Mexico and Mitochondrial Replacement Techniques: What a Mess

Abstract

Background

The first live birth following the use of a new reproductive technique, Maternal Spindle Transfer (MST), which is a Mitochondrial Replacement Technique (MRT), was accomplished by dividing the execution of the MST procedure between two countries, the US and Mexico. This was done in order to avoid US legal restrictions on this technique.

Sources of data

Academic articles, news articles, documents obtained through freedom of information requests, laws, regulations, and national reports.

Areas of agreement

MRTs are new reproductive techniques that present novel ethical and legal challenges, since genetic material from three people is employed to create a child.

Areas of controversy

Could the first MST procedure that culminated in a live birth negatively impact reproductive medicine in Mexico?

Growing points

The US and Mexico need specific and clear legislation on MRTs, in order for such techniques not to be governed by prior existing legislation on assisted reproduction that is inadequate for dealing with the new challenges that these techniques present.

Areas timely for developing research

There is a pressing need for work to be done on the international governance of new reproductive techniques.

Keywords: Mitochondrial Replacement Techniques, Mitochondrial Replacement Therapy, Mitochondrial Donation, Mitochondrial DNA diseases, Mitochondria, three person IVF, Nuclear Genome Transfer

Introduction

On the 6th of April 2016 a boy was born in the US after a Mitochondrial Replacement Technique (MRT).[1] When the birth was reported, five months afterwards, the news left the scientific world astonished for two reasons.[2] First, this case was proof of concept that a live birth in humans could follow after an MRT, in this specific case Maternal Spindle Transfer (MST). Second, the scientists behind this case divided the execution of the whole procedure between two separate countries, the US and Mexico, in order to avoid US legal restrictions on MRTs. This review concentrates on the second point, and more specifically, on the

regulation of MRTs in Mexico and on how the first live birth following an MRT could adversely affect reproductive medicine in that country.

The review is divided in four sections. First, I put forward a basic description of mitochondria and mitochondrial diseases. Second, I present, in a schematic way, four MRTs that could allow women whose oocytes contain deleterious mutations of the mitochondrial DNA (mtDNA) to have healthy genetically related children. Third, I analyse how the first live birth following an MRT was presented in the media, and how this news could impact reproductive medicine in Mexico. Finally, I discuss why the way in which this MRT procedure was carried out should not be a blueprint for any further advances within reproductive medicine.

Mitochondria and Mitochondrial Diseases

Mitochondria are cellular organelles inherited via the maternal line. Their most important function known to date is to produce the energy, in the form of adenosine triphosphate, that the human body (i.e. cells, tissues and organs) needs to operate. Contrary to other organelles, mitochondria possess their own DNA: mtDNA. Thus, in all human cells (except erythrocytes, which do not possess DNA) there is nuclear DNA (nDNA) and mtDNA. Whereas nDNA constitutes, roughly, 99.9% of a cell's DNA, mtDNA comprises the other .1%.[3] There are many mitochondria in each nucleated cell; in the oocyte, more specifically, there are around 200,000 to 300,000 mitochondria.[4] And furthermore, there is not just one human mtDNA haplogroup; to this date 30 different mtDNA haplogroups have been discovered.[5] Finally, mitochondrial function does not solely depend on the expression of mtDNA, but rather it depends on the expression of genes both in the nDNA and the mtDNA. "There are more than 1500 mitochondrial proteins, most of which are nuclear-encoded, with only 13 encoded by mitochondrial DNA (mtDNA)."[6]

Mitochondrial diseases are a diverse group of diseases. They impact energy metabolism, and can be caused by mutations in the nDNA or the mtDNA. In this paper I will only focus on mitochondrial diseases caused by deleterious mutations of the mtDNA: mtDNA diseases. The severity of mtDNA diseases can range from mild to fatal, and their onset can occur during childhood or later in life. In fact, mtDNA mutations can cause miscarriage and stillbirth. To which degree human organs are affected by deleterious mutations of the mtDNA depends on their energetic needs. Organs with high energy requirements are those that are the most sensitive to such mutations of the mtDNA (e.g. the brain).

