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Workshop report

243rd ENMC international workshop: Developing guidelines for management of reproductive options for families with maternally inherited mtDNA disease, Amsterdam, the Netherlands, 22–24 March 2019

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The 243rd ENMC workshop met in Amsterdam, The Netherlands in March 2019 to discuss current perspectives and knowledge in reproductive options in patients with mtDNA-related mitochondrial disease. The 29 participants came from The Netherlands, UK, France, Germany, Spain, Austria, Belgium, Australia, USA and Brazil, and was multi-disciplinary, including patients, clinicians, basic scientists, ethicists, a sociologist, and representatives of industry and patient organizations (including the Lily Foundation, the Dutch Muscular Disease Association, International Mito Patients (IMP) and the LHON group of the Dutch Eye Association).

Genetic counselling is uniquely complicated in mitochondrial diseases, and the ENMC has played an important role in developing consensus guidelines for reproductive options [1,2]. As molecular characterisation has become routine, more options have become available. Pre-implantation genetic diagnosis (PGD, where routinely 1–5 cells are sampled from a pre-implantation embryo) is now robust and safety is established [3] for maternally inherited mtDNA disease, albeit that data remains relatively limited. Furthermore, great strides have been made in mitochondrial replacement therapy (MRT) [4,5]. In MRT the nucleus is removed from either a zygote (pronuclear transfer, PNT) or an oocyte (maternal spindle transfer, MST) and placed into a corresponding enucleated cell at the same stage, but from a donor with normal mitochondria. These techniques are being applied to a range of disorders beyond the purely mitochondrial, in which some investigators believe that cytoplasmic transfer [6,7] is useful for regeneration of poor quality oocytes. Patients are enthusiastic for these new options, but need appropriate, informed counselling regarding the risks and benefits of novel techniques such as MRT where clinical experience is limited. In the UK the HFEA have established a rigorous regulatory framework, with a detailed case-by-case review process for each MRT application. This consensus document is a response to the pressing need for internationally agreed guidelines on referral and counselling of couples seeking advice on assisted reproductive options for mtDNA disease.

1. Discussion of recent advances in the science surrounding mtDNA transmission that are relevant to clinical practice

1.1. Introduction

The goals of this workshop were to discuss, evaluate and summarise the available evidence of current reproductive options and to agree a core set of recommendations for ensuring the best possible standards of reproductive care and advice for women from families with mtDNA disease. Current knowledge of the different reproductive options was reviewed by the participants in the first half of the workshop, while

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the second half of the workshop was devoted to defining appropriate patient specific guidelines.

Families with members who have suffered from severe, maternally-inherited, mtDNA disease not only seek the opportunity to have healthy children, but may also wish to eradicate the disease. Prenatal diagnosis [8] and estimates of risk [9] are generally more complex for mitochondrial than Mendelian inheritance. Unique features of mitochondrial inheritance include heteroplasmy, that is, the co-existence of mutated (pathogenic) and wild type (normal) mtDNA within a cell; a "threshold effect" in most mtDNA diseases, with the level of heteroplasmy required for symptoms to become manifest varying from <10% [10] to 100% mutant mtDNA, depending on the mutation and tissue. In addition, there is a mtDNA bottleneck whereby dramatic and unpredictable fluctuations in the proportions of mutant and normal mtDNA may arise between generations [11]. In humans, significant fluctuations are already apparent in oocytes in both controls [12] and in carriers of mtDNA disease [13–15]. While these were initially held to be random [16] new data suggest some regulators. Furthermore, mitochondrial diseases are common. One in 400 individuals harbour the m.3243A>G mutation [17] which can be associated with a wide spectrum of clinical features and very severe, life-limiting disease in a minority. Hence the demand for interventions that reduce transmission could be substantial.

1.2. Basic biology: key issues for oocytes including the mitochondrial bottleneck

Mark Stoneking presented data from mtDNA analysed in blood from normal control trios (mother, father and children) [18]. He found that heteroplasmy was common and frequently differed between a mother and her offspring, consistent with a mtDNA bottleneck. Differences between siblings were smaller between identical than non-identical twins, suggesting that the genetic bottleneck occurs early in development, potentially before fertilisation. *De novo* variants showed evidence of selection, suggesting that the segregation is not entirely stochastic but is driven by mitochondrial function [18].

