

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(6): 892-900

www.biochemjournal.com

Received: 17-04-2026

Accepted: 21-05-2026

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Integration of microfluidics and ART a new frontier in gamete and embryo handling

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6k.8791>

Abstract

The scientific discipline of microfluidics which controls small-scale fluid manipulation has brought revolutionary changes to biomedical engineering applications including assisted reproductive technologies (ART). The success rate of ART depends heavily on precise handling of gametes and fertilization processes together with embryo culture requirements. The high-resolution physical and chemical microenvironment control through microfluidic systems enables advanced sperm sorting capabilities based on motility, chemotaxis, rheotaxis, and thermotaxis. Microfluidic approaches replace conventional sperm preparation methods such as swim-up and density gradient centrifugation because these traditional methods take too much time and cause damage to sperm DNA. The microfluidic system demonstrates its potential for automated oocyte handling as well as maturation and cryopreservation through stress reduction and in-vivo environment simulation. The combination of 3D culture systems with embryos leads to better developmental outcomes and cellular differentiation because it creates spatially accurate environments. Microfluidic technologies advance their scope in reproductive biology through recent developments of organ-on-a-chip systems and bioprinting that enable the creation of artificial reproductive tissues and embryo-maternal interaction models. The technology offers several benefits but faces ongoing challenges related to device standardization and clinical workflow integration and biocompatibility issues. The paper reviews microfluidics principles and ART applications and new developments while explaining how these developments can transform gamete and embryo manipulation. The integration of microfluidics technology with emerging platforms presents opportunities for developing novel *in vitro* embryo production methods which offer scalable efficient patient-specific reproductive healthcare solutions.

Keywords: Bioprinting, Chemotaxis, Cryopreservation, Microfluidics, Rheotaxis, Thermotaxis

Introduction

ART technology has progressed significantly to treat infertility in both humans and animals. The conventional gamete manipulation techniques as well as fertilization methods and embryo culture systems remain both labor-intensive and poorly standardized and operator-dependent which can negatively affect fertilization success rates and embryo development (Swain *et al.*, 2013) [42]. Microfluidics a discipline uniting physics with engineering and biology and chemistry represents an effective answer to existing problems. Microfluidics manipulates fluids within microliter to picoliter volumes through microscale channels which enables strict control over the physical and chemical microenvironment (Whitesides, 2006) [44]. The precise control enables better nutrient distribution while maintaining efficient waste removal and real-time observation of gametes and embryos under regulated settings (Berthier *et al.*, 2012; Sackmann *et al.*, 2014) [3, 35].

The ART field utilizes microfluidic platforms to perform sperm sorting procedures alongside oocyte handling and IVF processes and embryo culture applications. The systems function as sperm selection tools which evaluate motility as well as rheotaxis and chemotaxis and thermotaxis and surface charge properties that conventional swim-up and density gradient centrifugation fail to assess (Ricci *et al.*, 2009, Jahangiri *et al.*, 2024) [33, 20]. Through microfluidics scientists achieve delicate oocyte manipulation while decreasing mechanical stress which enhances both maturation and developmental potential (Lai *et al.*, 2015; Yanez

and Camarillo, 2017) [24, 47]. The integration of microfluidics with 3D culture systems and organ-on-a-chip platforms enables better replication of *in vivo* conditions through recent technological developments. These advanced models enable better studies of embryo-maternal interaction while providing cell differentiation in environments that duplicate natural reproductive tissues (Pensabene *et al.*, 2026; Stern-Tal *et al.*, 2020; Gargus *et al.*, 2020) [32, 40, 14]. This paper examines the fundamental elements of microfluidics together with its connections to ART practices. The discussion explores current applications in sperm and oocyte management and embryo cultivation and the joint implementation with new technologies including 3D matrices and bioprinting. The review examines present obstacles including standardization requirements and biocompatibility issues and clinical workflow integration while providing perspective on *in vitro* embryo production advances through microfluidic technology.

2. Basic Principles of Microfluidics

The field of microfluidics integrates microengineering with fluid mechanics and biological applications to manage tiny fluid volumes between picoliters and microliters through microchannel systems (Whitesides, 2006; Ma *et al.*, 2024) [44, 28]. The fabrication of these devices through PDMS glass and thermoplastics enables unmatched precision for cell and biofluid and gamete management under tightly controlled conditions. Fluids show laminar flow behavior at the microscale because their Reynolds numbers remain low which causes them to flow in parallel non-mixing layers. The ability to predict diffusion-driven transport exists because of this feature which also eliminates shear stress that occurs in turbulent systems (Stone *et al.*, 2004; Squires and Quake, 2005) [41, 39]. The small size of microchannels allows them to provide large surface-to-volume ratios which boost heat transfer rates and chemical exchange rates required to support stable microenvironments for cells and embryos (Berthier *et al.*, 2012; Xiao *et al.*, 2017) [3, 46].

