The role of automation within the ivf laboratory

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Summary

"Automation" is a notion already clear in the manufacturing sector and can be defined as "the use of self-regulating machinery, electronic equipment, etc. to make a manufacturing system or process operate at greater speed and with little or no human intervention" or "the use of equipment or devices, esp. computers, in any system or process so as to improve its efficiency or effectiveness".
Automation can provide energy, elaborate complex information and supply precise system control, in order to increase production and get better results. We all already use machines in our everyday life: in travel industry, banking sector, office automation, healthcare services, and in many more situations. Among all, a special mention goes to the medicine field, in which automation is increasingly used, from robotic surgery to clinical biochemistry. Reproductive medicine is not excluded from this on going mechanization, such that we can hypothesize to implement any assisted reproductive unit of the future with integrated automatic devices. Almost all the steps of the routine in vitro fertilization (IVF) laboratory work may be mechanized, and indeed some procedures are already performed automatically in a number of clinics: culture media preparation; sperm analysis and selection; oocyte insemination or microinjection; embryo culture and development monitoring; embryo biopsy; gametes and embryos cryopreservation; witnessing.
We will now discuss briefly about the introduction of the main automated technologies within an IVF laboratory, particularly focusing on their potential advantages and possible drawbacks, in order to give a simple overview of the present and future challenges depicted.

KEY WORDS: automation, computer-aided sperm analysis (casa), microfluidics, robotic icsi, electronic, witnessing.

Sperm analysis, preparation and selection

Computer-Aided Sperm Analysis

Even if there is still a substantial debate about its diagnostic role and prognostic value, traditional semen examination is still the first-line male evaluation in an IVF laboratory. It is one of the most operator-dependant analyses in reproductive medicine and this lack of standardization may account for estimation differences among centres.
One strong attempt to face this problem dates back in the late 1980s, with the introduction of Computer-Aided Sperm Analysis (CASA). Initially born to calculate automatically sperm kinematics, CASA consists in the acquisition of

several digital images of a sperm specimen on which it is possible to perform an automated reconstruction of individual sperm tracks. Thanks to the use of mathematical algorithms, CASA is then able to measure different motility parameters such as curvilinear velocity, straight-line velocity, average path velocity, amplitude of lateral head displacement, linearity, straightness and wobble (1). During the years, as the field developed, many scientists raised the interest in extending its application in sperm concentration measurement and morphology evaluation, so that many modern machines now include modules specifically designed for these purposes. Moreover, some of these available machines can perform functional testing on the spermatozoa, such as cervical mucus penetration, hyperactivation analysis, acrosome reaction test, vitality test, DNA fragmentation assessment (2).

However, several technical and biological factors may limit the computer-assisted analysis ability to provide accurate results, mainly sperm clumping, presence of background debris and artefacts due to collisions or disturbances in cells' trajectory, high viscosity and consequently inherent micro-heterogeneity of the sample, highly pleiomorphic sperm morphology. In the last decade many progresses have been made and diverse new-generation CASA systems can now address many of the original problems. Moreover, recently it has been proposed a new analysis method for sperm movement, as an extension of the 2D CASA measurements, namely high speed Digital Holographic Microscopy (DHM). DHM allows to track sperm head and flagellar movements in 4 dimensions gathering additional information about trajectories, chiralities, rolling and beating patterns (3). Nonetheless, the computer-assisted technology needs to be further improved, studied and definitively validated, in order to provide a reliable tool for routine clinical use superior to conventional procedures.

Novel automatic sperm preparation techniques

The ejaculate is a complex mixture of seminal plasma, germ cells precursors, multiple leukocytes subtypes and a variable amount of debris from which sperm cells must be separated. Currently, sperm preparation and selection is usually performed with multi-step manual mechanical methods such as swim-up or density-gradient centrifugation, with the main purpose to obtain a semen specimen enriched in viable, motile, normomorphic spermatozoa. These systems are often time-consuming and require the intervention of a skilled operator, not to mention the possible sperm sub-lethal damage deriving from inadequate sample handling such as centrifugation, which is considered one of the most iatrogenic origins of oxidative stress (4). Moreover, these methods hardly resemble the complex process of sperm migration and selection that occurs *in vivo*.