Jo Poulton showed mtDNA data from oocytes of controls and patients with maternally inherited diseases demonstrating mtDNA segregation during oogenesis and/or follicle development [11]. She and Louise Hyslop discussed two events that may contribute to this segregation. Firstly there may be clonal proliferation as mtDNA copy number increases ~1000 fold (to 100,000–400,000 copies/cell) while primordial germ cells (PGCs) develop into mature oocytes. Secondly, only ~400 of the 1000,000 oocytes present at birth are ovulated, the majority being degraded. It is not clear whether these events represent processes for selecting the best mitochondria or oocytes from declining ovarian reserve. Claudia Spits described substantial variation in heteroplasmy between embryos but stable between sister blastomeres of 3-day, 8-cell embryos. Levels of novel mtDNA variants could be explained by point mutations occurring during germline maturation.

Iain Johnston presented work combining mathematical models and data from mouse models to reveal the timing and mechanism of the mtDNA genetic bottleneck. He highlighted that the genetic mtDNA bottleneck (an increase or decrease in heteroplasmy between cells and/or offspring) is not identical to the physical mtDNA bottleneck (a reduction in mtDNA copy number per cell during development). The physical bottleneck contributes to the genetic bottleneck, but other physical processes like cell divisions and mtDNA turnover also generate variability. The genetic bottleneck is an effective genetic quantity, and different interplay between these physical processes can lead to flexibility in this effective "bottleneck size" [19]. Variance in germline heteroplasmy of two models increased linearly with maternal age, consistent with ongoing mtDNA turnover (and thus a decreasing "bottleneck size" with age) [20]. Iain stated that mitophagy is one of the ongoing processes that could underlie the changing bottleneck size and the involvement of mitophagy in determining the characteristics of the bottleneck could be mutation-specific. Prof Poulton showed mouse data consistent with a burst of mitophagy in mouse pre-implantation embryos and showed that this could be driven by activators of mitophagy [21].

Barbara Arbeithuber explained the rationale for and the superior quality of duplex sequencing for validating low levels of heteroplasmy, as well as to measure de novo mutations. She showed evidence for an increase in somatic and germline mutations in ageing mice compared to young controls, but with a smaller difference in oocytes.

Key points in normal mitochondrial inheritance

- Low level heteroplasmy occurs in healthy humans.
- The "bottleneck size" may vary between individuals, between mutations, and may decrease as individuals age.
- Key contributions to the bottleneck occur by the time that oocytes are mature, but changes continue thereafter. For instance in mouse, oocytes become increasingly variable with maternal age and survival of "unfit" embryos is reduced.
- There is selection against certain classes of mutations, that are not seen in live-born humans, but this does not eliminate the common mutations.

1.3. Animal models

1.3.1. POLG mutator mouse

Jim Stewart showed that there is a strong bias against inherited mutations that change nucleotides in the first and second codon positions in the POLG mutator mouse. In maternal lineages derived from mutator mice, mutation

detection using a threshold of 0.05% variant frequency revealed no signature of selection. However, when increasing the detection threshold to 5%, the codon mutation distribution then revealed the signature of purifying selection. Also, when using their mt-tRNA^{Ala} mouse model (a mouse model for mitochondrial disease), the frequency of this mutation never exceeded 83% [22], a further indication of selection in mice. Though a mutation affecting the corresponding base-pairing in the human mt-tRNA^{Ala} has been observed at homoplasmic levels in patients [23] suggesting that there are species-specific differences in selection or elimination of mtDNA mutations.

1.3.2. Oogenesis and bottlenecks in zebra fish

Bert Smeets showed that the zebrafish is an excellent animal model for studying mtDNA bottlenecks. The reduction of mtDNA copy number in PGCs is comparable to that in mammals. The copy number in oocytes was found to be relatively high with 19×10^6 mtDNA copies per oocyte. Study of the bottleneck in non-PGC cells revealed a more extreme reduction in mtDNA copy number. By controlling the expression of the mitochondrial transcription factor/histone-like protein, Tfam, mtDNA copy number could be modified and its effect studied. Zebrafish as model organism has considerable advantages such as access to unlimited oocytes/embryos [24,25].