Several microscale components that include channels mixers valves and chambers work with external actuation mechanisms like pressure gradients electrokinetics magnetophoresis or temperature fields to achieve fluid control according to Sackmann *et al.* (2014) [35] and Nosrati *et al.* (2014) [31]. The forces provide non-invasive handling of gametes and embryos through low reagent consumption. Modern microfluidic chips now include built-in sensors which measure temperature and pH and oxygen levels to provide continuous embryo monitoring during *in vitro* development (Zhang *et al.*, 2021) [50]. The fundamental importance of microfluidics in modern assisted reproductive platforms emerges from its automated precise and repeatable biological workflows (Swain *et al.*, 2013) [42].

3. Benefits and Motivation for Miniaturization in Microfluidics

The miniaturization of fluidic environments enables microfluidics to achieve multiple advantages above conventional macroscale systems. The microscale manipulation of fluids delivers improved operational efficiency along with reduced costs and heightened sensitivity and better control that benefits applications such as assisted reproductive technologies (ART).

3.1 Key Benefits of Microfluidics

- **Reduced Sample and Reagent Volume:** Microfluidic systems operate with minimal media or biological sample requirements of just several microliters thus decreasing expenses and protecting essential biological specimens including spermatozoa and oocytes and embryos (Sackmann *et al.*, 2014; Lee *et al.*, 2022) [35, 26].
- **High Sensitivity and Reproducibility:** The small size of microfluidic systems enables accurate control over their microenvironment which leads to better biochemical reaction sensitivity and dependable results (Ma *et al.*, 2024) [28].
- **Rapid Reaction and Analysis Time:** The short reaction time possible in microfluidic systems results from small sample volumes that allow for faster mixing and diffusion and thermal equilibrium (Xiao *et al.*, 2017) [46].
- **Enhanced Mass and Heat Transfer:** The high surface-to-volume ratio of microfluidic devices enables efficient transfer of nutrients as well as gases and heat which suits embryo culture requirements (Berthier *et al.*, 2012) [3].
- **Real-Time Monitoring and Integration:** The combination of optical thermal and electrochemical sensors in microfluidics enables real-time measurement of pH oxygen and embryo metabolism without affecting the culture environment (Zhang *et al.*, 2017) [50].
- **Minimized Contamination and Evaporation:** The closed-channel design protects against external contaminants and the low thermal mass allows rapid temperature and pH stabilization (Stone *et al.*, 2004) [41].
- **Single-Cell and Single-Sperm Manipulation:** Microfluidic systems achieve the separation and examination of individual gametes through their ability to evaluate their motility and surface charge properties and morphological features (Jahangiri *et al.*, 2024) [20].

3.2 Motivation behind Miniaturization in ART

ART applications benefit from miniaturization since it creates environments that closely resemble natural conditions. The human female reproductive system includes small fluid channels that display laminar flow together with specific chemical balance gradients. A microfluidic chip that replicates these conditions enables better physiological handling of gametes and embryos (Xiao *et al.*, 2017) [46]. The minimal mechanical stress in microfluidic systems protects fragile biological cells like oocytes and blastocysts while simultaneously boosting their survival rates and developmental outcomes (Lai *et al.*, 2015; Gargus *et al.*, 2020) [24, 14]. Automation becomes feasible through miniaturization which enables essential clinical reproducibility for IVF and sperm sorting in veterinary and human medical contexts (Sackmann *et al.*, 2014; Ferraz *et al.*, 2023) [35, 10].

4. Sperm Selection Using Microfluidics

Successful fertilization depends on the initial selection of spermatozoa that function properly. The ART laboratory uses two conventional sperm selection techniques known as swim-up (SU) and density gradient centrifugation (DGC) yet these methods present mechanical stress and DNA fragmentation and fail to replicate natural

sperm guidance processes izadeh *et al.*, 2020; Jahangiri *et al.*, 2024) [23, 20]. Microfluidics enables biomimetic functional selection which duplicates how sperm naturally choose the oocyte by utilizing cues including fluid flow chemical gradients and temperature signals. The technologies permit selection through motility rheotaxis chemotaxis thermotaxis boundary following and surface charge while keeping DNA integrity intact.

4.1. Motility-Based Microfluidic Selection

The quality of sperm depends heavily on its motility. Microfluidic systems from the past utilized sperm swimming against fluid flow and nutrient-rich areas to select motile sperm passively according to Cho *et al.*, 20 [6] and Gadelha *et al.* (2019) [13]. These platforms eliminate centrifugation steps, reducing reactive oxygen species (ROS) and DNA damage. Sperm motion consists of linear flagellar beating as well as multiple complex biomechanical

interactions which occur between the fluid environment. The properties of sperm motility enable microfluidic systems to extract sperm cells with the best possible motility characteristics (Norsati *et al.*,2014., Gadelha *et al.*, 2019) [31, 13].