Because mitochondria are transmitted via the maternal line "disease-causing alleles carried by a woman can be passed on to all her offspring with no ability for the father's (likely) wild-type alleles to compensate".[7] Dementia, deafness, stroke, blindness, Leber's hereditary optic neuropathy, and Leigh's syndrome (among other conditions and syndromes) all can occur because of deleterious mutations of the mtDNA. There are many mitochondria in each nucleated cell and mutations in the mtDNA can happen across all mitochondrial genomes,

known as homoplasmy, or they can occur in some mitochondrial genomes, known as heteroplasmy. In cases of heteroplasmy, the number of mitochondria with deleteriously mutated DNA can vary across cells and tissues. This happens because during the cell division process mitochondrial segregation occurs by means of the bottleneck effect.[8] Finally, it must be noted that at the present time there is no cure for mtDNA diseases; existing treatments aim at easing the gravity of the recurring symptoms.

Women who know that their oocytes have a significant amount of deleteriously mutated mitochondria have different reproductive options.[9] They can choose not to reproduce. They can choose to have a child by means of sexual intercourse, and risk having a child with a mtDNA disease. These women have other reproductive, and family making, options if they want to have a child *without* a mtDNA disease, depending on: what kind of parental link they want to share with their children, if they have access to specialized medicine, and the laws concerning assisted reproduction to which they are subject. These women can: adopt, adopt an embryo, or resort to a donated egg. These three options entail that *they would not be genetically* related to their children. In addition to the former, they can also seek to have a child with whom they share a genetic link but *without* a mtDNA disease. Those couples who already have a child with a mtDNA disease can resort to oocyte sampling, in order to assess the possible risk of recurrence. They can also, depending on the type of mtDNA mutation and its prevalence across the mitochondria, undergo chorionic villus sampling or amniocentesis, and afterwards choose for or against termination. Or they can opt for preimplantation genetic diagnosis (PGD) and then select to transfer only those embryos with a non-existent or very minor mtDNA mutated load (this option is not available for women with homoplasmic deleterious mutations). It must be noted that the prediction of a future mtDNA disease by means of PGD depends on the availability of data on the mtDNA mutation in question.[10] In conclusion, the use of the former techniques would guarantee that there is a genetic link between mother and child, but because of the nature of mtDNA diseases they cannot always be employed when the main aim is to have a child *without* a mtDNA disease.

Mitochondrial Replacement Techniques

Four techniques have recently been developed which can aid women whose oocytes contain deleterious mtDNA mutations to have genetically related children without a mtDNA disease. The successful use of these techniques (i.e. the creation of a child without a mtDNA disease) does not require specific knowledge about the type of mtDNA mutation, nor its prevalence across all mitochondria. These techniques have been collectively called Mitochondrial Replacement Techniques (MRTs), although this terminology has been contested.[11, 12] They are: maternal spindle transfer (MST), pronuclear transfer (PNT), first polar body transfer (PB1T) and second polar body transfer (PB2T). All these techniques work by rehousing the nuclear material of the intending mother (or intending couple) into an enucleated cell with healthy mitochondria. The following description of MRTs is schematic in nature; for a detailed

account see Greenfield et al.'s *Assisted reproductive technologies to prevent human mitochondrial disease transmission*.[13]

In MST, assisted reproductive techniques (ARTs) are used to obtain eggs from a healthy donor and the intending mother (i.e. the woman whose oocytes contain deleteriously mutated mtDNA). The nuclear material of both eggs (at the metaphase II stage) is extracted. The intending mother's enucleated cell is discarded, along with the donor's nuclear material. Afterwards, the intending mother's nuclear material is transferred to the donor's enucleated oocyte. The new oocyte, which possesses healthy mitochondria and the nuclear material from the intending mother, can now be fertilized in vitro and then transferred.[14]

In PNT, ARTs are employed to create two zygotes: one with the intending mother's egg and another one with a donated egg. The sperm can be provided by the intending father or a donor. After fertilization, but prior to the breakdown of the pronuclear membranes, the maternal and paternal pronuclei of both zygotes are extracted. The pronuclei of the zygote produced with the donor's egg are discarded, just as the enucleated zygote produced with the intending mother's egg. Afterwards, the pronculei that were contained in the zygote produced with the intending mother's egg are transferred into the enucleated zygote produced with the donated egg. Finally, the new zygote is transferred to the intending mother or a surrogate.[15]

Polar body transfer techniques are the newest MRTs. The developing oocyte, contrary to most human cells which contain two sets of 23 chromosomes, has already duplicated its chromosomes so it possesses four sets of 23 chromosomes. During the oocyte development process these four sets will separate, and half of them will end in a small daughter cell: the first polar body. When normal fertilization process takes place this first polar body will not be part of any resulting embryo. Now, the second polar body is produced during the fertilization process. The two remaining sets of maternally inherited chromosomes again divide in half. One set will become the maternal nuclear DNA, remaining within the just created zygote, whereas the other set will be stored within a small daughter cell: the second polar body.[16]

