fresh embryo transfer or vice versa from 1994 to 2008. It was determined that, singletons after FET have an increased risk of being born large for gestational age. This could not be solely explained by intrinsic maternal factors as it was also observed in sibling pairs, where the sibling conceived after FET had an increased risk of large for gestational age compared with the sibling born after FET.
- 1.14 A retrospective cohort study was conducted by Cobo et al (2014) to assess outcomes after egg vitrification on obstetric and perinatal outcomes compared with those achieved with fresh eggs. Children born after use of vitrified eggs and fresh eggs were included. Vitrification had no clinically relevant adverse effects on obstetric and perinatal outcomes after adjusting for potential confounders. Additionally, no differences were found between the vitrified and fresh egg groups in the rate of obstetric problems (including diabetes, pregnancy-induced hypertension, preterm birth, anaemia, and cholestasis), gestational age at delivery, birth weight, Apgar scores, birth defects, admission to neonatal intensive care unit, perinatal mortality,

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<sup>3</sup> Placental accreta is the term given when all or part of the placenta attaches abnormally to the myometrium (the muscular layer of the uterine wall).

and puerperal problems. It was highlighted that only a greater number of invasive procedures and a reduced occurrence of urinary tract infection were observed in the vitrified egg group.

- 1.15 Pelkonen et al (2014) carried out a register-based cohort study to assess whether there is a different risk for major congenital anomalies in children born after FET compared with children born after fresh embryo transfer. The study was focused on singleton births and included children born after FET, fresh embryo transfer and spontaneous conception. Data suggested that the risk for at least one major congenital anomaly of children born after FET was not increased compared with children born after fresh embryo transfer. Furthermore, no increased risks according to the organ system affected were found between these two groups. Notably, the risk of having at least one major congenital anomaly was moderately increased when comparing the children born after assisted reproduction against the reference group.

#### Impact

- 1.16 The potential benefits of using freeze-all cycles depend on the ability to eliminate, or at least minimise, the risks associated with fresh embryo transfer following a stimulated cycle. By waiting a stipulated period of time between egg collection (ie, stimulation) and the transfer of frozen embryos, the endometrium is able to return to its natural, and potentially more receptive and sensitive state. This may increase the implantation rates and reduce the risks associated with OHSS.

#### Level of work recommendation

- 1.17 The benefits of freeze-all cycles need to be understood through a thorough analysis of the current research in this area, and the correct advice for patients and the sector will need to be formulated by the Executive with guidance from the Committee. The Committee is, therefore, asked to consider whether there are any further studies or developments in the area.

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## 2. Mitochondrial function

### Background

- 2.1 Mitochondria are the energy-producing organelles in the cytoplasm of every mammalian cell. This energy is required for cells to synthesise proteins and other molecules, to move and to proliferate. Mitochondria have other important roles in cellular physiology, notably in programmed cell death (apoptosis) and steroid synthesis. They contain a small amount of DNA that is inherited exclusively from the mother through the mitochondria present in her eggs. Mutations in this mitochondrial DNA can cause a range of rare, yet serious diseases. As such, the mitochondrion and its genome are attracting increased attention in reproductive biology and medicine.
- 2.2 This briefing note provides a summary of recent studies that look into mitochondrial inheritance, its effect on egg and sperm quality, and embryo development and selection. Developments relevant to mitochondrial replacement techniques to avoid mitochondrial diseases are not outlined in this brief.

### Summary of developments

#### *Mitochondria and eggs*

- 2.3 A review by Schatten et al (2014) highlights that women affected by metabolic disorders involving mitochondrial functions, such as diabetes or obesity, and egg ageing are being offered treatment that aims to overcome the effects of these conditions and restore egg quality. The described method, known as the 'augment technique', aims to boost the quality of ageing eggs by taking mitochondria from egg precursor cells<sup>4</sup> and injecting them into a mature egg from the same woman at the time of intracytoplasmic sperm injection (ICSI). As egg precursor cells contain the same genetic information as the mature egg and have not stayed in a post-mitotic state, they are thought to have the optimal conditions for donating mitochondrial DNA. It is thought that a low amount of mitochondria from the precursor cells are sufficient to restore optimal mitochondrial function in the mature egg and increase egg viability<sup>5</sup>. As such, previous success for the augment technique has been reported. This technique differs from 'cytoplasmic transfer' – a discontinued technique – where cytoplasm containing mitochondria from a donor egg is injected into an egg with compromised mitochondria.