4.2. Spartan (Simple Periodic Array for Trapping and isolation): The innovative microfluidic system SPARTAN utilizes flagellar kinematics to differentiate sperm. The system contains periodic micropillar arrays that capture sperm with irregular flagellar movements or shapes yet permits normal motile sperm to pass through (Nosrati *et al.*, 2014) [31]. SPARTAN-selected sperm show higher DNA integrity, better morphology, and improved fertilization potential. The method operates without labels or centrifugation agents which makes it appropriate for clinical settings.

Table 1: Comparative Overview of Advanced Sperm Selection Techniques Based on Motility and Functional Characteristics

Method	Principle	Advantage	References
Motility-based	Self-propulsion	Non-invasive, preserves DNA integrity	Bhat <i>et al.</i> ,2024 [4]
SPARTAN	Flagellar biomechanics	Removes abnormal sperm mechanically	Riordan <i>et al.</i> , 2019
Rheotaxis	Flow-guided swimming	Mimics <i>in vivo</i> transport	Miki and Clapham, 2013 [29]
Chemotaxis	Response to attractants	Functional sperm selection	Eisenbach and Gjojals, 2006 [7]
Thermotaxis	Heat gradient	Reflects sperm functional maturity	Li <i>et al.</i> , 2014 [27]
Boundary following	Wall-guided motion	Selects robust swimmers	Nosrati <i>et al.</i> , 2014 [31]

4.3. Rheotaxis-Based Sperm Selection

Sperm exhibit rheotaxis as a natural trait through their ability to swim opposite to fluid flow when found in oviduct environments (Miki and Clapham, 2013) [29]. The artificial simulation of directional fluid flow in microfluidic devices enables the separation of highly mobile sperm with improved DNA quality and better structural integrity (Kantsler *et al.*, 2014; El Sherry *et al.*, 2023) [22, 9]. Modern rheotaxis-based chips use structured channels or corrals that induce flow gradients. The collection zone remains accessible only to sperm which possess strong motility along with rheotactic properties while all other sperm get eliminated by the process (Zaferani *et al.*, 2019) [49]. The devices provide sperm selection with non-invasive capabilities while being label-free and capable of high throughput operations. Microfluidic sperm sorting platforms utilize physiological guidance mechanisms such as rheotaxis, chemotaxis, thermotaxis, and surface charge-based separation to improve functional sperm selection (Figure 1)

4.4. Chemotaxis-Based Sperm Selection

The biochemical guidance mechanism chemotaxis enables sperm to detect chemical gradients that come from the oocyte and cumulus cells (Eisenbach *et al.*, 2025) [8]. Microfluidic chips generate gradients to separate the sperm subpopulation which demonstrates maximum sensitivity to these cues and shows optimal fertilization potential within the range of 10-15%. Channels receive attractants such as progesterone and acetylcholine and heparin to create conditions similar to the body's natural environment. Sperm selection based on chemotaxis improves the capacitation process and acrosome reaction and zona binding capacity (Eisenbach *et al.*, 2025) [8]

4.5. Thermotaxis-Based Sperm Selection

The oviductal isthmus and ampulla experience a small temperature variation (0.5-2°C) during ovulation. The

process of thermotaxis helps sperm cells to migrate toward warmer temperature zones for successful fertilization (Bahat and Eisenbach, 2006) [2]. These microfluidic systems create stable temperature differences across their channels through their design. The sperm selection process using thermotaxis results in better DNA quality and improved fertilization outcomes according to research conducted with thermal microfluidic chips (Li *et al.*, 2014; Wu *et.*, 2024) [27, 45].

4.6. Boundary Following

Sperm cells exhibit boundary following as a wall-guided behavior by showing alignment and movement along the walls of microchannels (Samuel *et al.*, 2018) [36]. Sperm head-wall interactions along with flagellar dynamics determine the way sperm behave in this context. The fallopian tube conditions are replicated by microfluidic chips containing circular or spiral microchannel arrays to find sperm that are stable without needing centrifugation (Khodamoradi *et al.*, 2021) [23]. Some samples may experience decreased efficiency because of wall-related clogging or debris accumulation.

4.7. Surface Charge (Zeta Potential)

The sperm membrane carries negative charges from sialic acid composition that scientists measure as zeta potential. The zeta potential values of functionally competent sperm differ from those of immature sperm cells or apoptotic sperm cells according to Esfahani *et al.* (2016) [30]. Sperm sorting occurs through dielectrophoresis and electrostatic interactions which function on microfluidic devices that use electric fields to separate sperm based on their surface charge. The testicular sperm extraction (TESE) process can benefit from these methods to extract non-motile sperm in asthenozoospermia cases (Iranzo *et al.*, 2025) [19].