In recent years, automatic microfluidics technology seems to offer a promising alternative to standard sperm preparation methods, taking advantage from microscopic pressure-driven flow of fluids used to move sperm down the channels of a miniaturized device. The chip can be designed mimicking the physical and chemical environment of the female reproductive tract in order to obtain a highly throughput and highly selective separation process. The microfluidics approach may be both active and passive, depending on the starting choice of using or not external forces to drive sperm sorting. Whilst passive mechanisms use viscous media to mimic the sperm path *in vivo* and the selection is driven solely by sperm motility, active systems include the use of flow (motile spermatozoa could be separated from non-motile ones on the basis of their capability of exiting actively the streamline onto a separate outlet), chemical gradients (spermatozoa are separated through chemotaxis towards the egg), electrophoresis and dielectrophoresis (spermatozoa are separated from other cells contaminating the ejaculate on the basis of their different size and charge) (5). All these methods allow optimizing sperm recovery, with a seemingly efficient and reliable separation of a sub-population of motile and morphologically normal spermatozoa presenting low level of DNA fragmentation. Notwithstanding, the commercial distribution and the clinical application of the chips have been slow, mostly

due to technical difficulties related to material compatibility and the lack of clear demonstrated reproducibility and standardization.

Oocyte insemination

Oocyte insemination remains the principle technique in an IVF laboratory. It can be performed incubating overnight the retrieved cumulus-corona-oocyte complex (CCOC) with a previously prepared sperm suspension (classic IVF), or microinjecting one single selected spermatozoon directly into the ooplasm, by means of a modified microscope with a micromanipulation tool (ICSI: IntraCytoplasmic Sperm Injection). These manual methods have been unchanged for decades, even if some attempts have been made during the years to develop the inherent technology.

Taking advantage from microfluidics, it is possible to perform an automatic classic IVF by means of a special chip accomplishing egg precise positioning, sperm screening and selection by chemotaxis, fertilization and subsequent embryo culture (6). What is more, this innovating technology may allow oocyte denuding in preparation for an ICSI procedure. The CCOCs should be allocated in a specifically designed device and manipulated through directional fluid flow into narrow channels. The separation of granulosa cells (GCs) from the egg can be accomplished thanks to a combination of appropriate suction force and geometrical channels disposition that retains the oocyte in one position while clustering the GCs at opposite poles (7).

The ICSI procedure is the most challenging technique to mechanize, due to its complexity. In fact, it involves sperm identification and selection, sperm immobilization, sperm aspiration into a micropipette and the injection into the oocyte. As to the first step, it has been proposed a special microfluidics chip able to select a single spermatozoon in an automatic and time-effective manner: the fluid flow drives the sperm cells from a main reservoir into side connected narrower channels in which spermatozoa are single-trapped (8). This method may be combined with microrobotic technologies that perform automated sperm immobilization by means of computer-assisted sperm tracking and, eventually, microinjection (9, 10).

Unfortunately, as for the chips for microfluidics-based sperm preparation, all these technologies are still at an experimental level and are regarded with reservation from the experts.

Embryo culture and monitoring

Culture of pre-implantation embryos is considered one key element of an IVF laboratory, largely contributing to the success of assisted reproductive treatments. However, a consensus is still missing on the best culture conditions, even in the basic principles, and the literature is full of controversies that open an active debate toward proficient optimization (11).

Again, microfluidics currently represent the most intriguing new technology in aid of this complex issue. With the use of computer-controlled valves and micropumps and a complex circuit of channels and chambers, microfluidics devices allow gradual continuous media replacement, seemingly in accordance with the *in vivo* dynamic conditions to which gametes and embryos are exposed (12). The further application of nanosensors to the chip may help measuring in real-time some key parameters such as pH, oxygen and nutrients consumption, embryos secretions (metabolomics) and more (13, 14), possibly leading in the future to a real individualized embryo culture. Moreover, apart from these potential advantages, microfluidics technology permits to significantly diminish the manipulation timing outside the controlled environment of the incubator, since it renders unnecessary the manual change of media. It is, in fact, well known that strictly controlled culture conditions are essential for embryo development, and any oscillation of pH, temperature and oxygen tension may impair the outcome.