1.3.3. Four heteroplasmic strains of mice: implications for haplotype matching

Joerg Burgstaller presented mtDNA segregation data in somatic tissues of four heteroplasmic mouse lines. He concluded that segregation is common when two genetically distinct mtDNA haplotypes are mixed in a mouse model. This has potential implications for human reproductive medicine in cases where mtDNA heteroplasmy is created with genetically divergent mtDNA haplotypes. He suggests that mtDNA haplotypes should be matched in these therapies as a precautionary measure [26].

1.3.4. Alterations in gene expression and metabolism in heteroplasmic mice

Tonio Enriquez presented a detailed analysis of the metabolic consequences and health issues of mtDNA heteroplasmy in a mouse model. Segregation of mtDNA was influenced by the nuclear genetic background, implicating mito-nuclear interactions and specifically reactive oxygen species, as key determinants of mtDNA composition. Furthermore, shifts in mtDNA heteroplasmy lead to differences in OXPHOS-function. Gender specific impact of mtDNA heteroplasmy was also present in the model. Nutrition was also found to lead to shifts in mtDNA heteroplasmy [27].

1.4. Mitochondrial replacement therapy

1.4.1. Segregation and mitochondrial dynamics in mitochondrial replacement therapy

Marcos Chiaratti showed that the morphology in oocytes differs from somatic cells: In oocytes, the architecture of mitochondria is immature, with a fragmented network. This likely results from increased fission compared to fusion. He presented data on mouse models in which the expression of mitochondrial pro-fusion proteins mitofusin 1 and 2 was knocked out. Fertility and oocyte development are substantially impaired in these models, showing that mitochondrial dynamics are critically important in maintaining oocyte health [28].

Justin St John showed that Somatic Cell Nuclear Transfer (SCNT), where a whole donor cell (e.g. fibroblast) is transferred into an enucleated oocyte, is often accompanied by segregation of donor mtDNA (which may populate the embryo with levels of 0% to ~59% in sheep, cow, and pig), indicating the involvement of selection. In these models, somatic cell mitochondria can have negative effects on the development of the embryo. This issue can be overcome by depleting the donor cell of its mtDNA. He also showed that mitochondrial supplementation of oocytes that have a low mtDNA copy number - which has a negative impact on fertility - is possible by using sister oocytes to provide extra mitochondria. Even though this supplement comprised only ~800 mtDNA copies this still results in better embryo development [29]. Nevertheless, supplementation of oocytes with a normal mtDNA content may be detrimental [30], as shown in studies using egg precursor cell mitochondria as the donor source. Indeed, some have questioned the feasibility of using egg precursor cell mitochondria given the lack of benefit seen in a recent clinical trial [7]. The use of autologous mitochondrial supplementation in humans is distinct from cytoplasmic transfer as mtDNA haplogroups were mixed when cytoplasm was transferred from donor oocytes to recipient oocytes as another means to treat recurrent fertilization failure, embryos arrest and implantation failure [31]. Although the results have not been reported in detail, some abnormalities have reported [32].

2. Ethics, regulation and clinical approach to MRT

2.1. Ethics

An overview of the ethical issues associated with mitochondrial replacement therapy was presented by Sarah Chan. This considered the personal and societal implications of having genetic contributions from three parents and the ethical challenges that novel biotechnological breakthroughs often present. One specific example in relation to pronuclear transfer is the requirement to fertilise two eggs to create a single embryo with donated mitochondria. Dr Chan also explored the nature of donation and the anonymity afforded to maternal mitochondrial donors in the UK, contrasting this with the ‘right’ to know the identity of one’s gamete donor.

2.2. Mitochondrial replacement therapy: practical issues and mtDNA segregation

Dr Hyslop presented the science and technology behind the pronuclear transfer technique, illustrating this with a

video recording of the process. Dr Hyslop explained with regard to clinical service delivery, it is impractical to perform pronuclear transfer with fresh donor and patient eggs. Carryover of mutant mtDNA from the patient to the donor fertilised egg was minimised using fresh rather than vitrified (frozen) donor eggs, and so the care pathway has evolved to include several cycles of ovarian stimulation to harvest and vitrify approximately 20 patient eggs. These are then thawed and utilised when fresh donor eggs are available. HFEA confidentiality requirements prohibited a discussion on the success of pregnancies achieved to date using this technique.