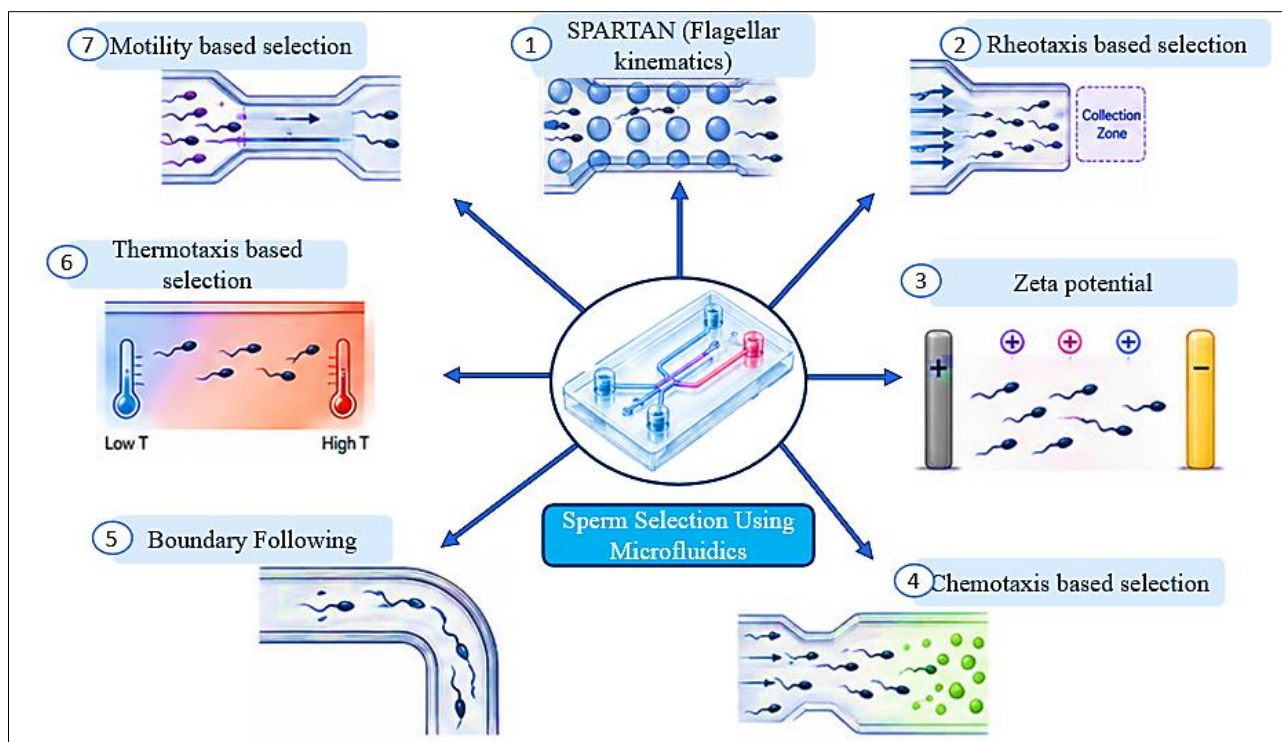


Fig 1: Microfluidic-based sperm selection techniques used in assisted reproductive technologies (ART).

The figure illustrates the major microfluidic approaches employed for functional sperm selection in assisted reproductive technologies. These biomimetic systems mimic natural physiological guidance mechanisms present within the female reproductive tract and enable the isolation of highly competent spermatozoa with improved fertilizing ability and DNA integrity. The illustrated techniques include: (1) SPARTAN (Simple Periodic Array for Trapping and Isolation), which separates sperm based on flagellar kinematics and motility patterns using micropillar arrays; (2) rheotaxis-based selection, where motile sperm swim against controlled fluid flow toward the collection zone; (3) zeta potential-based selection, which separates sperm according to surface electrical charge characteristics associated with membrane maturity and functional competence; (4) chemotaxis-based selection, where sperm migrate toward chemical attractants that mimic oocyte-derived signals; (5) boundary-following behavior, in which sperm preferentially navigate along microchannel walls resembling movement within the female reproductive tract; (6) thermotaxis-based selection, which utilizes temperature gradients to guide sperm toward warmer regions associated with fertilization sites; and (7) motility-based selection, where progressively motile sperm are separated through controlled microfluidic flow dynamics. Collectively, these techniques provide non-invasive, centrifugation-free and physiologically relevant alternatives to conventional sperm preparation methods.