As to this final consideration, a special mention goes to the new computerized systems now available to continuously monitor embryo development avoiding culture exposure to atmospheric conditions, i.e. when checking fertiliza-

tion, cleavage or blastulation at the microscope. In this case, the technology involves a time-lapse imaging of the developing embryos, thanks to periodic illumination by means of a special micro-camera mounted in the incubator and connected to a software for subsequent image processing. Beside the clear advantage of never exit the culture from the incubator, time-lapse allows to study more in detail embryo development leading to the characterization of new morphokinetics parameters potentially associated with the final clinical outcome (pregnancy and implantation rates), such as timing of cell divisions, duration of cell cycles and synchrony of the cleavage (15-17). Nevertheless, since the embryos status depends mostly upon the culture conditions, different results may be obtained by different clinics (18) and a consensus is still missing. Recently, the use of Artificial Intelligence (AI) based on an algorithm associated with an artificial neural network has been used in order to objectively classify blastocysts regarding their morphological grade, demonstrating promising performances: the study has been conducted on bovine blastocysts and the technology is being implemented in human IVF (19, 20).

Embryo biopsy

Standard embryo evaluation, by means of morphological or morphokinetics parameters, is still not sufficient to predict the clinical outcome. Complementary methods imply the use of the so-called OMICS technology (Genomic, Transcriptomic, Proteomic and Metabolomic), which allows investigating the differences among embryos at a molecular, and presumably more informative, level (21). Among these, the Genomic analysis is currently the most extensively used in many clinics, although a general consensus has not yet been reached. The early identification and selection of embryos with a correct chromosomal status and/or specific monogenic diseases should assist the embryologists in the choice of the "better" embryo to transfer in a single-embryo transfer (SET) policy, potentially improving the clinical results per transfer while minimizing the risk of multiple pregnancy.

The Genomic analysis implies the need of an embryonic biopsy, that is the removal of 1-2 blastomeres at the cleavage stage or 3-5 cells of the trofoectoderm at the blastocyst stage. This procedure requires high skilled operators and can be a possible source of lethal or sub-lethal damage to the embryos if inaccurately performed. This potential dramatic pitfall has contributed to the design of an automated high precision equipment for cellular micro-extraction, which combines the unique configuration of fluidic channels and the precise piezoelectric cut to effectively immobilize, perforate and biopsy embryos (22). However, the system must still prove its effectiveness and safeness and more studies are needed.

Gametes and embryos cryopreservation

Cryopreservation of gametes and embryos is a major technique in reproductive medicine, contributing to the overall live birth rate after assisted reproductive treatments. In summary, it consists in the exposure of the biological material to an optimized concentration of cryoprotectants in order to safely maintain the biological status under subzero temperatures (liquid nitrogen, -196°C). Currently, when we talk about cryopreservation we refer to vitrification, rather than slow-cooling methods, because data from available randomized controlled trials suggest the superiority of this technique in terms of cryosurvival and, at some extent, clinical outcomes (23, 24). This procedure is relatively time-effective, since it takes only 15-20 minutes, but it requires the intervention of high skilled embryologists. In fact, it involves multiple steps of stringently timed pick-and-place operations for processing the biological material and fined controlled addition of cryoprotectants, which are potentially toxic. The entire manual process is not reproducible and cell survival can vary significantly among operators.

Automation in this field may lead to a significant standardization, helping the laboratory to be reliable and consistent in each single proce-

dure. Automated vitrification systems are already available (25-27), basically consisting of in a machine performing cryo media automatic replacement by means of robotic arms, guaranteeing consistent volume pipetting and precise incubation timing. It is also possible to implement the instruments with a micromanipulator device able to recognize the material and to move it on a specific spot of the cryopreservation support (26). Unfortunately, very few evidences are currently available on the effective clinical advantages of using automated vitrification devices and further studies are needed.