Another form of mitochondrial replacement therapy, maternal spindle transfer (MST), was discussed by Shoukhrat Mitalipov. In MST an oocyte is removed from the recipient, and when it is in the metaphase II stage of cell division, the spindle-chromosome complex is removed; some of cytoplasm comes with it, so some mitochondria are likely included. The spindle-chromosome complex is inserted into a donor oocyte from which the nucleus has already been removed. This egg is fertilized with sperm, and allowed to form a blastocyst. In his experiments, Chinese and Indian rhesus monkeys with different mtDNA haplotypes were used to create embryos containing meiotic spindles from Chinese origin rhesus and Indian origin donor cytoplasts and vice versa. This was followed by intracytoplasmic sperm injection, with embryo culture to blastocyst stage prior to transfer into 9 females. This resulted in 3 pregnancies and 4 live births of healthy young who have gone on to live healthy lives and reproduce. These monkeys have maintained a near constant mtDNA heteroplasmy (carryover) of approximately 2% in blood and skin [33], consistent with 1–3% carryover in a range of his experiments. He described how segregation of pathogenic mutant mtDNA, after spindle transfer using human embryonic stem cells as a donor, can increase to high levels in 15–20% of transfers [4]. He emphasized that for some mtDNA mutations even a relatively small percentage of carryover followed by even a modest degree of reversion to mutated genotype may become clinically relevant. He commented that differences in replication rate might be at least as important as OXPHOS efficiency in driving segregation [4].

The potential for partial reversion was also seen in studies by Hyslop et al. [5] using PNT rather than MST. This was found in a similar proportion of human embryonic stem cells following prolonged culture, but was not observed in the embryos themselves. This reversion phenomenon was not eliminated when karyoplast and cytoplast donors belonged to the same mtDNA haplogroup [5] or when carryover was $<2\%$.

Thus, if mtDNA haplogroup in humans replicates the mtDNA segregation seen between inbred strains of mouse [4,26], it might be an issue in cases where there is substantial mtDNA carry over [5]. Levels of mtDNA variation in human populations are comparable to those found in mouse strains where substantial segregation bias is observed [34]. However, results from human oocyte material suggest that features beyond coarse-grained genetic distance are important in determining the potential for “reversion” or segregation bias [4]. A further question on this topic is how the in vitro risk of reversion in cultured stem cells would translate to in vivo embryos, where development is regulated differently.

The potential risks from “selfish” mtDNAs [35] might be reduced by haplotype matching, that is, using donors who harbour mtDNA that is closely related to the recipient. However, such a regime would be difficult to implement. However, there is a tradeoff between minimising the potential for undesired segregation bias and the ease of identifying appropriate donors, particularly for families carrying rare haplogroups [34]. Future studies may help clarify this issue. Currently, haplogroup matching is recommended as a consideration but not required by the regulator. A recent study of naturally occurring nuclear-mitochondrial mismatch seen in humans including 2504 individuals across 26 populations [36] and a meta-analysis of a range of organisms [37] both concluded that nuclear-mitochondrial mismatch is unlikely to jeopardize the safety of mitochondrial donation. However they recommended more research to establish what degree of variation between donor and patient mitochondrial DNA haplotypes is acceptable to ensure ’haplotype matching [37].

2.3. Patient experiences of reproductive choices in mitochondrial disease

Rebecca Dimond, then facilitated a session where we were able to hear the personal experiences of patients and parents of patients from the Netherlands and the UK. Some of these parents had made reproductive choices including sex selection for female embryos to reduce the risk of developing symptoms of LHON. Others had campaigned in the UK for a change in the law that would enable MRT to be offered for severe mtDNA disease and shared their experience of the robust discussion and opposite viewpoints it raised in a very public arena under intense media scrutiny. We also heard how debilitating the disease can be on a day-to-day basis and the devastating loss that some families have endured. All of the speakers in this session expressed agreement that it was important to develop safe reproductive options for women with maternally inherited mitochondrial disease.