5. Oocyte Handling, Maturation, and Cryopreservation using Microfluidics

5.1 Oocyte Handling, Maturation, and Cryopreservation

The implementation of microfluidic technology enables oocyte manipulation by delivering precise and automated and physiologically relevant solutions beyond standard laboratory methods. These systems enable different ART procedures including denudation (cumulus cell removal), oocyte selection, *in vitro* maturation (IVM),

and cryopreservation (Lai *et al.*, 2015; Yanez and Camarillo, 2017) ^[24, 47].

a. Microfluidic Denudation and Handling

The removal of cumulus cells becomes crucial because it allows ICSI procedures and embryo evaluation to take place. The combination of micropillar arrays and flow-shear interfaces in microfluidic devices enables the efficient removal of cumulus cells through mechanical methods which replaces both enzymatic digestion and pipetting stress (Han *et al.*, 2010) ^[16]. The mechanical denudation technique protects oocytes from damage and maintains their zona pellucida structure. These platforms also automate the capture and positioning of oocytes which decreases both operator dependence and the chances of contamination (Yanez *et al.*, 2016) ^[48].

b. Oocyte Selection and Maturation

Oocyte selection methods in traditional practices depend on manual visual assessment of morphological characteristics. Modern microfluidic systems now integrate optical and mechanical sensors to conduct real-time measurements of oocyte dimensions and zona pellucida thickness as well as cytoplasmic texture and shape (Yanez and Camarillo, 2017) ^[47]. A microfluidic chamber that includes cumulus or granulosa cells enables the simulation of natural ovarian conditions which improves oocyte maturation (Han *et al.*, 2010) ^[16]. This method enhances the success of fertilization by improving both meiotic development and cytoplasmic maturation.

c. Microfluidics in Cryopreservation

Osmotic shock together with ice crystal formation represents the main challenges during conventional cryopreservation methods. The microfluidic chip technology enables sequential exposure to cryoprotectants through precise control of flow rates and temperature gradients and CPA gradients (Lai *et al.*, 2015; Guo *et al.*) ^[24]. These

devices enable the reduction of oocyte shrinkage and they enhance post-thaw survival as well as spindle integrity and cleavage rates. Microfluidics-enhanced vitrification through murine and bovine models produced superior results in terms of morphology and membrane integrity and developmental competence (Aydin *et al.*, 2024) ^[1].

6. In Vitro Fertilization, Embryo Culture, and Analysis using Microfluidics

1. Microfluidics in In Vitro Fertilization (IVF)

IVF microfluidic systems duplicate the spatial-temporal dynamics of female reproductive tract to enable controlled gamete interactions. The systems decrease polyspermy occurrences while maintaining sperm-oocyte synchrony and minimizing shear stress that directly affects zygote development (Richards *et al.*, 2024) ^[34]. IVF chips that consist of microwell arrays enable direct observation of fertilization events and prevent the zygote overcrowding effect. The advanced devices possess the capability to control sperm numbers and concentrations when introducing sperm to each oocyte especially useful during cases of low sperm count (Gurkan *et al.*, 2024) ^[15].

2. Embryo Culture in Microfluidic Systems

Traditional culture systems often fail to mimic the in vivo uterine environment. The microfluidic platforms generate stable microenvironments that replicate all in vivo mechanical forces and pH conditions and nutrient flow patterns. The systems require fewer medium exchanges and sustain optimal embryonic environments through continuous perfusion (Lee *et al.*, 2021; Ren *et al.*, 2015) ^[26, 51]. Embryos cultured in microfluidic systems show improved blastocyst development, enhanced cell number and differentiation, better mitochondrial activity and epigenetic stability (Zhao *et al.*, 2015) ^[51].

3. Real-Time Embryo Monitoring and Metabolic Assays

The built-in sensors in microfluidic embryo culture chips provide non-invasive real-time monitoring of embryo viability. The use of electrochemical or optical sensors in these systems enables continuous tracking of glucose, lactate, pH and oxygen tension to evaluate embryo metabolic activity and developmental capacity (Heo *et al.*, 2010; Swain *et al.*, 2013) ^[17, 42]. The analytical microfluidic systems enable the selection of high-quality embryos without the need for biopsy thus supporting safer and more accurate embryo transfer decisions. Microfluidic IVF offers several advantages over conventional IVF systems because it more closely mimics the physiological microenvironment of the female reproductive tract. In microfluidic IVF, the culture medium volume is typically maintained at very low levels (1-5 μ L) compared to conventional IVF systems that generally require 100-500 μ L of media. This reduced volume enhances the accumulation of embryo-secreted autocrine and paracrine factors while simultaneously decreasing reagent consumption (Heo *et al.*, 2010) ^[17]. In addition, gamete handling in microfluidic systems occurs within enclosed microchannels and is largely automated, thereby minimizing manual pipetting and centrifugation procedures commonly used in conventional IVF laboratories. As a result, microfluidic platforms reduce mechanical stress, oxidative stress, and DNA damage to gametes and embryos (Swain *et al.*, 2013) ^[42]. Furthermore, these systems provide

controlled fluid dynamics, continuous nutrient exchange, and opportunities for real-time, non-invasive monitoring of embryo development through integrated sensing technologies (Bhatia and Ingber, 2014) ^[5]. Overall, microfluidic IVF systems more effectively reproduce in vivo reproductive conditions and therefore have substantial potential to improve fertilization outcomes, embryo quality, and developmental competence (Xiao *et al.*, 2017) ^[46].