#### *Mitochondria and sperm*

- 2.4 Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen that are formed as a natural by-product of the normal metabolism of oxygen. They have important roles in cell signalling and homeostasis; however, during times of environmental stress, ROS levels can increase dramatically, which may result in significant damage to cell structures. Marques et al (2014) conducted a study to

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<sup>4</sup> Egg precursor cells are immature egg cells that are found inside the ovarian lining.

<sup>5</sup> White YA, Woods DC, Takai Y, et al. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat Med 2012; 18(3): 413-421

detect mitochondria-specific reactive oxygen species (mROS) production in human sperm samples using flow cytometry. The group showed that human ejaculates are heterogeneous<sup>6</sup> with regards to mROS production, comprising of sperm that produce differing amounts of mROS. The sperm subpopulation producing the lowest amount of mROS represented the most functional subset of male sperm, as it was correlated with the highest amount of live and non-apoptotic sperm. This data suggests that mROS may provide a way to evaluate sperm samples and isolate the most functional sperm for use in assisted reproductive techniques.

- 2.5 Hurtado de Llera et al (2014) investigated the functional effects of AMP-activated kinase (AMPK)<sup>7</sup> activation in boar sperm. It found that AMPK activation modifies essential sperm processes such as motility, viability, mitochondrial membrane potential, acrosome membrane integrity, and organisation and fluidity of the plasma membrane. It was concluded that balanced levels of AMPK activity are essential for regulating sperm function; namely, motility which is essential for their role in fertilisation.

#### *Mitochondria and embryo selection*

- 2.6 Over the last decade, efforts in improving non-invasive embryo assessment have looked into the material secreted by embryos into the culture medium ('spent medium'). Human embryos release genomic DNA (gDNA) and mitochondrial DNA (mtDNA)<sup>8</sup>, and the ratio between the two is thought to be significantly correlated with embryo fragmentation. A study by Stigliani et al (2014) investigated whether the mtDNA/gDNA ratio in spent medium is correlated with blastulation<sup>9</sup> potential and implantation. It was found that embryos which successfully developed into blastocysts exhibited a significantly higher mtDNA/gDNA ratio in the culture medium compared with those that ceased to develop, and mtDNA/gDNA, combined with morphological grading, has the potential to predict blastulation better than morphology alone. Moreover, mtDNA/gDNA ratio was higher in the media from good-quality embryos that reached the full blastocyst stage on Day 5 compared with those that developed more slowly. With respect to blastocyst morphology, higher trophectoderm quality was associated with a higher mtDNA/gDNA ratio in the culture medium. Additionally, a high mtDNA/gDNA ratio in spent medium was associated with the successful implantation outcome of good-quality embryos. In summary, In turn, it was concluded that the mtDNA/gDNA ratio in the Day 3 embryo spent medium, in combination with morphological grading, may be a novel, non-invasive, early biomarker to improve identification of viable embryos with high developmental potential.

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<sup>6</sup> Genetic heterogeneity is where a single phenotype or genetic disorder may be caused by any one of a multiple number of alleles or non-allele (locus) mutations.

<sup>7</sup> AMP-activated kinase (AMPK) acts a sensor and regulator of cell metabolism.

<sup>8</sup> It is unclear as to why embryos release genetic material into the culture media; DNA fragmentation is hypothesised to be one cause.

<sup>9</sup> Blastulation follows the morula stage in embryo development and consists of a single, spherical layer of cells enclosing a hollow, central cavity.

- 2.7 Konstantinidis et al (2014) sought to develop a microarray platform that allows simultaneous assessment of aneuploidy and quantification of mitochondrial DNA in human polar bodies and embryos. The fully optimised microarray was estimated to have an accuracy of  $\geq 97\%$  for the detection of individual aneuploidies and to detect 99% of chromosomally abnormal embryos; it was also shown to accurately determine relative quantities of mtDNA. As such, it was concluded that this technique may be able to provide information on several aspects of egg and/or embryo genetics, and could lead to improved strategies for identifying viable embryos, thereby increasing the likelihood of successful implantation.

#### Impact

- 2.8 The potential ability to use the mitochondrial genome as a marker for quality may allow for the selection of more viable eggs, sperm and/or embryos; therefore improving the ability to increase fertilisation and implantation success rates.

#### Level of work recommendation

- 2.9 The viability of these techniques, and resulting success rates, need to be monitored. The Committee is asked for their views on the safety and efficacy of the techniques outlined in studies summarised within this briefing document, including their potential in clinical practice. Additionally, the Committee is asked whether the literature and/or techniques mentioned should be further explored or monitored as part of their workplan.