2.4. Commercial interests in reproductive choices for mitochondrial families

Dagan Wells provided an industry perspective on PGD (increasingly referred to as PGT - preimplantation genetic testing). He noted that several commercial laboratories offering PGD services have declined to accept patients with mtDNA disorders. This may be related to worries about medical liability, since the transfer of embryos with low (but not zero) mutant load could be associated with a risk of clinical symptoms. The lack of commercial PGD provision might also be related to the relatively high cost to develop PGD protocols for the comparatively small pool of patients at high risk of transmitting an mtDNA disorder. Despite these concerns, Prof Wells was of the opinion that commercial providers should offer PGD for mtDNA disorders and that it

would be his intention to do so in his laboratory. He pointed out that estimates of heteroplasmy based on polar body sampling are inaccurate. Results from blastomeres appear to be more reliable for PGD of mtDNA disorders. Despite some controversy in the literature, his experience suggests that trophectoderm biopsy, performed at the blastocyst stage [38] might also be suitable for diagnostic purposes, although additional work is needed to verify this. He showed data suggesting that measurement of mtDNA content of embryonic cells provides an insight into embryo quality, increased mtDNA copy number being associated with aneuploidy and failure of euploid embryos to successfully implant in the uterus [39]. The potential relevance for infertility makes this of more commercial interest (see caveats above) than MRT, which he holds is specialised and infrequently required for the indication for which it is currently licensed in the UK.

2.5. Regulation and social impact of MRT

The session was launched with an overview regarding the legal issues pertaining to MRT delivered by Mair Crouch. Dr Crouch was keen to point out that the techniques involved in MRT actually required physical transfer of the nuclear genetic material rather than the mitochondria and in that sense the term used in the UK regulations, "Mitochondrial Replacement Therapy", was something of a misnomer and the technique might be more aptly named as "Nuclear Replacement Therapy". Dr Crouch argued that this also played down the importance of mtDNA in determining personal identity. Dr Crouch also asserted that the HFEA consultation regarding MRT placed little emphasis on the welfare of the child, even though there is clear guidance on this issue in the Human Fertilisation and Embryology Act of 1990. She expressed concerns regarding unproven safety of the technique and the related issue of mtDNA carryover as posing possible risks to the welfare of children born as a result of this technique. Furthermore, Dr Crouch highlighted the potential risk of healthy children born following MRT becoming carers for their progressively affected mothers; a point which raised considerable disquiet and challenge from the audience [40]. Dr Crouch also endorsed the case-by-case approach that has been adopted by the HFEA, so that a licence is not issued for a condition, but for a particular applicant. Finally, Dr Crouch also covered aspects related to the rights of the egg donor and of the resulting child in relation to the 'donation'. At present a child born following MRT, upon reaching the age of 16 years, will be able to apply for non-identifying information regarding the mitochondrial donor, but will not be able to learn their identity. Dr Crouch's viewpoint was that there is an increasing recognition of the importance of genetic identity and recent legislation in the Australian state of Victoria and Europe regarding the prospective waiver of anonymity (*retrospectively* in Victoria) for gamete donors might have future implications for MRT egg donors [41,42].

Dr Dimond then presented the findings of her project interviewing patients about their experiences of mitochondrial disease and their thoughts about mitochondrial donation. She found that most of the participants agreed with legalising mitochondrial donation, identifying it as potentially useful in future for young female family members, or to help those who had more severe disease. Although participants recognised why there might be objections to the technology, almost all strongly felt that the technology was not 'immoral'. They believed that the benefits of having a healthy child generally outweighed potential risks, but they also recognised the benefits of engaging the children in 'follow up' care. Most participants felt that the term 'three parent baby' was misleading and the donor should not be considered a 'parent' to the child. Although it was clear that the majority of participants supported legalisation in principle, Dr Dimond concluded by asking whether patients would use mitochondrial donation in practice? Here Dr Dimond highlighted several questions which show the complexity of reproductive decision making, including when and how reproductive risk information is communicated in families and how they make sense of a variable and often late onset genetic disease.