7. 3D Culture Systems for Gametes and Embryo Manipulation

The conventional 2D culture systems restrict cells to flat rigid surfaces which lack physiological significance. In contrast, 3D culture systems allow gametes and embryos to interact in all three dimensions, mimicking the extracellular matrix (ECM) and in vivo reproductive environments more closely (Laronda *et al.*, 2017; Jensen and Teng, 2020) ^[25, 21]. The early stages of embryonic development require precise mechanical cues and spatial orientation and cellular signaling which 2D systems fail to provide adequately (Sittadjody *et al.*, 2017; Hoffman, 2018) ^[38, 18]. Different biomaterials such as type I collagen, gelatin, and alginate hydrogels have been employed for 3D embryo culture. Soft scaffolds made from collagen at a stiffness of 1 kPa support better cleavage rates along with improved blastocyst development and hatching and cell lineage differentiation (Zhao *et al.*, 2015) ^[51]. The biocompatible and non-cytotoxic alginate hydrogels derived from brown algae allow for embryo elongation and adhesion and morphogenesis and are tunable (Zhao *et al.*, 2015; Zhao *et al.*, 2015) ^[51, 51]. Porous alginate scaffolds also enable co-culture with oviductal or endometrial cells to simulate implantation environments (Stern-Tal *et al.*, 2020) ^[40]. The platforms enhance embryo viability while also improving morphology and trophoblast differentiation which are essential for implantation success and postnatal outcomes.

The 3D matrices provide suitable environments for in vitro growth of follicles and maturation of oocytes. Hydrogel-based ovarian follicle encapsulation techniques have been utilized across mice and human and cattle and dog species for ex vivo oocyte production (Gargus *et al.*, 2020) ^[14]. They offer Controlled stiffness to support antral follicle expansion, improved oxygen and nutrient diffusion, Co-culture support with granulosa cells or hormones to mimic follicular fluid conditions. Compared with conventional two-dimensional (2D) culture systems, three-dimensional (3D) culture platforms provide a more physiologically relevant microenvironment for gamete and embryo development. In 2D culture, cells grow on flat rigid surfaces with limited spatial organization and restricted cell-cell interactions, often resulting in altered embryo morphology and suboptimal developmental outcomes. In contrast, 3D culture systems mimic the native extracellular matrix and support dynamic multidirectional cellular interactions, allowing embryos to maintain natural spheroid or tubular morphology. These systems also facilitate implantation-like processes through co-culture approaches and improve embryo viability, trophoblast differentiation, and placental development after transfer (Hoffman, 2018; Jensen and Teng, 2020) ^[18, 21]. Biomaterials such as collagen and alginate hydrogels have been widely used to create supportive 3D reproductive microenvironments that better

resemble *in vivo* conditions (Zhao *et al.*, 2015; Stern-Tal *et al.*, 2020) [51, 40].

8. Organ-on-a-Chip and Bioprinting in Reproductive Technologies

The technology of Organ-on-a-chip produces microengineered 3D models which reproduce the structure and functional behavior of actual tissues. The technology unites microfluidics with cell culture methods along with sensor systems to generate organ-scale physiological and disease-related behavior (Bhatia and Ingber, 2014) [5]. ART researchers and reproductive biology scientists utilize organ on a chip platform to recreate and investigate ovarian follicle growth, menstrual cycle hormone dynamics, embryo-maternal interactions, implantation and endometrial receptivity (Xiao *et al.*, 2017; Pensabene *et al.*, 2026) [46, 32]. Applications of organ-on-a-chip and bioprinting technologies in reproductive biology and ART are summarized in Figure 2.

Key Reproductive Organs Modeled on Chips

- **Ovary-on-a-Chip:** The device supports the *in vitro* growth of both primordial and antral follicles through periodic hormone treatments. These chips enable the process of oocyte maturation together with steroidogenesis (Gargus *et al.*, 2020) [14].
- **Endometrium-on-a-Chip:** A device duplicates either a receptive or non-receptive uterine lining condition.

