# State-of-the-art in Reproductive Bench Science: Hurdles and New Technological Solutions

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**Abstract:**

Infertility is a growing issue in modern society, being the fifth highest serious global disability according to the World Health Organization. To study infertility and other reproductive system complications, bench science still relies on 2D and animal studies, which regularly have been criticized due to their inability to mimic the human body. Particular challenges in 2D studies include the inability to mimic fluid dynamics, gametes modulation and their crosstalk, hormonal patterns as well as the low quality and viability of gametes and embryos. Animal models also present other drawbacks, namely the absence of menstruation, making it difficult to establish a reliable predictive model for the human system. Additionally, reproductive studies should not be limited to the fallopian tube as the sole responsible for most infertility cases, but instead the research spectrum should be widened to the whole reproductive system given the tight interconnectivity between each and every organ. In the last few decades, new *in vitro* technologies have been developed and applied to the study of reproductive system complications. These systems allow to create complex three-dimensional structures, which are therefore able to more closely resemble specific microenvironments and

provide more realistic physical and biochemical cues. 3D (bio)printing, organoids and organs-on-chips are some of the dynamic technologies which are replacing conventionally employed static 2D culture. Herein, we provide an overview of the challenges found in conventional 2D and animal models of the reproductive system and present potential technological solutions for those same challenges.

**Keywords**: Infertility; Reproductive challenges; Biofabrication; Additive Manufacturing; Organs-on-chips.

## Introduction

Infertility is defined as the inability to achieve clinical pregnancy after one year of regular unprotected intercourse and poses as a global public health issue [1]. According to the World Health Organization (WHO), about one in every four couples have been affected by infertility in developing countries [2]. This problem has a major impact not only on public human health, but also on the livestock industry, animal husbandry, and world food production. In this respect, abnormal ovulation and tubal obstruction are the main problems in female infertility, which lead to deficient fertilization as the oocyte is not able to correctly await fertilization in the fallopian tube [3]. Cancer is also correlated to infertility due to the gonadotoxic properties of some anticancer treatments [4].

The female reproductive system is mainly composed by ovaries, fallopian tubes, oviduct, uterus, and cervix [5]. Each organ is dynamic, responding to fluctuating hormonal concentrations driven by the pituitary gland and ovaries. This influences the ovulation, fertilization, embryo implantation, and placentation, making the reproductive system a

highly complex system to study [6]. The main research and clinical reproductive advances were achieved so far by means of bi-dimensional (2D) *in vitro* culture studies, which will be later discussed. However, this type of model presents problems related to the lack of mimicry of the physiological system. Operative conditions such as pH, osmolarity, light availability as well as availability of specific cues and factors are among the factors that make 2D *in vitro* studies inadequate [7]. Inappropriate culture conditions and resulting inadequate models can be detrimental and be a limitation in this research field. To our best knowledge, currently, most studies have a limited scope, focusing on studying mainly the oviduct and neglecting the bigger picture. However, it is indispensable to have a global perspective, to be able to understand the whole process and comprehend its dynamics.

Gamete/embryo handling and cell culture media can also be a limiting factor. For instance, the medium typically employed in 2D culture of embryos made by *in vitro fertilization* (IVF) procedures can effectively impact birth weight [8]. Despite all the advances in reproductive biotechnology, current operative conditions do not entirely mimic interactions and hormonal patterns observed in the natural morpho- physiological environment [9]. It is clear that *in vitro*-produced embryos differ markedly from those that have been developed *in vivo,* due to embryo manipulation and due to the static nature of cultures [10].

Nonetheless, when studying human fertility, animal models are not sufficiently useful either, since a reliable comparison cannot be made between these two systems. Animal models continue to face several challenges given that they are not entirely predictive of the human body function and there still are some safety and efficacy issues related to them [7]. Additionally, these models are significantly different from the human *in vivo* conditions, since most of them do not menstruate nor have the same regulatory patterns

or specific hormones [11]. Rodents in particular are also insensitive to certain classes of chemical compounds, compromising the conclusiveness of any toxicological risk assessment intended to predict effects in humans [11].