2.6. Pre-implantation genetic diagnosis (PGD): practical issues

Since the first report of PGD for a mtDNA mutation [43], it has been applied in a few PGD centres: Paris (France) [43–45], Maastricht (the Netherlands) ([46–48], Melbourne (Australia) [49], Newcastle (UK), New Jersey (America) [38], Ghent (Belgium) [50] and Oxford (UK). Representatives from Paris (Julie Steffann), Maastricht (Suzanne Sallevelt), Oxford (Dagan Wells) and Newcastle (Robert McFarland) attended the ENMC meeting and presented data from their centres while David Thorburn presented data from Dr Sharyn Stock-Myer at Melbourne IVF. Overall, a very small number of patients (<100) have been treated in these centres, and less than 20 babies have been born to date. PGD for mtDNA mutations is usually performed on day 3 or 4 (cleavage-stage) after fertilisation, and one or two blastomeres are sampled. Most data suggest there is little variation in heteroplasmy between blastomeres at the 8-cell stage. Two main factors drive the decision of whether to biopsy a single or two blastomeres. Biopsying two blastomeres maintains the highest possible diagnostic certainty (reported to be fairly high for one blastomere as well [48]) but seems to impair the implantation potential of the embryo [51]. All centres agreed that mutant loads are quite stable among sister blastomeres at this stage. Postnatal results from babies born after cleavage-stage PGD are so far in good concordance with PGD data. Very limited data are available at blastocyst stage [38,50], but in one case, the mutant loads in preimplantation embryo (blastocyst biopsy) and samples taken postnatally were strikingly different [38,52]. The cause of this discrepancy is not yet clear, but different laboratory techniques may have played a role.

One major limitation of PGD is the availability of transferable embryos. A consensus on mutant load thresholds

under which embryos are considered for uterine transfer is complex and was discussed at length. For mtDNA mutations where relatively large datasets are available, specific thresholds may be applied [46,53]. Often however, data on a given mtDNA mutation are often very limited. David Thorburn commented that m.13513G>A is a common mutation with an unusually low threshold of ~35%. A generic threshold of 18% has been proposed [53,54] as it would be expected to prevent most embryos from severe illness. That said, it is critical to evaluate this on a case-by-case basis for every couple opting for PGD, as disease thresholds [10] and transmission patterns vary with the nature of the mutation. Joana Bengoa discussed another important factor that may influence on the chosen transfer threshold, namely, the couple's own views about what level of residual risk they can accept. While there are scanty published data, most centres agree that high maternal mutant loads decrease the chance of having at least one transferable embryo and some centres may refuse some patients because the maternal mutant load is too high for a trial of PGD. PGD therefore appears to be a good reproductive option for many mtDNA mutation carriers, but cannot be offered to women harbouring homoplasmic or near homoplasmic load of pathogenic variant.

3. Consensus: Clinical pathways for MRT and PGD

3.1. Developing guidelines based on the current clinical indications

We are obliged to reconsider current strategies in the light of changes in legislation and recent scientific advances. The best genetic management of maternally inherited mtDNA disorders will depend on the specific mutation and the family involved.

Grainne Gorman pointed out that individuals undergoing PGD and MRT for mtDNA disease need to be sufficiently fit to undergo superovulation and pregnancy. Dr Gorman has developed a care pathway for mitochondrial reproductive decision-making that includes screening for diabetes and cardiac problems, including both ECHO and ECG. Moreover, patients with symptomatic mitochondrial disease need even more detailed clinical evaluation before embarking on pregnancy. Those whose mitochondrial disease causes additional problems such as epilepsy or end stage renal disease requiring transplantation need particularly careful appraisal. Furthermore, many of the women with mitochondrial disease currently taking advantage of PGD have previously eschewed pregnancy knowing that the risk of transmission of serious mitochondrial disease was high. Consequently, many are now at a stage in their reproductive lives where diminished ovarian reserve is an important consideration – antral follicle count and serum levels of anti-Mullerian hormone are therefore key elements in the PGD assessment, particularly for women aged 35 years and older. For successful IVF the woman should be young, have a low BMI and be a non-smoker. Hence, in the UK, NHS funding is only available for PGD for non-smoking couples, where the woman is under 40 years of age and has a BMI between 19 and 30.

Prenatal diagnosis and oocyte donation are tried and tested options that may well be appropriate and should be considered at an early stage, with particular reference to the sporadic or inherited nature of the mtDNA mutation. While PGD is not appropriate for most homoplasmic patients, embryonic sex selection has been used in the Netherlands and elsewhere, to reduce the risk to carriers of LHON mutations. In this disorder, males are 2–5 times more likely to develop symptoms than females, hence female offspring have a lower risk [55].