PB1T is similar to MST. In PB1T, ARTs are used to obtain eggs from a healthy donor and the intending mother. The nuclear material from the donated egg is extracted, and the first polar body from the intending mother's egg is extracted too. The intending mother's egg is discarded, just as the donor's nuclear material. Then, the first polar body is transferred to the donor's enucleated oocyte. The new oocyte, which possesses healthy mitochondria and the nuclear material from the intending mother (which came from the first polar body) can now be fertilized in vitro and then transferred.[16]

PB2T is similar to PNT. In PB2T, ARTs are employed to create two zygotes, one with the intending mother's egg and another one with a donated egg. After fertilization, the second polar body is removed from the zygote created with the intending mother's egg. The *maternal* pronucleus and the second polar body of the zygote produced with the *donor's egg* are also removed, thus only the paternal pronucleus remains within the cell. The maternal pronucleus and the second polar body of the cell produced with the donor's egg, and the zygote produced with the intending mother's egg (now without the second polar body) are discarded. Afterwards, the second polar body is transferred into the zygote produced with the donor's egg, which at this point only contains the paternal nuclear material. The new zygote is transferred to the intending mother or a surrogate.[16] It is worth mentioning that PB1T can be performed in conjunction with MST, and that PB2T can be performed in conjunction with PNT. This is possible since an oocyte will contain a maternal spindle and a PB1, and a zygote will contain a maternal pronucleus and a PB2, see [Fig. 1].

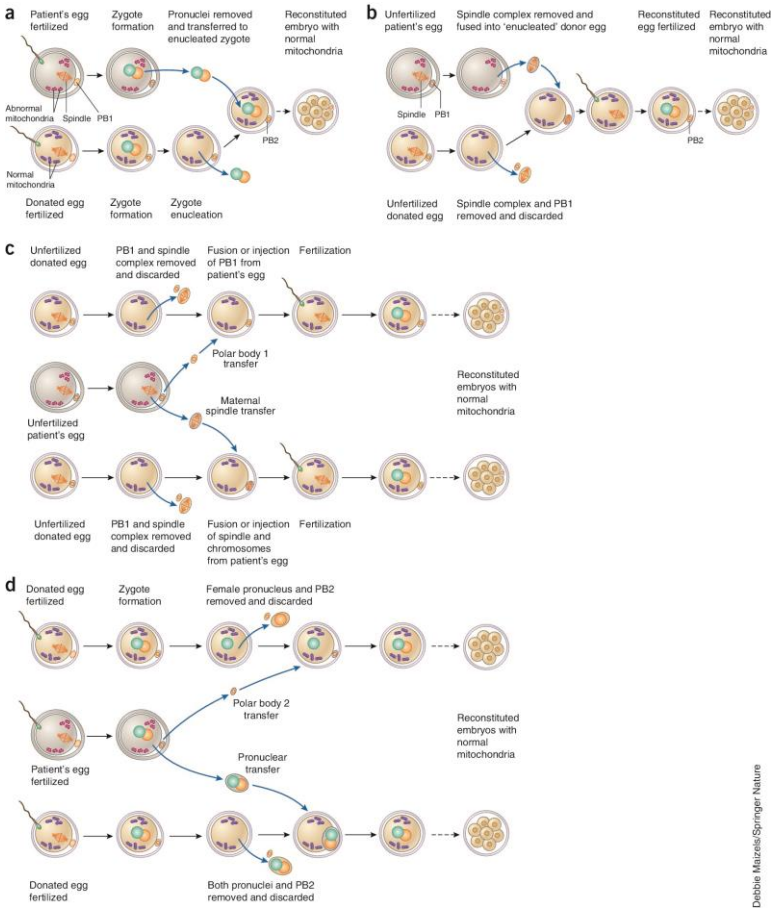

Figure 1: Protocols for mitochondrial replacement therapy. (a) Schematic of PNT. (b) Schematic of MST. (c) PB1T and MST. A combination of both techniques can be used to create two reconstituted donated oocytes from one oocyte carrying mutated mitochondria. (d) PB2T and PNT. As in c, the genetic material from one patient oocyte could be used in two donated oocytes to create two reconstituted embryos with normal mitochondria. (Reprinted by permission from Springer Nature Terms and Conditions for RightsLink Permissions Springer Customer Service Centre GmbH: Springer Nature, NATURE BIOTECHNOLOGY, Assisted reproductive technologies to prevent human mitochondrial disease transmission, 2017.)