In the present work we review various limitations encountered in reproductive research, as well as various novel technological solutions which may help advance the field.

## Conventional 2D cell culture and common hurdles in reproductive research

*In vitro* static 2D cell culture is the most widely used strategy in the study of the female tract. It is a low cost, simple and convenient approach which also enables easy downstream processing [12,13]. A considerable number of related assays and techniques were also extensively developed turning 2D cell culture into a flexible and quick platform that can be easily employed in reproductive studies. In conventional 2D culture, cells are seeded on a plastic surface and allowed to grow in a bidirectional manner, having access to both media and growth factors [12,13]. The plastic surface can also be further functionalised with different materials and proteins in order to resemble certain microenvironments [14].

However, various studies alerted the scientific community to the sub-optimal conditions provided by 2D cell cultures. Particularly, 2D cell culture models can compromise the viability and reliability of experiments by influencing fundamental cellular features and in this way affect the correct understanding of the whole organ function [12,13]. During bidimensional growth, cells can suffer membrane receptor loss, modifications and alterations in several characteristics, such as genetic patterns,

hormonal responses, cell differentiation and proliferation rates as well as responses to different stimuli and secretions [15]. Additionally, during manipulation of biological samples, these can suffer several manipulations, causing excessive stress to oocytes or embryos (temperature, light, pH and osmolarity) and compromising their potential [8,16]. Furthermore, *in vitro* 2D cultures showed to not be adequate for mimicking natural cellular environments given that the employed cellular monolayer suffers a quick transformation, making it difficult to maintain nutrient concentration and prevent metabolite accumulation [16,17]. This phenomenon is observable in cuboidal–columnar oviduct epithelial cells which change their phenotype into flattened cells with a complete loss of cilia and a reduced secretory ability, when compared to the normal physiology (figure 1) [16]. Inadequacies may also be found in 2D ovary cultures, which are not able to promote ovary follicle maturation due to the inexistence of follicular architecture. Another limitation found in 2D cell cultures is related to the inability to create a sufficient air-liquid interface, given that high volumes of medium are needed to supply proper conditions and most current embryo culture systems are static. Therefore, these systems often lose cell viability, compromising the experimental efficacy [10].

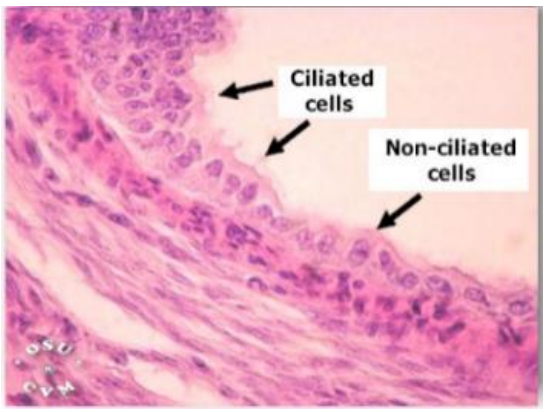



Figure 1 - Histological characterization of rat oviduct. Source: [78]

One of the most compromising aspects of the bidimensionality found in conventional cell culture is the lack of naturally occurring interactions between cells and organs. The oviduct is a good example of a non-reproducible system via 2D cell culture. This organ is characterised by highly complex interactions, being where the fertilization occurs and where the embryo develops before migrating to the uterus as a morula. It has a specific microenvironment that activates gametes, helps avoid polysemic fertilization, promotes embryo nourishment and provides developmental stimuli. Also, the oviduct wall is able to recognize the arrival of spermatozoa and even alter their proteome, due to these interactions [7,18-21].

