New frontiers in human assisted reproduction - from research to clinical practice: Several considerations (Review)

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Received September 22, 2015; Accepted August 22, 2016

DOI: 10.3892/mmr.2016.5749

Abstract. In the era of very late, or advanced, motherhood, in which 'egg banks', 'social' egg-freezing, egg donation and surrogacy represent a potential solution to a number of obstacles to human reproduction, what is the role of scientists and clinicians involved in assisted reproduction? In light of the apprehension that, in the future, through fertility treatment infertility may be passed on to the offspring, boundaries of medical vs. 'social' infertility are being created. Scientists and clinicians are joining forces in a synergistic effort to improve the effectiveness of infertility care by introducing novel therapeutic protocols with the intent of customising care and improving cost-effectiveness, testing novel drugs and formulations, and searching for novel markers (for estimating biological age) and nomograms (to optimise the yield of a controlled ovarian hyperstimulation cycle). On the other hand, political, social and health institutions are doing little to educate young women with respect to disinformation and to increase their awareness regarding age as the predominant factor that contributes towards the decline in fertility. Nevertheless, despite the great advances that have been made, 38 years after the birth of the first baby via *in vitro* fertilisation, the intricate road leading from the antral follicle to the fully developed baby continues to be designated as being too 'expensive', 'empirical', 'mysterious' or 'bound by ethics', with few significant improvements in terms of real cost-effectiveness.

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Abbreviations: ARTs, assisted reproductive technologies; COS, controlled ovarian hyperstimulation; HPV, human papilloma virus; IVF, *in vitro* fertilisation; PGD, pre-implantation genetic diagnosis; PGS, pre-implantation genetic screening; TLM, time-lapse monitoring

Key words: assisted reproduction, biotechnology, cost-effectiveness, counselling, ethics, genetic screening, health care policy, pre-implantation genetic screening, metabolomics, morphokinetic, embryos

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1. Introduction

In an era of advanced, or very late, motherhood, strategies including 'social' egg-freezing, surrogacy and commercialisation of fertility treatments represent a potential solution to overcoming obstacles to human reproduction, while simultaneously turning infertile couples into potential consumers. Leaving aside the potential ethical and obstetrical problems associated with an advanced maternal age [as consequences of *in vitro* fertilisation (IVF) and, in particular, of heterologous fertilisation], which certainly warrant further investigation (1-4), if assisted reproduction technologies may be considered as a 'pendulum swinging' between the health care service and industry in offering solutions against time, what then are the roles to be adopted by scientists and clinicians involved in human reproductive care? What are their responsibilities, and what effects will developments in the future have?

Caution must always be exercised in the interpretation of evidence generated by investigations on infertility care to avoid the generation of 'speculative' fears, as, for example, in the case of the hypothetical 'transmission of infertility to the offspring' of fetuses grown in infertile couples who underwent IVF treatments (5). Nevertheless, in developed countries, the increasing prevalence of infertility warrants a further investigation of the mechanisms that are involved, and rigorous boundaries between medical vs. social infertility, which are gradually being created, will be of vital importance in the near future. Scientists and clinicians are joining forces in a synergistic effort to improve the effectiveness of infertility care by introducing novel therapeutic protocols for the purpose of customising care and improving cost-effectiveness, for example, by adopting

different formulations of gonadotropins according to different cohorts of patients and the IVF setting (6,7), testing novel drugs (for example, introducing recombinant luteinising hormone) and formulations (e.g. long-acting gonadotropins), and searching for novel markers for estimating biological age (8,9), and novel nomograms to optimise the yield of a controlled ovarian hyper-stimulation cycle. On the other hand, political, social and health institutions are doing little to educate young women with respect to disinformation and to increase their awareness regarding age as the predominant factor that contributes towards the decline in fertility.

2. Critical overview of the state of the art

In developed countries, the number of couples referred for assisted reproduction due to age-associated infertility is growing exponentially due to career priorities, financial concerns and the increasing trend in postponing marriage and childbearing (10).

It remains to be demonstrated whether increased awareness of the interplay between age and fertility should lead to a lesser degree of postponement of motherhood, since the question of a woman's 'biological clock' is, indeed, an important socio-political issue that is influenced by several broader factors, among which are the rise in higher education among women, the demands of the labor market, technological developments and financial interests stemming from the 'reproduction industry'. However, even though the immense size of the problem could erroneously induce a sense of powerlessness in terms of finding a solution, it would appear to be mandatory to encourage educational efforts by schools, the health care system and the media to eradicate the fertility 'myth' (i.e. the mistaken belief that, in the field of reproduction, modern medicine is already able to overcome the barriers imposed by biological age), particularly among young people, as subsequently acting on the misinformation may result in a reliance on costly, unpleasant techniques with potentially disappointing outcomes. In so doing, access to improved biological/medical information may indeed raise the awareness of the decline in age-associated fertility (11).