One of the reasons why scientists have looked into the latter two techniques (PB1T and PB2T) is because the polar bodies contain very little cytoplasm and thus the possibility of mutated mitochondrial carryover appears to diminish greatly.[17] This is important since if there were to be a large carryover of mutated mitochondria during any of the MRT processes a mtDNA diseases could manifest.[18] In a recent experiment, mitochondrial reversion was observed in human embryonic stem cell lines; in it the carried over deleteriously mutated mitochondria expanded preferentially during cell division.[19]

Finally, it must be clear that any offspring product of an MRT would possess DNA from three different persons – nDNA from two persons and mtDNA from a third person – and that the third party mitochondria will be passed down to future generations via the maternal line. The former has sparked a lively philosophical debate on whether such children indeed have three biological/genetic parents or not.[20–22]

A Baby is Born

On the 27th of September 2016 Jessica Hamzelou, writing for New Scientist, broke the news about the first live birth following an MRT, in this case MST. Let us remember that the baby was born on the 6th of April 2016. In her article Hamzelou did not mention where the baby was born, but stated that he was born to a Jordanian couple and that the treatment had happened in Mexico: "the birth of the child, whose Jordanian parents were treated by a US-based team in Mexico" and "[n]either method [MST and PNT] has been approved in the US, so Zhang went to Mexico instead".[2] Up to that point the Jordanian couple had lost two children to Leigh syndrome, and the woman had had multiple miscarriages.

The news impacted the scientific community because it was the first proof of concept that MRTs could end in a live human birth.[23] They were also shocking in that the scientific community presumed that the first MRT birth, if there was going to be any, would occur in the UK. This was a common assumption since work on MRTs, and their potential, had been explored in the UK since the 2000s.[24] And because by February 2015 the House of Lords and the House of Commons had voted in favour of passing the *Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015*, which came into force the 29th October 2015.[25]

In order to lawfully perform an MRT in the UK: a) a clinic requires that its licence be amended in order for MRTs to be carried out there, by the Human Fertilisation and Embryology Authority's (HFEA) license committee, and b) the individual in question requires approval by the HFEA's statutory approvals committee.[26] Once these two hurdles have been surpassed an MRT procedure can take place. Newcastle Fertility Centre was the first centre whose licence was amended in order for it to carry out MRTs; and in February 2018 two women were granted approval to undergo an MRT procedure there.[27] It must be noted that in the UK

only MST and PNT can be legally employed, and that they can only be used to prevent the transmission of serious mtDNA diseases, their use for treating infertility is not allowed.

Returning to our case, the lead scientist behind the US/Mexico international MRT case was US based Dr. John Zhang. Dr Zhang is a fertility expert, who is also the Founder/CEO of the private fertility clinic New Hope Fertility Center, in New York City. Before aiding the Jordanian couple Zhang had already carried out an MRT procedure in 2003, in China.[28] On that occasion he employed PNT to aid a woman with a non-mtDNA disease infertility problem (she had had two failed IVFs after embryo arrest) and a triple pregnancy ensued. No live delivery from that pregnancy followed.

When Hamzelou broke the news about the live delivery there had not been any press release on behalf of the family or scientists, nor any scientific paper published on this case. In fact, the timing of publication of the New Scientist article suggests that Hamzelou got her scoop from the program of the 2016 American Society for Reproductive Medicine (ASRM) Scientific Congress & Expo, which was available online since the 31st of August 2016.[29] The former is relevant because up to the publication of Dr. Zhang et al.'s paper - and an accompanying critical editorial - on the 3rd of April 2017 all the information known about this case was limited to that which appeared in: a) the ASRM abstract; b) the media interviews that Dr. Zhang and the Mexican physician involved in this case, Dr. Alejandro Chavez-Badiola, gave; and c) a video recording of Dr. Zhang's presentation at the ASRM congress.[30,31]

At this point we can confidently assert that this novel case in reproductive medicine had significant shortcomings in terms of science transparency and communication. Mainly, that after the news broke it took Zhang's team more than five months to publish the whole account of this case, and by that moment harm had already been done to the political stance of reproductive medicine in Mexico.[32,33] I will expand on this point next. While it is true that publishing in scientific journals takes months, and sometimes years, it is also true that the baby was already five months old when the news broke; and that up to that moment Zhang and his team had more than enough time to produce a press release - one that clarified what was done and where. Doing the former, after the news broke, would have aided in cooling down the international political animus in terms of the governance of new reproductive techniques.