*In vivo* hormonal patterns are also impossible to be fully emulated in 2D cell cultures. The long- and short-range hormonal signals that are established between the gametes or embryo and the female tract during pregnancy are a good example of such hormonal patterns. These signals promote a unique dialogue and generate multiple signalling cascades and a complex interactome, which may influence the maturation and transport of gametes, coordinating a successful fertilization and assuring embryo viability [20].

Genetic patterns are another hurdle found in 2D cell culture, given that many critical interactions that impact cells at a molecular level cannot be mimicked. The oviduct in particular has a specific role in embryonic genome activation and reprograming. These crucial interactions are responsible for creating somatic epigenetic methylation, which in turn modifies the gene expression by silencing or activating their expression through the epigenetic marks which later influence the blastocyte's transcriptome [22-24]. Ferraz *et al*. performed a comparative study between *in vitro* and *in vivo* embryos which showed that active DNA demethylation was higher in *in vivo* zygotes [15].

Naturally occurring mechanical forces resulting from ECM stiffness and other mechanical stresses are also factors that cannot be mimicked in 2D *in vitro* assays. When sensed, these forces are converted by cells into inner biochemical signals which influence their development. In particular, embryonic and extra-embryonic fluids have a critical role in the embryo behaviour since they modulate tensional and frictional stress and hydrostatic pressure [23]. Shear stress is one of the mechanical stresses that result from fluid flow, peristaltic tubal compression and kinetic friction between embryo and cilia, and which influence early development of the embryo [25-27]. This mechanical property is responsible for inducing cell-cell communication, positively affecting the embryo by refreshing the surrounding fluid, eliminating metabolites produced by the embryos and influencing genetic factors. Furthermore, mechanical events, such as pulsating muscle contractions, cilia beating and sperm motility, act in a positive way by increasing the dispersion and availability of hormones and nutrients [26]. Shear stress can modulate several important developmental mechanisms in pre-implanted embryos and should be recreated in *in vitro* experiments.

Overall, 2D models are inefficient in promoting these normal environmental cues along the tract and the interaction between the different female organs. Such limitations may affect not only the gametes but also the embryo in their DNA methylation dynamics or hormonal signalling. As such, all these experimental conditions may impact the embryo's outcome and consequently promote sub-optimal conditions in the reproductive system studies.

***3D cell culture approaches***

Recent biotechnological advances brought about new techniques and more complex approaches to investigate the female reproductive system. These allow to more closely mimic the human body, having the potential to address the limitations previously mentioned. Three-dimensional (3D) cell culture, organoid models and organ-on-chip are some of these new advanced systems, which will be discussed below.

3D cell culture systems emerged as a way to overcome the hurdles reported above, given their ability to more closely mimic the macro- and micro-architecture of tissues and organs, and to stimulate cells with the appropriate biochemical and biomechanical cues. The transition from 2D to 3D models represents a breakthrough in cell biology and related areas since it can lead to levels of tissue organisation never seen before. In these cell culture systems, cells grow and organize themselves in a 3D architecture, promoting a more complex structures and representing a more *in vivo*-like biological microenvironment. In this way, the three-dimensionality improves and promotes communication between cells, replicating cell and tissue physiology, mimicking mechanical cues, allowing communication between the cell and its matrix and taking into account the spatial organization of the tissue [12-14]. Moreover, 3D approach allows cells to maintain the basal-apical polarity, as well as to retain their genetic and epigenetic patterns. However, these models may in certain cases still face some challenges involving deficient non-homogenous distribution of cells, nutrients and oxygen, inefficient removal of waste, lack of vascularisation and therefore limited reliability and repeatability [28]. The characteristics as well as pros and cons of several promising 3D-enabling technologies will be described below.