#### References

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### 3. New technologies in genetic testing

#### Background

- 3.1 Various techniques exist to test embryos for genetic abnormalities before they are used in treatment. These embryo testing techniques currently fall under two main categories: preimplantation genetic diagnosis (PGD)<sup>10</sup> and preimplantation genetic screening (PGS)<sup>11</sup>. Techniques that are categorised as PGD help to identify embryos carrying a specific genetic abnormality and PGS – also known as aneuploidy screening – identifies embryos carrying common chromosomal abnormalities.
- 3.2 The clinical use of new techniques for embryo testing, such as karyomapping<sup>12</sup> and next generation sequencing (NGS)<sup>13</sup>, simultaneously screens embryos for both genetic and chromosomal abnormalities without the need to develop any disease-specific test. This briefing note updates the Committee on recent developments in these techniques and their validation.

#### Regulatory mechanisms for the use of new technologies

- 3.3 In 2011 the HFEA's Compliance Committee approved a model which sought to define the procedures for clinics to introduce new and novel processes, defining them in the following way:

- Novel processes<sup>14</sup>

If centres wish to use a novel process in clinical practice, they are required to apply to the Authority to seek permission for its use. The application is discussed by the relevant committee, who subsequently advise the Authority's Statutory Approvals Committee (SAC) on the safety and efficacy of the process. SAC will then decide whether to add the process to the

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<sup>10</sup> Preimplantation genetic diagnosis (PGD) techniques help to identify embryos carrying a specific genetic mutation by developing a condition-specific test, which can take weeks or months and often delays patient treatment. Embryos that are shown to carry the specific genetic mutation are not transferred and therefore do not result in a child being born.

<sup>11</sup> Preimplantation genetic screening (PGS) – also known as aneuploidy screening – are techniques that help to identify embryos carrying an abnormal number of any of the 23 pairs of chromosome. Embryos that are shown to carry a common chromosomal abnormality are not transferred and therefore do not result in a child being born.

<sup>12</sup> Karyomapping is a technique that simultaneously screens embryos for both genetic mutations and chromosomal abnormalities without the need to develop any disease-specific test. It presents the ability to generate additional information on genetic conditions or chromosomal abnormalities that are not being specifically tested for, and to concurrently conduct PGD and PGS at the same time. It is achieved by analysing the DNA sequence in parents and affected family members, and comparing their sequence with that inherited by the embryo. This is achieved using microchip technology known as microarray. The technique analyses the inheritance of genetic defects in the embryo without any prior patient or disease-specific test development.

<sup>13</sup> Next generation sequencing uses numerous technologies that involve mapping the DNA of an individual's entire genome. A cheaper – yet still effective – variation of this technique involves mapping specific, selected sequences of DNA. This approach may be clinically relevant to embryo testing to identify Mendelian inherited conditions; particularly, those that are uncommon.

<sup>14</sup> A novel process is one that is not on the authorised list and has not been conducted in the UK.

authorised processes list. Both PGD and PGS are previously established, authorised processes for the activity of embryo testing.

- New processes<sup>15</sup>

If a licensed centre intends to conduct a new process (i.e. an established process which is new to that centre) they are required by General Directions 0008 to notify the HFEA.