Mexico and MRTs

In the New Scientist article Dr. Zhang was quoted as saying that he and his team went to the neighbouring country because in Mexico "there are no rules".[2] This quote was very controversial, and it ended up being repeated in the press over and over again. However, a quote such as this is not very informative because it does not tell us what there are no rules about. If we accept its most charitable interpretation then we can presume that Zhang's

comment was aimed at the regulatory landscape of MRTs in Mexico. In addition to the supposed lack of applicable legislation on MRTs, a foreign destination seemed more than convenient for carrying out this procedure, given that in the US MRTs are *de facto* banned.[34] In the US it is impossible to legally carry out MRTs, because the *Consolidated Appropriation Act* of 2016, approved by Congress, prohibits the Food and Drug Administration from even reviewing applications to carry out research "in which a human embryo is intentionally created or modified to include a heritable genetic modification."[35] In recent work Eli Adashi and Glenn Cohen have argued that this moratorium, that was in fact aimed at nuclear germline modifications, should be revised and MRTs allowed.[36] It should be remembered that the US Institute of Medicine concluded, in their report on MRTs (that was released on the 3rd of February 2016), that it was ethically permissible to move ahead with the first-in-human clinical trials of MRTs, but on the condition that only male embryos were transferred.[37] This condition, which is not part of the UK regulations, was attached so as to prevent third party mitochondria being passed to further generations.

The fact that this MRT procedure *supposedly* happened in Mexico took the Mexican scientific community by surprise, and the timing of the news, in terms of the regulatory landscape of reproductive medicine, could not have been worse. Just six days before the news broke – on the 21st of September 2016 – the Health Commission of the Mexican Chamber of Deputies approved an amendment to the Mexican General Health Law, the highest federal level legislation in matters of health, heavily restricting assisted reproduction and banning all embryo research.[38] Fortunately for Mexican scientists, physicians, and fertility patients, the bill was stopped before it was voted on the floor. This was very good news, since the reproductive rights of such fertility patients, for example, were not affected by ill-thought legislation. Stopping this legislation was achieved after substantive activism from academics, scientists, and human rights activists. Although at this point the bill is 'frozen' it can still be submitted for a vote any day. Now, even though the bill preceded the MRT news, it seems that any further attempt to pass it into law will include some provision banning MRTs. This seems the case given that, when asked about MRTs, the federal deputy (from the conservative political party National Action Party) who proposed the new amendments to the General Health Law asserted that they will seek to "prohibit pronuclear transfer techniques that allow for three parent embryos, since many abuses can happen at the moment of carrying out these experiments".[39]

Now, starting from the assumption that the MRT procedure happened in Mexico, Palacios-González and Medina-Arellano examined the legal standing of MRTs in such country.[40] They reached three conclusions. First, in nine states - out of 32 - PNT is prohibited beacuse state laws protect human life from the moment of fertilisation. And in the State of Mexico City (formerly the Federal District) PNT is prohibited if the would-be-enucleated embryo is first created for a non-reproductive purpose. If the embryo is first created for a reproductive purpose and then it is used for an MRT then that would not violate such state law.

Second, even though in Mexico there are no federal laws concerning assisted reproduction, there are regulations that do apply to MRTs: the Regulations of the General Health Law on Health Research. According to Palacios-González and Medina-Arellano's interpretation of the law, and what was publicly known at that time, they concluded that Zhang's team violated Article 56 of aforementioned regulations. They reached this conclusion because Article 56 establishes that research on assisted fertilization: "will only be admissible when it is applied to solve *sterility problems that cannot be solved otherwise* [emphasis added], respecting the couple's moral, cultural, and social point of view, even if these differ from those of the researcher".[41] And in this case Zhang's team helped a woman who according to Mexican legislation is not sterile, and thus do not have sterility problems, to have a genetically related child. Tetsuya Ishii[42] has argued that this woman in fact suffered from sterility problems, however Palacios-González and Medina-Arellano[43] have defended that sterility under Mexican legislation should be understood following the WHO's definition of primary infertility:

> When a woman is unable to ever bear a child, either due to the inability to become pregnant or the inability to carry a pregnancy to a live birth she would be classified as having primary infertility. Thus women whose pregnancy spontaneously miscarries, or whose pregnancy results in a still born child, without ever having had a live birth would present with primarily infertility.[44]