**Spheroids** are simple 3D models that take advantage of the capability of adherent cells to aggregate. These cell aggregates are able to mimic the microenvironment of various tissues as well as their cell-cell and cell-ECM interactions. However, the generation of necrotic cores is generally a considerable challenge of this technique, due to the lack of nutrients, oxygen and waste diffusion to/from the center of the cell aggregate. Due to its simplicity and mimicry properties, spheroids are widely used in drug screening assays [28]. Lawrenson *et al* have successfully developed a fallopian tube spheroid model composed of primary fallopian tube secretory epithelial cells. The employed spheroids were able to restore the three-dimensional *in vivo* architecture although at a low proliferative rate. In this way, it was possible to show that spheroid technologies are able to generate relevant models to study the quiescent status of normal secretory epithelial cells [29].

**Microcapsules** are mainly used as 3D cell carriers, and have been employed in the delivery of cells for the treatment of conditions such as cancer and diabetes [30-33]. Microcapsules are small sized systems, ranging from 100 µm to 750 µm, generally made of natural or synthetic polymers and able to encapsulate hundreds to thousands of cells. The reduced capsule size allows to increase oxygen, nutrient and waste diffusion and allow delivery of cells and therapeutics via small incisions or catheters, therefore avoiding major surgeries [32, 33]. Dorati *et al* developed a 3D barium-alginate microcapsule for enriching the medium employed in *in vitro* embryo production from cryopreserved domestic cat vitrified oocytes. The results of this study showed that while using vitrified oocytes 3D culture *per se* did not capacitate the viability of vitrified cat oocytes. However,

206 when using a culture condition enriched with 3D alginate microcapsules, it was possible
207 to promote maturation and embryo development [34].
208
209 **Organoid models** are composed of 3D aggregates of cells, formed either from
210 pluripotent stem cells or multipotent organ-specific adult stem cells [35]. These 3D
211 models have a higher degree of complexity, and, therefore, are able to more reliably
212 mimic tissue's histology, functionality and physiology, when comparing to the classical 2D
213 *in vitro* cell culture. Additionally, organoids are able to retain tissue phenotypical and
214 functional properties [36]. In a study by Kessler and co-workers it was possible to
215 successfully produce a fallopian tube organoid made from stem cells derived from the
216 human fallopian tube epithelium [37]. This model could mimic the normal physiology and
217 anatomy of the human fallopian tube epithelium, maintain the phenotypic patterns for
218 several months and differentiate epithelial cells into secretory and ciliated cells. Finally,
219 the authors were able to observe a high degree of similarity between the organoid and
220 its human counterpart. Also using an organoid model, Buretto *et al* created an
221 endometrium model that could efficiently reproduce the tissue physiology and allow
222 long-term expansion [36]. Despite all, organoid morphology and architecture are still a
223 limitation of this type of 3D cell culture technique. For example, in oviduct organoids,
224 gametes and embryos can only gain access by means of a micro-puncture to the organoid,
225 which can be invasive and damaging to the model. Additionally, this procedure is
226 performed manually, therefore increasing the susceptibility to error [16].
227
228 **Scaffolds** are support constructs that foster cell and tissue growth. They can also
229 further influence cell and tissue development by providing topographical cues and/or

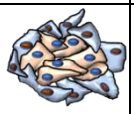
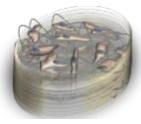
various biochemical cues, such as growth factors and drugs [38]. Scaffolds can be created using different physical and/or chemical approaches and composed of a wide range of materials, either natural, synthetic or hybrid. Some of the most promising scaffolds are composed of hydrogels and manufactured by means of biofabrication techniques [39-41].

**Hydrogels** are three-dimensional networks composed of physically or chemically crosslinked hydrophilic polymers. These are biocompatible and, although being water insoluble, can uptake high amounts of water or other liquids and may have their physical conformation tuned in terms of mechanical and morphological properties [42,43]. The utilization of hydrogels may however be limited given that some of them are created employing harmful crosslinkers and most lack adequate mechanical and degradability properties. These limitations are usually overcome by combining both natural and synthetic polymers in order to obtain hydrogels with tailored properties [42-44]. Joo *et al* used a collagen-based hydrogel for seeding oocytes and investigate the hormone patterns and oocyte maturation. The employed hydrogel was shown to indeed contribute to the maintenance of follicle native function and its phenotypic patterns when cultured *in vitro* [45].