### Summary of recent developments

#### *Aneuploidy screening*

- 3.4 Chromosomal aneuploidy has been reported to be a contributing factor in implantation failure and spontaneous miscarriage. Fluorescence in situ hybridization, used to test for a limited number of chromosomes, has been used to carry out PGS; however, this has generally been side-lined for microarray techniques that test all 24 chromosomes. Fiorentino et al (2014) aimed to validate a NGS-based method for 24-chromosome aneuploidy screening and to investigate its applicability to PGS. The retrospective blinded study analysed around 5,000 chromosomes, 402 of which carried a copy number imbalance; NGS specificity for aneuploidy was 99.98% with a sensitivity of 100%. The group suggests that the study accurately validates NGS-based comprehensive aneuploidy screening on single cells. As the level of consistency was noted as being similar to established methodologies (eg, array-CGH), NGS was determined as ready for clinical application in reproductive medicine with potential advantages of reduced costs accuracy.
- 3.5 The same group, Fiorentino et al (2014), followed up their study by developing a prospective trial involving a double-blind parallel evaluation using both array-CGH and NGS. Following trophoctoderm biopsy, NGS specificity for consistency of chromosome copy number assignment was 99.98% with a sensitivity of 100%. Despite one discordant result, NGS specificity and sensitivity for 24-chromosome diagnosis consistency were both 100% since the discordant sample presented several other aneuploidies. It was concluded that this study shows extensive application of NGS-based comprehensive aneuploidy screening on embryos at blastocyst stage in a clinical setting versus array-CGH as test of reference. As per their previous study, the group noted that NGS has demonstrated a reliable methodology, with the potential to improve chromosomal diagnosis on embryos especially in terms of high-throughput, automation and ability to detect aneuploidy.
- 3.6 Wells et al (2014) aimed to develop a cost-effective method for preimplantation aneuploidy screening. A rapid NGS protocol was developed with consumable cost reported to amount to two-thirds of the 'most widely used method for embryo aneuploidy detection'. The method was applied clinically, assisting in the selection of euploid embryos in two IVF cycles, producing healthy children in both cases. The NGS approach had sensitivity and specificity of 100%, and was also able to reveal specified mutations in the nuclear or mitochondrial genomes in parallel with

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<sup>15</sup> A new process is one which has already been authorised by the HFEA but may be new for a particular licensed centre.

chromosome assessment. Notably, elevated mitochondrial DNA content was significantly associated with aneuploidy which was highlighted as a suggestive link between mitochondria and chromosomal malsegregation.

- 3.7 Konstantinidis et al (2014) sought to develop a microarray platform that allows simultaneous assessment of aneuploidy and quantification of mitochondrial DNA in human polar bodies and embryos. The fully optimised microarray was estimated to have an accuracy of  $\geq 97\%$  for the detection of individual aneuploidies and to detect 99% of chromosomally abnormal embryos; it was also shown to accurately determine relative quantities of mtDNA. As such, it was concluded that this technique may be able to provide information on several aspects of egg and/or embryo genetics.

#### *Single gene disorders*

- 3.8 Conventional PGD identifies embryos carrying a specific genetic mutation by developing a condition-specific test, which can take weeks or months and often delays patient treatment. New technologies in genetic testing have allowed for the development of a comprehensive linkage-based test, which has been proposed to create a genome-wide mapping, or karyomap, of the parental origin of each chromosome in the embryo.<sup>16</sup> Natesan et al (2014) aimed to compare the accuracy of family- or disease-specific targeted haplotyping with direct mutation-detection strategies by using karyomapping. The group concluded that karyomapping is highly accurate without the need for customised test development. The same group, Natesan et al (2014), used karyomapping in another study to confirm the results of an existing PGD case, detecting both chromosomal abnormalities and a disorder, simultaneously. The study confirmed a live birth after PGD with confirmation by karyomapping.

#### Impact

- 3.9 The benefits of NGS and karyomapping as new technologies in genetic testing mean that chromosomal abnormalities and single gene disorders can be detected in the same test, using a single biopsy sample. Consideration should be given, however, to the notion that these technologies present the ability to generate extra genetic information.

#### Level of work recommendation

- 3.10 The advantages and disadvantages of NGS and karyomapping need to be understood through a thorough analysis of the current research in the area. The Executive will present a paper to the Authority in May 2015 to seek views on the legal boundaries for testing embryos for genetic conditions and chromosomal abnormalities using new methods of embryo testing. In turn, this should provide the

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<sup>16</sup> In April 2014, the Authority's Ethics and Standards Committee (ESC) discussed and considered whether karyomapping should be considered a 'novel' or 'new' technology; and whether centres could use karyomapping to simultaneously test for specific gene mutations and chromosomal abnormalities. ESC concluded that karyomapping is a new technology that does not differ from alternate embryo testing technologies that are already considered authorised processes. It was also highlighted that an additional paper should be taken to ESC to discuss additional developments in technology for embryo testing.

basis for the Executive to advise centres, and formulate correct and up-to-date patient advice on the area, with guidance from the Committee.