It is important to emphasize that with the publication of Zhang's paper describing this case, and the accompanying critical editorial, it became known that the MRT process *did not* happen in Mexico, but rather that it happened in the US.[33,43] In Mexico only the embryo transfer procedure took place. This means that they imported a modified embryo across the border.[45] It is also important to note that Dr. Alejandro Chavez-Badiola, the Medical Director of New Hope Fertility Center Mexico, which is part of Zhang's fertility clinics, did not clarify this point. He did not do so even when in TV interviews he was explicitly asked about the role of Mexico in the development of MRT technology and how this live birth was accomplished.[30]

The third, and final, conclusion is that MRT research can still *legally* happen in Mexico, both for avoiding mtDNA diseases, and for helping non-mtDNA disease related infertile women or couples. In order for this to happen legally scientists must follow: the laws and regulations governing human medical experimentation; article 56 of the Regulations of the General Health Law on Health Research; and, when appropriate, state laws that protect life from the moment of fertilization.

We now have a clear timeline regarding how this MRT case developed, how it could affect *future* legislation on assisted reproduction in Mexico, and how MRTs can be legally carried

out in Mexico. We must now investigate whether Mexican authorities had any role in this case. Through freedom of information requests we asked specific Mexican authorities if Zhang's team had approached them to seek advice prior to carrying out the MRT-embryo transfer. According to the information we received, through such requests, the following institutions were not consulted, or aware, of the MRT procedure: the National Council on Bioethics; the Commission for the Protection Against Sanitary Risks of the State of Jalisco, the National Centre for Gender Equality and Reproductive Health; the Secretary of Health of the State of Jalisco; and the Secretary of Health. The Federal Commission for the Protection Against Sanitary Risks, on the other hand, told us that they in fact were consulted on the legality of this case, but they did not reveal what was their stance on its legality. It is important to point out that the fact the Federal Commission for the Protection Against Sanitary Risks was consulted on this matter makes little sense from a legal standpoint. This is because the Commission's remit in regards to reproductive clinics is just to certify the working spaces, and not the procedures that take place in them. Furthermore, from a regulatory standpoint the Commission would not be able to comment on the legality of such procedure without breaching the regulations that govern it, since legal advisory functions on matters of reproductive medicine fall outside the Commission's regulatory remit.[46]

In terms of Mexican legislation, Zhang's team was not required to seek advice from the Secretary of Health on this matter. However the fact that they did not do so shows a disregard for the socio-political situation of reproductive medicine in Mexico. Mexico is a country where Catholicism is still the dominant religion and where the vast majority of society is conservative in terms of reproductive medicine, and thus it should have been obvious that this MRT case would cause a great stir. It did so to the point that it dominated the Mexican news cycle immediately after the news broke and for the following week. Seeking advice from the Secretary of Health, either at a state level or a federal one, and then communicating explicitly about this would have mitigated any claim that Mexico is a lawless country, and that Mexico and other nations are in need of an urgent *reactive* prohibitory legislation on MRTs and other new reproductive technologies.[47] Eli Adashi and Glenn Cohen have asserted that regulatory legislation should strive to be "timely in its enactment, transparent in its prosecution, evidence-grounded in its rationale, deliberative in its process, civic-minded in its outreach, representative in its inclusiveness, and measured in its scope".[36] Predictably, a novel use of a reproductive technology, like Zhang's, short-circuits this process and instead helps those groups that want to restrict reproductive medicine in general.

Conclusion

There were three main problems with Zhang's MRT procedure, at least within the context of reproductive medicine in Mexico. First, they did not keep the adequate Mexican authorities, at the state and federal level, informed about the procedure, and its relevance within the international reproductive scientific landscape. Second, there was very poor science

communication about this case. There was no press release on behalf of the scientists involved in this procedure, at least not one in Spanish. As a result, misinformation spread about this MRT case, in which Mexico was presented as a lawless place. Third, there was an utter disregard for the medico-political landscape of reproductive medicine in Mexico. In the end it is not only that this case could lead to the banning of MRTs in Mexico, but that it could help in advancing a federal restrictive legislation that would affect most patients in need of reproductive medicine, violating their reproductive rights. Importantly, recall here that not all patients can travel abroad to receive reproductive care. In conclusion, Dr. Zhang's actions should be considered as the antithesis of a blueprint for carrying out a new reproductive technique.

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