**Advanced scaffolds** are constructs typically manufactured by means of technologies such as electrospinning, among others [46] . Due to the precision of these technologies, scaffolds can be manufactured with tailored morphology and mechanical properties, maximizing the biomimetic properties of these systems [39,47]. Furthermore, by employing these technologies it is possible to achieve greater levels of repeatability and reproducibility, when compared to other cell culture approaches [48]. However, similarly

to other scaffolds, limited nutrient, oxygen and waste diffusion in large scaffold volumes, heterogeneous cell distribution and architectural challenges are among the drawbacks that may eventually be observed in these systems [48,49]. In a study by Liverani *et al* employing electrospun fibrous scaffolds for culture of porcine follicles, it was possible to observe that scaffold morphology and composition had crucial roles in enabling the ovary to maintain its normal function and follicle morphology, given that the scaffold could closely mimic the *in vivo* tissue [50].

*Table 1 - Advantages and disadvantages of the different 3D cell culture techniques*

| *3D MODELS* | *ADVANTAGES* | *DISADVANTAGES* | *REFERENCES* |
|---|---|---|---|
| **SPHEROIDS**  | -Simple<br>-Mimics *in vivo* interactions | -Necrotic cores<br>-Size variability<br>- Long-term culture difficulty<br>-Simplified architecture | [29,51-54] |
| **ORGANOIDS**  | -Emulate interaction between cells of different tissues<br>-Higher degree of complexity<br>-Can be patient specific | -Necrotic cores<br>-Size variability<br>-Need validation | [54-56] |
| **HYDROGEL SCAFFOLDS**  | -Emulate *in vivo* ECM interaction<br>- Physical or chemical crosslinking<br>- Highy reproducible | -Inefficient exchange of substances<br>-Simplified architecture<br>-Difficult cell recovery | [43,54] |

| | | | |
|---|---|---|---|
| **BIOFABRICATED SCAFFOLDS**  | -Mimics the *in vivo* microenvironment<br>- High porosity<br>-Tailored morphology and mechanical properties | -Heterogeneous cell distribution<br>- Limited diffusion | [54,57,58] |

***Advanced technological solutions***

Nowadays there are new advanced technologies that are capable of further replicating the complex heterogeneity found in the reproductive system in a more precise manner and therefore being able to closely resemble *in vivo* microenvironments. These technologies include 3D printing and bioprinting, as well as microfluidic systems and organ-on-chips, which will be discussed below.

**3D printed scaffolds and devices.** 3D printing technology is based on the fabrication of 3D structures by consecutive deposition of layers of material. It was first used in 1986 by Charles W. Hu, who first developed stereolithography [59]. By using computer-aided design (CAD) software and medical imaging, simple or complex physical shapes can be obtained with an unprecedented speed and detail. The development and optimisation of new materials, combined with reduced manufacturing costs and advanced printers, enabled the use of 3D printing in research labs and industrial settings [60-62]. This technology can be adopted for a broad range of applications, from aviation and car industries to healthcare. In the medical field, the use of biocompatible materials, such as polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS) allowed to produce scaffolds that could be used in certain medical scenarios [60-62]. Additionally, 3D printing presents a tremendous potential for scaffold production due to the increased reliability

and reproducibility, when compared to other methods. Post-manufacturing treatments, such as heat treatments and hot isostatic pressing, are used to tailor the properties of scaffolds, by modifying their microstructure or surface roughness [62].

**3D Bioprinting** (a very specific type of 3D printing) also applies a layer-by-layer concept however, unlike standard 3D printing, it relies instead on the deposition of cell-laden filaments or cell-containing droplets into specific substrates in order to generate three-dimensional biological structures [59].