Stromal and epithelial cell co-culture under hormonal exposure generates implantation models that are realistic (De Bem *et al.* Stern-Tal *et al.*, 2020) [40]

- **Embryo-maternal interface:** The technology allows researchers to examine blastocyst adhesion as well as hatching and nutrient exchange when applying flow conditions. These platforms establish a connection between artificial embryo cultivation and the successful implantation process.
- **Bioprinting in reproductive research:** 3D bioprinting uses bioinks made from cells combined with hydrogels and ECM components to build tissue layers one by one. The reproductive technology application of this technology involves three uses i.e., folliculogenesis takes place on printed ovarian scaffolds, imitation of individual endometrial environments through printing for implantation studies and the technology allows for enduring hormone-sensitive tissue development. Bioprinting technology helps create species-specific reproductive models that veterinary ART and conservation biology benefit from as reported (Heo *et al.*, 2010; Laronda *et al.*, 2017) [17, 25].
- **Organ on a Chips and Bioprinting present multiple advantages for ART procedures:** These testing platforms are beneficial for evaluating embryo culture media while also assessing toxicant effects and individualized fertility treatments.

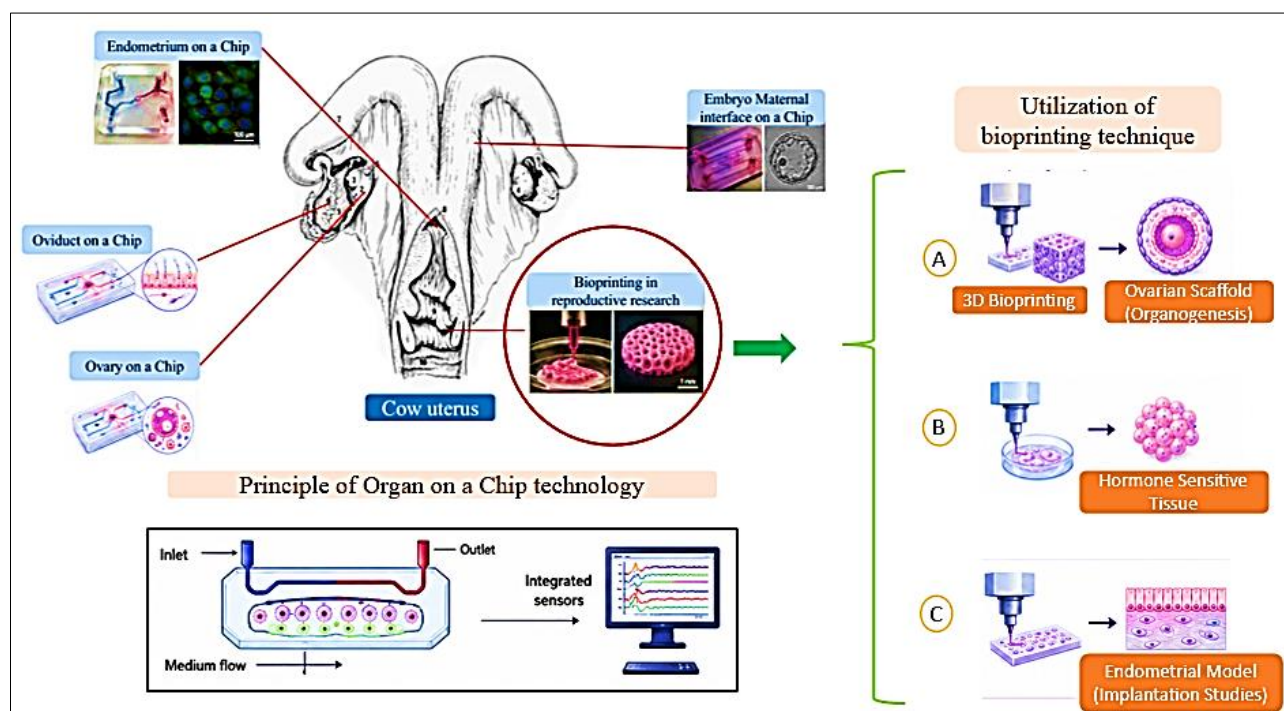


Fig 2: Organ-on-a-chip and bioprinting applications in reproductive technologies.

The figure illustrates the integration of organ-on-a-chip technology and three-dimensional (3D) bioprinting approaches in reproductive biology and assisted reproductive technologies (ART). Organ-on-a-chip systems combine microfluidics, cell culture platforms, and integrated biosensors to recreate physiologically relevant reproductive microenvironments under controlled flow conditions. The left panel depicts reproductive organ models developed on microfluidic chips, including ovary-on-a-chip, oviduct-on-a-chip, endometrium-on-a-chip, and embryo-maternal

interface-on-a-chip systems, which are designed to simulate folliculogenesis, fertilization, embryo transport, implantation, and endometrial receptivity. The lower panel demonstrates the basic principle of organ-on-a-chip technology, where culture medium flows through microchannels containing living cells and integrated sensing systems for real-time physiological monitoring. The right panel highlights the utilization of 3D bioprinting techniques in reproductive research. Bioprinting employs bioinks composed of cells, hydrogels, and extracellular matrix

components to fabricate reproductive tissue constructs layer-by-layer. Applications illustrated include: (A) fabrication of ovarian scaffolds for folliculogenesis and artificial ovary development, (B) generation of hormone-sensitive reproductive tissues, and (C) development of printed endometrial models for implantation and embryo-maternal interaction studies. Collectively, these advanced platforms provide physiologically relevant and species-specific reproductive models that have significant potential in fertility research, embryo development studies, toxicological screening, and personalized reproductive medicine.