Electronic witnessing

Biological sample mix-up is a dramatic, even fortunately rare, event in IVF. As a consequence, the presence of a witness to double-check any samples handling is strongly recommended (28). However, this policy implies the dedication of an operator which otherwise may be appointed to other tasks. Moreover, the mere manual mechanism of control has shown to be vulnerable to human errors, such as conscious or involuntary automaticity, ambiguous accountability and stress (29), resulting in checking omission, non-contemporaneous checking, incomplete or wrong checking. Alternative options include barcode-based systems, silicon barcodes for gametes/embryo direct tagging and radio frequency identification technology (RFID) (29).

Among these, RFID-based electronic witnessing (EWS) is certainly the most advantageous. It functions by means of RFID tags attached to any container with gametes or embryos, specifically recognized by the electronic system. The labels are matched with an identity card, previously provided to the patients and that accompanies them throughout the treatment, used to safeguard the beginning and the end of any cycle. This system provides electronic objective witnessing, it prevents embryologist working on more than one patient at a time and omitting key tasks in the process. In fact, any step is registered and monitoring is constant, so that identity check can never be overlooked. Currently electronic witnessing is mandatory in the UK; however, only few centres all around the world have actually implemented the technology. The main reason that impedes its routine application is maybe represented by the elevated costs. Moreover, even if animal studies provide assuring data, it must be fully determined the potential risk of using radio frequencies in the laboratory. Notwithstanding, EWS is perceived as a trusted strategy not only in an experts' perspective but also in a patients' perspective (30).

Conclusion

Automated standardization is a hot topic in today's healthcare industry. Its controlled use allows to higher the standard quality of a process, increase and shorter productivity, guarantee a more efficient use of materials and lower lead times. The automation of the whole IVF procedure is a realistic perspective, although requires multidisciplinary efforts and further investment (31) (Figure 1). Nevertheless, the general impression is that clinical users don't believe in the ability of automated standardization to positively impact the patients outcome. This attitude underlines the irrefutable fact that evidences are still few and without any clear proof of the automation impact on quality improvement and cost-effectiveness there is little hope of encouraging operators to introduce it routinely.