PGD is usually performed on day 3 or 4 after fertilisation when the embryo has reached an 8-cell stage. One or two blastomeres are sampled, most centres moving towards sampling just one for m.3243A>G mothers with a low mutant load, because of a very close correlation of mutant load between sister blastomeres. For common mutations there is sufficient data on which to base the threshold for implantation. However, it is always important to consider the family history and the heteroplasmy levels of members known to be clinically affected or unaffected; a task which is often hampered by issues of patient confidentiality. For rare mutations it is even more difficult and patients frequently struggle with the uncertainties. While there is a robust correlation between the mutant load in individual blastomeres and the whole embryo at the cleavage stage, there are fewer data on trophoblast samples. Most centres attempt to estimate the chance of obtaining a transferrable embryo from maternal mutant load, but this can only rarely be based on oocytes and is hence of limited accuracy.

Only one centre is licensed for MRT in Europe (Newcastle upon Tyne). The UK regulator, HFEA, states [56] it can be carried out in families:

> "where (i) there is a particular risk that any egg extracted from the ovaries of a woman named in the determination may have mitochondrial abnormalities caused by mitochondrial DNA; and (ii) there is a significant risk that a person with those abnormalities will have or develop serious mitochondrial disease".

In practical terms, most centres reserve discussions about MRT to patients in whom the other options are unsuitable and who would not contemplate oocyte donation. The HFEA regulations state, "The centre should only offer MST or PNT to patients for whom PGD is inappropriate or likely to be unsuccessful and who exhibit (or are predicted to exhibit) high levels of germ line heteroplasmy or homoplasmy". These include patients who are homoplasmic for mtDNA mutants of proven pathogenicity, such as m.4300A>G, m.9185T>C and LHON mutations such as m.11778G>A for whom PGD is largely inappropriate because selection of low-risk embryos is impossible when all embryos will harbour 100% mutation load. In practice, couples for whom PGD fails because of insufficient embryos with an acceptable mutant load may generate sufficient zygotes for MRT from which the nucleus can be removed for transfer.

3.2. Establish counselling guidelines for the new techniques

Counselling should be tailored closely to the family's needs, depending on their molecular diagnosis and health. The regulations clearly state that to be licensed the centre must have a multidisciplinary team with expertise in mitochondrial disease and assisted reproductive technologies including experienced medical, nursing and laboratory personnel. Informed consent to these procedures must be ensured, emphasizing that clinical experience of outcomes is limited. Counselling of patients therefore needs to include "information about the process, procedures and possible risks involved in mitochondrial donation, including the risks for any child that may be born following the mitochondrial donation, and the risks of IVF treatment" to promote informed choice and informed decisions whilst respecting patients own beliefs and values. Prioritizing patient autonomy through patient-centred care and shared and informed decision-making is essential for all assisted reproductive technologies.

Psychological aspects need to be considered and support offered if necessary. The HFEA required that the centre also needs to offer "information about prenatal testing following treatment (in these circumstances, the patient should be counselled about the specific additional risks associated with prenatal testing) [56]. Patients may feel empowered by genetic counselling, which will encourage autonomous decision-making. In addition, it is important that the oocyte donors are counselled to enable them to understand the purpose and implications of their donation. Follow-up counselling and support is needed and confidentiality must be maintained.

In addition, the HFEA regulations [56] state that patients should be given the following information, all which is part of routine genetic counselling for mitochondrial assisted reproduction:

(a) genetic and clinical information about the mitochondrial disease
(b) the possible impact (if known) of the mitochondrial disease on those affected and their families
(c) the importance of telling any resulting children of the assisted reproductive treatment
(d) information about treatment and social support available, and
(e) information from a relevant patient support group or the testimony of people living with the condition, if those seeking treatment have no direct experience of it themselves.

Finally, the consensus opinion of those present at the ENMC meeting was that patients should be informed that MRT does not necessarily eradicate the risk of mitochondrial DNA disease to future generations.

4. Conclusion

This consensus statement documents internationally agreed guidelines on referral and counselling of couples seeking advice on assisted reproductive options for mtDNA disease. The guidelines were prompted by the availability of mitochondrial donation therapy, a first in Man technology that holds the potential to prevent transmission of mtDNA disease. As we remain in the early stages of regulated introduction of this particular technique it also seemed pertinent to review the criteria, referral pathway and success of Preimplantation Genetic Diagnoses for mitochondrial disease. In producing these guidelines we acknowledge that revision of their content may be required periodically as centres gain experience in both the care pathway and outcomes of treatment, particular for Mitochondrial Replacement Therapy.

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