Regarding the application of 3D printing in the study of the reproductive system, the best example can be found in a study by Laronda *et al*. describing the development of a bioprosthetic ovary. In this case, a 3D printed microporous scaffold was able to provide space and nutrition diffusion for follicle survival and maturation and enabled the growth of an ovary-like vascularization, which is fundamental for the circulation of hormones around the follicle. This system also enabled the development of a *corpus luteus* to produce hormones, after the ovulation, and egg release without mechanical manipulation or digestion [63].

**Microfluidic systems** are another strategy available to study the reproductive field in a more complex manner. Microfluidic systems are small devices with a single or a set of sub-millimetric channels. These micro-channels allow the injection of liquids or gases through inlets connected to tubing and/or syringe/pump adapters. Such devices can be used for several purposes not only in the biomedical field but also in chemistry and other areas. The utilization of microfluidics for reproductive purposes has increased in the past years due to their ability to mimic the reproductive system in a more efficient and physiological way [19]. Microfluidics has already been used on sperm motility tests

allowing to study sperm rheotaxis and chemotaxis [64,65], gamete and embryo culture and evaluation [23, 66], transportation of embryos and respective monitoring [66,67] and to remove cumulus cells and zona pellucida [17, 68]. For example, Angione *et al* were able to develop a microfluidic device that not only allowed perfusion and live imaging of the living system but also a precise and flexible handling of individual oocytes and embryos [69]. Another example is the device developed by Yin *et al*. which could mimic an *in vivo*-like 3D placenta in a chip [70]. In this study, the construct was designed with two parallel cell channels separated by a middle matrix channel, and with a perfusion flow that could generate a near-physiological dynamic microenvironment. In this way, it was possible to successfully build a device that allowed to study placental responses to toxicological environments.

**Organs-on-chips** are complex microfluidic devices, designed for emulating the architecture, function and dynamic environment of organs (or portions thereof). Organs-on-chips may consist of very simple devices or highly complex device systems, often integrating components such as valves or pumps into the design itself. These systems can for instance allow fluid mixing, as well as generate fluid gradients and microdroplets [71]. Furthermore, the integration of flow perfusion and the ability to co-culture cells in a controlled manner makes these devices more able to resemble *in vivo* conditions.

Organs-on-chips are highly suitable for laboratory assays since they are small, employ low volumes of reagents being therefore less expensive to operate, and are able to generate and maintain a stable microenvironment with precise control over spatial and temporal dynamics. Additionally, these systems enable a greater capability for batch sample processing and greater screening scope [19, 72]. These systems are already being

used for medical and pharmaceutical purposes, namely in drug screening and pharmacokinetic and pharmacodynamic tests [5]. These devices are also able to better simulate natural environments which are useful for embryo culture optimization. Organs-on-chips can also mimic naturally occurring physical and mechanical stimuli, enabling cells to experience relevant physiological cues that directly affect their biological function [73]. Additionally, medium perfusion allows to improve nutrient supply, clearance of waste products and metabolic profiling [73]. Furthermore, organs-on-chips are adequate for profiling the cell secretome in culture medium and, therefore, used for searching specific biomarkers [74]. In the future, this technology may be used as a fertilization platform, improving the *in vitro* fertilization rate and quality, as well as simultaneously integrate a variety of functional tests that supplement the information about embryos or gametes. Finally, by providing an environment with controlled osmolality, temperature, and pH, organ-on-chip devices could eventually reduce the stress typically imposed on embryos and enable spermatozoa and oocyte interaction to take place in *in vivo*-like environments, generating embryos more suitable for implantation [19]. The most advanced examples of organ-on-chip devices directed at reproductive studies were developed by Ferraz *et al*. [21] and Xiao et al. [6].