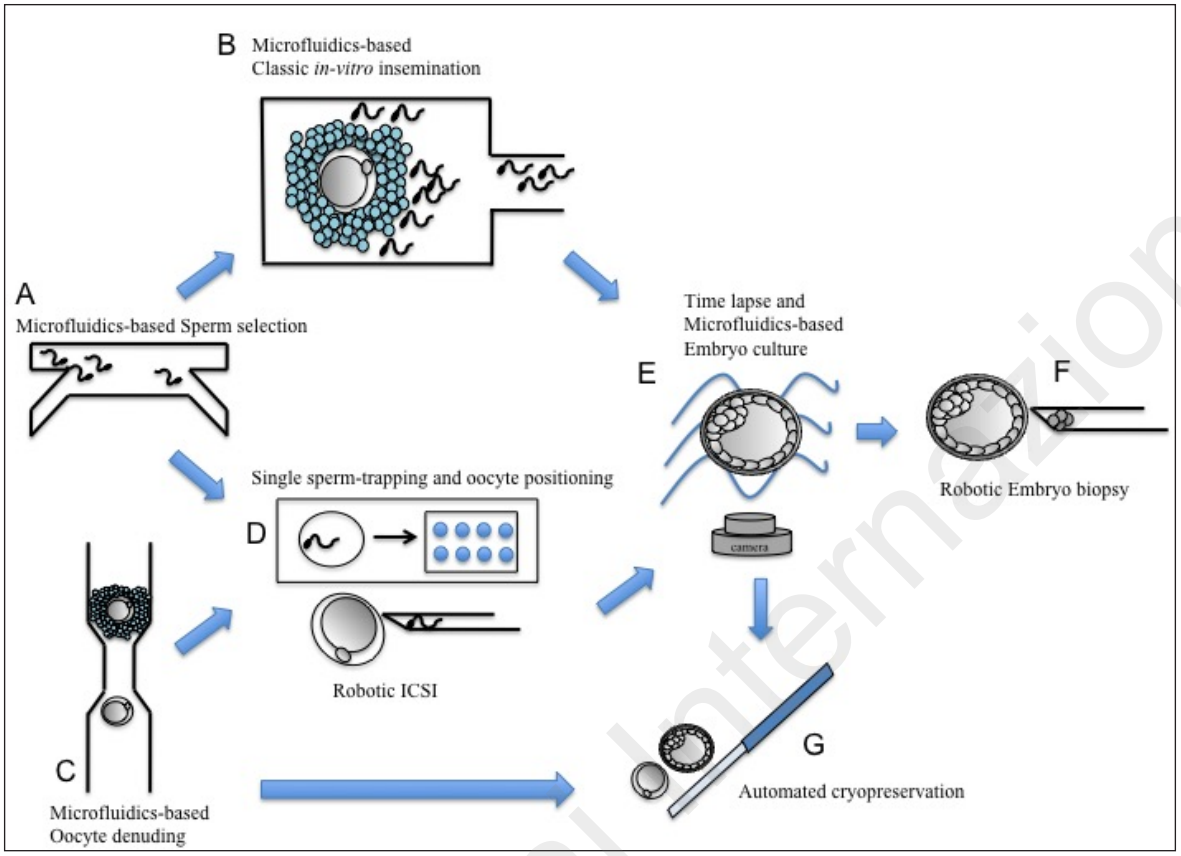

Figure 1 - Schematic overview of the IVF processes that can be mechanized.
A: Simplified view of a microfluidics chip for sperm selection. Sperm recovery can rely both on passive or active mechanisms, aimed to concentrate the final suspension with normomorphic motile intact spermatozoa.
B: Simplified view of an automated classic IVF by means of a microfluidics chip. The oocyte, correctly positioned by fluid flow in a specifically designed microwell, can be reached and inseminated by spermatozoa automatically selected through chemotaxis.
C: Simplified view of oocyte denuding in a microfluidics chip. The separation of Granulosa Cells (GCs) can be accomplished by a combination of fluid flow, progressively narrower channels and appropriate suck force.
D: The denuding oocytes can be injected by means of robotic microinjection of automatically selected and single-trapped spermatozoa.
E: The obtained embryos can be cultured in microfluifics-based systems that guarantee gradual continuous media replacement and real-time measurements of key parameters of nutrients consumption and embryo development.
F: The obtained blastocysts can be biopsied by means of a special automated high-precision equipment for cellular microextractions.
G: The oocytes and embryos can be efficiently cryopreserved by the use of automated machines that guarantee consistent volume pipetting, precise incubation timing and eventually allocation to a precise position on the cryo support.

References

1. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. WHO press. 2010.
2. Mortimer ST, van der Horst G, Mortimer D. The future of computer-aided sperm analysis. Asian J Androl. 2015;17:545-553.
3. Muschol M, Wenders C, Babcock D, et al. Four-dimensional analysis of sperm flagellar waveform as an extension for CASA. Selected oral communication at ESHRE. Session. 2017;36:0-132.
4. Mortimer D. Sperm preparation techniques and iatrogenic failures of in-vitro fertilization. Hum Reprod. 1991;2:173-176.
5. Nosrati R, Graham PJ, Zhang B, et al. Microfluidics for sperm analysis and selection. Nat Rev Urol. 2017;14:707-730.
6. Ma R, Xie L, Han C, et al. In vitro fertilization on a single-oocyte positioning system integrated with motile sperm selection and early embryo development. Anal Chem. 2011;83:2964-2970.
7. Zeringue HC, Beebe DJ. Microfluidic removal of cumulus cells from Mammalian zygotes. Methods Mol Biol. 2004;254:365-374.
8. De Wagenaar B, Berendsen JT, Bomer JG, et al. Microfluidic sperm entrapment and analysis. Lab Chip. 2015;15:1294-1301.