Nonetheless, despite the great advances that have been made, 38 years after the birth of the first IVF baby (Louise Brown on July 25, 1978), the intricate road leading from the antral follicle to the fully developed baby continues to be designated as being too 'expensive', 'empirical', 'mysterious' or 'bound by ethics', with few significant improvements in terms of real cost-effectiveness.

Regarding costs, rational intuition should lead us to reflect upon and act on the variable costs (predominantly due to drugs and stimulation protocols) rather than on fixed costs (attributable to clinicians, the laboratory and research). It is a concern that variable costs are frequently unduly influenced by a consideration of the time involved, or the 'inappropriateness' of the stimulation protocols.

In addition to the efforts made by scientists to increase the number and level of sophistication of treatment options so as to improve the personalisation of care and overall cost-effectiveness, clinicians should promote both primary (i.e. counselling regarding age-associated infertility) and secondary prevention (for the early diagnosis of disease potentially able to compromise and/or delay the search for a first pregnancy, including common female/male endocrine issues, immunological infective disorder or female specific fertility compromising disease like endometriosis, polycystic ovarian syndrome, uterine/tubal organic pathologies), improving the estimation of pretreatment prognoses in order to choose the most appropriate controlled ovarian stimulation (COS) protocols. Expanding research programs will be able, in the near future, to resolve a large proportion of the confusion regarding the empirical prescription of drugs with orphan designations in IVF, whose positive (or maybe, negative) effects have yet to be confirmed from either the clinical and/or the molecular point of view (12-15). Certainly, the empirical approach will never be eradicated from clinical practice, at least, not prior to the clarification of certain myths, mysteries and misconceptions (16,17). It remains our conviction that scientists, with their novel discoveries, should be called upon first of all to solve the problems associated with empirical treatments. However, research requires a level of economic investment that is rarely made in low-cost-treatment infertility centres, which therefore prolongs the application of the empirical approach in the clinic, and the negative consequences that may ensue from this.

Selecting one example as a suitable allegory, the sheer quantity of speculation regarding luteal phase support and endometrial receptivity following cycles of IVF (both fresh and thawed) (18), and the molecular interactions that are formed between the blastocyst and the endometrium following embryo transfer, may be compared to our understanding of Egyptian hieroglyphs prior to Jean-François Champollion's studies on the Rosetta Stone. While the sheer availability of resources would prevent the publication of a voluminous scientific literature on the topic, further experimental research is required prior to beginning clinical trials. Furthermore, in approaching experimental questions on human gametes and embryos from the perspective of performing the basic research, scientists may be confronted by the 'gatekeepers' or 'custodians' of ethics, who place huge obstacles in their path, as is the case, for example, with research on genetic pre-implantation in numerous countries (19).

These limitations may be partly considered as the driving forces behind the provision of anachronistic misinformation and general ignorance regarding topics of concern, including human papilloma virus (HPV) infection and idiopathic infertility (20,21), fertility preservation in patients suffering from malignancy (22,23) and the applicability of gonadal stem cells in infertility (24), issues which may potentially represent the future frontier of human reproductive medicine. Nevertheless, even if, in certain situations, ethics may be a barrier, modern scientists and those of the future will be dutifully obligated to navigate a way through, rather than avoid, these issues. Despite the fact that the solution to this dilemma may be less simple than it appears, it may be asserted that, in the era of '-omics', it is not an arduous task to circumvent the obstacles. Since the inception of IVF, selection of the most competent embryos for transfer has been a primary focus of investigation. As the field has progressed, an increasing number of studies have concentrated on developing more advanced technologies, invasive [such as pre-implantation genetic diagnosis (PGD) and pre-implantation genetic screening (PGS)] and non-invasive [such as time-lapse monitoring (TLM) and metabolomics/proteomic approaches], to select the most

competent embryos with the highest potential of implantation for transfer (25,26).

Political scientists, doctors and bioethics experts have discussed the need for governments to improve the regulation of research and the clinical use of ARTs (assisted reproductive technologies). Certain experts and stakeholders argue for the adoption of novel legislation that will allow scientists to achieve the potential benefits of reproductive technologies that may be applicable to human health. Conversely, others see legislation as necessary to prevent scientific exploration into ethically unacceptable areas. Others again, of course, question the need for any government involvement in this regard (27).

A clear example of the existing confusion may be seen in the application of PGD, a technique currently performed at the blastocyst stage as PGS that was originally developed as an alternative approach to prenatal diagnosis for couples who present a high risk of transmitting a genetic defect, and which has been subsequently confirmed as a useful technology for improving the cost-effectiveness of IVF (28).

Concerning PGD, complex issues associated with ethics and equitable access to embryonic genetic testing have become even more complicated and controversial in legislative debates. Although several initiatives have attempted to harmonise legislation across Europe and intercontinentally, a diversity of health care systems and the presence of cultural differences have hampered attempts to achieve this goal (28).