In the first case, a polydimethylsiloxane (PDMS) microfluidic oviduct-on-chip device was developed, comprising two independent and perfusable 370 µm deep compartments separated by a porous membrane. To mimic the oviduct, a confluent oviduct epithelial cell layer was grown on the top of the membrane. The compartments were designed to ensure shear stress and to entrap the oocytes in an apical compartment. This device was built with a thin design so that the apical compartment of the device would allow live imaging of the epithelial cells, gametes, and embryos inside the chip [21]. This 3D model

allowed a deeper understanding in the early maternal-gamete/embryo interaction with production of zygotes highly resembling *in vivo* zygotes, within a microenvironment closely resembling *in vivo* conditions.

In the latter case, a complex multi-organ on chip (called EVATAR) was composed of five different tissues (cervix, fallopian tube, ovaries, uterus, and liver) connected by a complex microfluidic device circulating flow between all tissues. Given that the use of microfluidic devices enable the control of flow rates and real-time monitoring of metabolites, drug compounds, signalling molecules and hormones [5,65], this device could more realistically mimic the complexity of the reproductive female system, when comparing to 2D petri dishes or animal models. Additionally, this organ-on-chip was able to mimic the 28-day *in vivo* human follicular and luteal phase hormone synthesis and hence enable the provision of steroid and peptide hormones [6]. Such systems represent the next step in the study of the female reproductive system and may become an important tool for personalized medicine purposes in the future. Furthermore, with the rise of induced pluripotent stem cells (iPSC) such systems could also become patient-specific and therefore emulate even more closely the dynamic human tissue interactions [75,76].

In general, such complex microfluidic platforms can help reveal more biological insights more reliably than usual lab assays, since they can recreate the physiological microenvironment, replicating many features such as chemical gradients, fluid dynamics, surface interactions and morphologies [75]. However, before widespread adoption, it is important to make sure that these devices are fully tested and characterized in order to safeguard the reliability of results obtained. As an example, the material most commonly

used in the manufacture of microfluidic and organ-on-chip devices – PDMS – undesirably binds to some classes of molecules and some additive manufacturing materials may be toxic to cells [76].

## Conclusions and future perspectives

Conventional 2D static culture methods are clearly uncapable of fully mimicking the natural environment where mammalian embryos typically develop under constant exposure to complex combinations of stimuli, crosstalk and cascade reactions. Advanced technologies such as organoids, bioprinting and organs-on-chips show the potential to address these limitations by ultimately enabling the creation of 3D devices and constructs where embryos and micro replicas of various reproductive organs and tissues may be grown in close communication with each other. The ability to spatially control the three-dimensional positioning of all these elements as well as the ability to accurately control their ability to communicate amongst themselves provides a powerful means to perform systematic in-depth studies where the role of each of these elements can be elucidated and quantified as the embryo development progresses. Apart from simply mimicking normal physiological conditions, it may also become possible to induce abnormal stimuli and conditions, therefore enabling the study and understanding of diseased or abnormal states which, apart from rarely occurring in nature, may also be extremely difficult or even impossible to properly study in conventional *in vivo* and *in vitro* conditions.

Despite the unquestionable potential of these new technologies, it is however important to keep in mind that they are fairly recent and are still rarely employed in reproductive studies. In that sense, there is still a long and arduous way to go until such

technologies become routinely employed for reproductive purposes. Not only the technology needs to be further developed in order to achieve its full potential, but it also needs to become standardized so that results obtained from different studies can be adequately combined and compared. Furthermore, and given the dynamic complexity of the biological systems generated in this way, it will be equally important to assure the quality and speed of acquisition of all sensorial and analytical methods employed.

Overall, the new technologies herein described show the potential to revolutionize reproductive research as well as human and animal reproduction as a whole, although much work still needs to be done so that these technologies may become standardized and routinely adopted in the field.

**Acknowledgements**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 798014.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 828835.

The authors are members of the COST Action CA16119 In vitro 3-D total cell guidance and fitness (CellFit).

**Conflict of Interest**

Dr. Costa is CEO, CTO, and shareholder of the company Biofabics Lda.

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