9. Advancements, challenges and future directions in microfluidics for assisted reproductive technologies

The evolution of microfluidics technology led to the development of comprehensive reproductive platforms which now perform automated gamete handling (Lai *et al.*, 2015) ^[24]. The platform performs *in vitro* fertilization and tracks embryos (Heo *et al.*, 2012) ^[17]. Previous studies used real-time metabolic monitoring techniques. Hormone-responsive organ-on-a-chip models for ovarian and uterine tissues (Gargus *et al.*, 2020; De Bem *et al.*) ^[14]. The integration of 3D culture systems with microenvironmental sensors and AI-based embryo scoring makes microfluidic ART systems more precise and clinically beneficial (Lee *et al.*, 2022; Ren *et al.*, 2023) ^[26]. Significant progress has been achieved but multiple key limitations remain unaddressed.

Despite major advances in microfluidic-assisted reproductive technologies, several important challenges continue to limit their large-scale clinical and veterinary application. One of the primary concerns is biocompatibility, particularly in polydimethylsiloxane (PDMS)-based devices, as PDMS can absorb small hydrophobic molecules including steroid hormones and toxicants, thereby altering media composition and cellular responses (Toepke and Beebe, 2006; Berthier *et al.*, 2012) ^[43, 3]. Another major limitation is workflow integration, because most IVF laboratories still rely on manual and highly variable protocols, making integration of automated microfluidic systems challenging within existing ART procedures (Swain *et al.*, 2013) ^[42]. In addition, the lack of standardized fabrication methods, chip geometries, and validation criteria creates difficulties in reproducibility and inter-laboratory comparisons (Whitesides, 2006) ^[44]. Some advanced platforms also require specialized technical expertise and clean-room fabrication facilities, restricting their routine adoption in reproductive laboratories (Sackmann *et al.*, 2014) ^[35]. Furthermore, species-specific optimization remains limited in veterinary ART, as many currently available devices are primarily designed for human reproductive systems and have not yet been fully validated for bovine or porcine applications (Ferraz *et al.*, 2018) ^[12]. The integration of on-chip sensors to track embryo pH levels and oxygen consumption and glucose concentration in real-time enables non-invasive embryo viability predictions. AI and Machine Learning The combination of embryo development metrics with AI technology will enhance embryo selection performance especially when it uses time-lapse imaging (Simopoulou *et al.*, 2018) ^[37]. Portable microfluidic devices that use sperm assessment on farms or in patients' homes will transform livestock reproductive technology and fertility testing. Patient-derived endometrial or ovarian cells in bioprinted

chips could help scientists develop individualized fertility treatments and sustainable materials the development of bioinert and biodegradable chips will solve issues related to environmental and biological toxicity.

Conclusions

Microfluidic technology has emerged as a transformative tool in assisted reproductive technologies by enabling precise, automated, and physiologically relevant manipulation of gametes and embryos. Compared with conventional ART procedures, microfluidic systems provide superior control over fluid dynamics, nutrient exchange, temperature gradients, and cellular microenvironments while minimizing mechanical and oxidative stress. The integration of sperm sorting platforms, microfluidic IVF systems, embryo culture devices, and cryopreservation modules has significantly improved the efficiency and reproducibility of *in vitro* embryo production. Recent advances in three-dimensional culture systems, organ-on-a-chip platforms, and reproductive bioprinting have further expanded the scope of microfluidics by enabling realistic simulation of ovarian, endometrial, and embryo-maternal interactions. These biomimetic systems provide valuable opportunities for studying reproductive physiology, implantation biology, toxicology, and personalized fertility treatments in both human and veterinary medicine. Despite substantial progress, several challenges remain, including device standardization, large-scale manufacturing, biocompatibility of materials, integration with existing laboratory workflows, and species-specific optimization for veterinary applications. Future developments involving artificial intelligence, biosensors and portable lab-on-chip platforms are expected to accelerate the clinical and field-level adoption of microfluidic ART systems.

Overall, the convergence of microfluidics with reproductive biotechnology represents a promising next-generation approach for improving fertility management, embryo production, and reproductive research in animals and humans.

Acknowledgements

The authors extend their gratitude to the ICAR-National Dairy Research Institute, Karnal, for providing the necessary support. Special thanks to colleagues and staff for their valuable insights and technical assistance.

Competing interests

Authors have declared that no competing interests exist.

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