9. Leung C, Lu Z, Esfandiari N, et al. Automated sperm immobilization for intracytoplasmic sperm injection. IEEE Trans Biomed Eng. 2011;58:935-942.
10. Lu Z, Zhang X, Leung C, et al. Robotic ICSI (Intracytoplasmic Sperm Injection). IEEE Trans Biomed Eng. 2011;58:2102-2108.
11. Vajta G, Rienzi L, Cobo A, et al. Embryo culture: can we perform better than nature? Reprod Biomed Online. 2010;20:453-469.
12. Suh RS, Phadke N, Ohl DA, et al. Rethinking gamete/embryo isolation and culture with microfluidics. Hum Reprod Update. 2003;9:451-461.
13. Amatore C, Klymenko OV, Oleinick A, et al. In situ and on-line monitoring of hydrodynamic flow profiles in microfluidic channels based on microelectrochemistry: optimization of channel geometrical parameters for best performance of flow profile reconstruction. ChemPhysChem. 2007;8:1870-1874.
14. Urbanski JP, Johnson MT, Craig DD, et al. Noninvasive metabolic profiling using microfluidics for analysis of single preimplantation embryos. Anal Chem. 2008;80: 6500-6507.
15. Lemmen JG, Agerholm I, Ziebe S. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. Reprod Biomed Online. 2008;17:385-391.
16. Meseguer M, Herrero J, Tejera A, et al. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011;26:2658-2671.
17. Cruz M, Gadea B, Garrido N, et al. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. J Assist Reprod Genet. 2011;28:569-573.
18. Herrero J, Tejera A, Albert C, et al. A time to look back: analysis of morphokinetic characteristics of human embryo development. Fertil Steril. 2013;100:1602-9.e1-4.
19. Rocha JC, Passalia FJ, Matos FD, et al. A Method Based on Artificial Intelligence To Fully Automatize The Evaluation of Bovine Blastocyst Images. Sci Rep. 2017;7:1-10.
20. Rocha J, Matos F, Basso A, et al. Using artificial intelligence to improve blastocyst morphology evaluation. Selected oral communication at ESHRE. Session. 2017;46:0-162.
21. Seli E, Robert C, Sirard MA. OMICS in assisted reproduction: possibilities and pitfalls. Mol Hum Reprod. 2010;16:513-530.
22. Bahadur I, Mills J. Overview of the automation of embryo biopsy for pre-implantation genetic diagnosis. Int J of mechantronics and automation. 2011:1:201-212.
23. Glujovsky D, Riestra B, Sueldo C, et al. Vitrification versus slow freezing for women undergoing oocyte cryopreservation. Cochrane Database of Systematic Reviews. 2014;9:CD010047. Doi: 10.1002/14651858. CD010047
24. Rienzi L, Gracia C, Maggiulli R, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2017;23:139-155.
25. Pyne DG, Liu J, Abdelgawad M, et al. Digital microfluidic processing of mammalian embryos for vitrification. Plos One. 2014;9:e108128.
26. Liu J, Shi C, Wen J, et al. Automated vitrification of embryos: a robotics approach. IEEE Robotics and automation magazine. 2015;22:33-40.
27. Roy TK, Brandi S, Peura TT. Chapter 20: Gavi-Automated vitrification instrument. Methods Mol Biol. 2017;1568: 261-277.
28. Brison DR, Hooper M, Critchlow JD, et al. Reducing the risk in the IVF laboratory: implementation of a double witnessing system. Clin Risk. 2004;10:176-180.
29. De los Santos MJ, Ruiz A. Protocols for tracking and witnessing samples and patients in assisted reproductive technology. Fertil Steril. 2013;100:1499-1502.
30. Forte M, Faustini F, Maggiulli R, et al. Electronic witness system in IVF- patients perspective. J Assist Reprod Genet. 2016;33:1215-1222.
31. Rienzi L, Vajta G, Ubaldi F. New culture devices in ART. Placenta. 2011;32:S248-S251.