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## 4. Safety of embryo biopsy

### Background

- 4.1 Embryos may be genetically tested prior to transfer in order to avoid the inheritance of serious inherited conditions; to screen for problems with the genetic material in an embryo; or to have a sibling child that could provide a potentially life-saving transplant to another child who suffers from a serious condition.
- 4.2 Scientists are able to remove either one or two cells from an embryo created by assisted reproduction between periods in its development. These biopsied cells are then genetically tested, while the remaining cells within the embryo have the potential to differentiate and develop further.
- 4.3 However, it is important to monitor the safety of this process and determine the optimal process, to ensure that the resulting child will not suffer from any adverse events. Researchers have examined the health, growth and development of these children, compared to children conceived by assisted reproduction without the need for embryo biopsy, and naturally-conceived children.
- 4.4 In the last briefing note on this topic to the Committee<sup>17</sup>, it was noted that while the long-term studies concerning embryo biopsy are reassuring, the Executive should continue to monitor research in this area as part of the horizon scanning process; particularly, where papers highlight the proportion of embryos that fail to reach blastocyst stage (if biopsied at the 8-cell stage) or implant, versus embryos that are not biopsied. This briefing note provides an update on recent studies published in this area.

### Summary of developments

- 4.5 Trophectoderm biopsy with comprehensive chromosome screening (CCS) has been suggested to increase implantation and pregnancy rates. Some patients desire CCS on previously cryopreserved blastocysts, resulting in blastocysts that are thawed/warmed, biopsied, vitrified and then warmed again. The effect of two cryopreservation procedures and two thawing/warming procedures on outcomes, however, has not been effectively studied. A study conducted by Taylor et al (2014) sought to investigate the effect. Cycles were divided into two groups: group 1 patients underwent a cryopreserved embryo transfer with euploid blastocysts that were vitrified and warmed once; group 2 patients had a cryopreserved embryo transfer of a euploid blastocyst that was cryopreserved, thawed/warmed, biopsied, vitrified and warmed. Blastocyst survival in group 1 and the survival of the second warming in group 2 was significantly different; however, no difference between biochemical and clinical pregnancy rates, implantation rate and live birth/ongoing pregnancy rate was observed between groups. Although it is occasional to thaw/warm, biopsy, revitrify and rewarm blastocysts for cryopreserved embryo transfer, the results indicate that outcomes are not compromised.

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<sup>17</sup> The Scientific and Clinical Advances Advisory Committee: Prioritisation of issues identified through the horizon scanning process [SCAAC(02/14)02]. February 2014. Available at: [www.hfea.gov.uk/8733.html](http://www.hfea.gov.uk/8733.html)



- 4.6 A prospective follow-up cohort by Eldar-Geva et al (2014) sought to examine whether embryo biopsy for PGD influences neonatal outcomes. Children born after PGD, ICSI, and spontaneous conception, were matched for maternal age, parity, and BMI. For singletons, the mean birth weight was higher after spontaneous conception compared with ICSI but not compared with PGD. Mean gestational ages were lower after PGD and ICSI compared with spontaneous conception. The low birth weight and intrauterine growth restriction rates were 4.4%, 12.0%, and 5.7% and 5.1%, 9.5%, and 5.5% for PGD, ICSI, and spontaneous conception, respectively. Similar results were found when controlled for the number of embryos transferred and cryopreservation. The results for twins exhibited similar but less statistically significant trends. Polar body and blastomere biopsies provided similar outcomes. The data suggested that embryo biopsy itself does not cause intrauterine growth restriction or low birth weight compared with spontaneous conception, despite lower gestational ages with PGD, and that the worsened outcomes in ICSI, compared with PGD pregnancies, may be due to the infertility itself.
- 4.7 Another study that looked into the effects of embryo biopsy for PGD was conducted by Winter et al (2014). Although a small study group, the study sought to assess whether cognitive and psychomotor development differs between PGD children and children born after ICSI and spontaneous conception. The cognitive abilities and motor skills of 5- to 6-year-old singletons born after PGD were assessed in comparison with ICSI and spontaneous conception children in a prospective, case-controlled, matched follow-up study. It was concluded that the cognitive development of PGD children was comparable to children born after ICSI and spontaneous conception, however, motor development differed between ICSI and spontaneous conception groups.

#### Impact

- 4.8 While the long-term effects are reassuring, it continues to be important to monitor the safety and optimal process for embryo biopsy. This is particularly due to changes in clinical practice, such as freeze all cycles, which make papers like Taylor et al (2014) – the outcomes of two cryopreservation procedures and biopsy on an embryo – key.

#### Level of work recommendation

- 4.9 The Committee is asked to consider whether there are any further studies or developments in the area; whether they wish to make any recommendations to the Authority regarding the use of embryo biopsy in assisted reproduction; and whether the Executive should continue to monitor research into the effect of embryo biopsy on resulting child health.

#### References

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#### **Prioritisation of issues identified through the horizon scanning process**

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