Thus, in each country, PGD is used with a specific approach that should reflect the views held by scientific groups, professional societies, legislators and society itself on the appropriate use of this technology. Although several countries have banned the use of PGD, others are discussing novel applications and regulatory strategies of this technology in order to maintain the method's reliability by defining standards and responsibilities for professionals performing PGD, and by protecting the rights of those involved. In other countries, there are no direct regulations for the technique, and, instead, professional guidelines for practicing service providers are consulted (29).

Those involved in ARTs have tried to solve the existing disparities between the potential capability of technologies and their clinical applicability by drawing from past experience in the use of non-invasive technologies. As a result, TLM has been implemented in numerous clinics worldwide. The proposed benefits of the method when compared with culture in a standard incubator and fixed time-point evaluation include uninterrupted culture, a flexible workflow in the laboratory and improved embryo selection. The latter is based on the reasonable assumption that more frequent observations will provide substantially more information on the association of development, timing and embryo viability. Although several retrospective studies (30,31) have confirmed an association between TLM parameters and embryo viability evaluated by developmental competence, aneuploidy and clinical pregnancy, TLM should prove to be useful for laboratories specialising in IVF in prospective randomised studies. However, given the relatively high expenses associated with acquiring an instrument and the ancillary products, the verification of the cost-effectiveness of TLM will be a matter of future developments, competition and the judgment of the individual clinics (32).

It is quite likely that, in the near future, TLM may generate promising results if it is associated with PGS, a technology that offers the advantage of biopsy of the blastocyst trophectoderm on day 5 or 6.

The introduction of PGS opens up the era of 'delayed' genetic investigations: Firstly, a greater amount of genetic material may be retrieved from biopsies of blastocysts compared with embryos at the cleavage stage (i.e. a greater number of cells facilitates genetic analysis, providing more accurate results, which thereby enables an easier detection of genetic and chromosomal abnormalities). Secondly, biopsy of the trophectoderm on day 5 post-fertilisation involves embryos that have successfully negotiated the initial steps of cell differentiation, and for this reason these embryos have the highest implantation potential. Thirdly, several recent studies (33,34) have also shown that the rate of aneuploidy is markedly lower in blastocysts compared with cleavage-stage embryos, and finally, biopsies performed on cleavage-stage embryos are more likely to be damaging compared with those performed on blastocysts (35).

On the one hand, the progress made in genomic research (whole-genome sequencing) may lead to whole-genome analysis and the potential for the diagnosis of diseases for which the genetic background has not yet been (or is insufficiently well) elucidated; on the other hand, the lack of well-defined and globally accepted guidelines regarding the application of genome-wide diagnostic testing in IVF, in addition to the heterogeneity of the numerous figures involved (clinicians, scientists, jurists, ethicists, patients, organisations and policy makers), may increase the complexity of the issues that are prolonging the debate. However, all is not lost, since in this progressive era of '-omics', the option of acquiring genetic information without direct genome analysis is now a concrete possibility, and this could be applicable in the clinical practice.

Metabolic profiling, or metabolomics, is the analysis of various molecular metabolites within cells and fluids through the use of various forms of spectral and analytical approaches, and it attempts to determine the metabolites associated with physiological and pathological states. It offers a significant advantage over the use of genomic, transcriptomic and proteomic approaches. Smaller variations in gene expression and protein synthesis result in an amplified change in the metabolite profile, known as the 'metabolome', and this information may be used to detect subtle cellular events (36).

Metabolic turnover is crucial for a pre-implantation embryo to grow and result in a successful pregnancy. Accordingly, preliminary studies and pioneering investigations into the nutrients and metabolites present within culture media and biological fluids (blood, urine, follicular fluid and endometrial supernatant) are yielding fascinating results, which are likely to ensure that this candidate technology is a most promising tool for predicting embryo quality without invasiveness (37,38).

An understanding of the early effects of genetic abnormalities through a metabolomics approach may allow us to collect, during the pre-implantation stage and perhaps in the pre-fertilisation period, more accurate information without the invasiveness and the ethical barriers pertaining to current techniques (39-41).

3. Concluding remarks

Ultimately, the key message for readers of the present review, and those with a keen vested interest in this area, would be to

offer encouragement to produce more compelling evidence based on novel discoveries obtained by rigorous investigations in order to improve the overall success of infertility care. Only a correct methodology in approaching this topic may eradicate several myths and misconceptions, solve a large part of the dilemma and propose novel treatments that would be able to really improve the prospects for 'infertility care users' (42-44).

While fully sharing Einstein's thought that 'we cannot solve the problems with the same thinking we used when we created them' (45), it is our considered opinion that often problems do not require a solution to solve them; instead, they require maturity to outgrow them.

Acknowledgements

We would like to thank the whole team of the Assisted Reproduction Unit of the University of Padova.

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