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REVIEW

Mechanobiology of the female reproductive system

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Abstract

Background: Mechanobiology in the field of human female reproduction has been extremely challenging technically and ethically.

Methods: The present review provides the current knowledge on mechanobiology of the female reproductive system. This review focuses on the early phases of reproduction from oocyte development to early embryonic development, with an emphasis on current progress.

Main findings (Results): Optimal, well-controlled mechanical cues are required for female reproductive system physiology. Many important questions remain unanswered; whether and how mechanical imbalances among the embryo, decidua, and uterine muscle contractions affect early human embryonic development, whether the biomechanical properties of oocytes/embryos are potential biomarkers for selecting high-quality oocytes/embryos, whether mechanical properties differ between the two major compartments of the ovary (cortex and medulla) in normally ovulating human ovaries, whether durotaxis is involved in several processes in addition to embryonic development. Progress in mechanobiology is dependent on development of technologies that enable precise physical measurements.

Conclusion: More studies are needed to understand the roles of forces and changes in the mechanical properties of female reproductive system physiology. Recent and future technological advancements in mechanobiology research will help us understand the role of mechanical forces in female reproductive system disorders/diseases.

KEYWORDS
Embryo, Female reproductive system, Mechanobiology, Ovary, Uterus

1 | INTRODUCTION

The field of mechanobiology focuses on how the responses of cells, tissues, and organs to mechanical cues, resulting from both intracellularly generated and externally generated forces, contribute to development, differentiation, physiology and disease via the integration of medicine, biology, engineering, and physics.[1-3] How living cells can sense their environment and adequately respond in terms of morphology, migration, proliferation, differentiation, and survival requires understanding at multiple scales, from molecules to single cells, tissues, and organs.[1-3]

More than a century ago, mechanical forces were proposed to drive embryogenesis and bone structure.[1-3] The importance of mechanical forces for biological regulation was recognized in the field of developmental biology at the beginning of the twentieth century.[1-3] However, there were no experimental tools available to directly test

the role of mechanical forces in biological regulation.[1-3] The greatest challenge in mechanobiology is quantifying how biological systems sense, transduce, respond to and apply mechanical signals.[1-3] With the recent advent of biophysical and molecular technologies, today mechanobiology is an emerging multidisciplinary field ranging from cell and developmental biology to bioengineering, material science, and biophysics.[1-3]

The impact of biochemical and genetic factors in biology has been studied extensively in the 20th century. Professor Ingber from the Wyss Institute, who has made major contributions to the mechanobiology field, stated, "The time has come to recognize that all of these factors—biochemical, genetic and mechanical—are equally important for biological control, and that to truly understand living systems, we must break through our silos of knowledge and create a fully integrated explanation of how our bodies work.[3]"

The female reproductive system is a complex multi-organ system with multiple closely regulated functional processes. It is clear that we need to understand how biochemical, genetic, and mechanical factors act together to control the physiology of the system and how perturbation of these factors results in pathological consequences, to truly understand the physiology, disorders and diseases of the female reproductive system. However, mechanobiology in the field of human female reproduction has been extremely challenging technically and ethically. More studies are needed to understand the roles of forces and changes in the mechanical properties of female reproductive system physiology, and little is known about how mechanical perturbations result in the related disorders.[4-10]

However, given the importance of mechanobiology in human biology and development,[2] the present review aimed to provide the current knowledge on mechanobiology of the female reproductive system. I focus on the early phases of reproduction from oocyte development to early embryonic development, with an emphasis on current progress. Because of the limited knowledge in humans, I include evidence from animal studies, both in vivo and in vitro. I also provide a perspective on the critical challenges in the field of mechanobiology of the female reproductive system.

2 | MECHANICAL SIGNALING AND MECHANOTRANSDUCTION

Here, I provide a brief introduction on mechanical signaling and mechanotransduction. Please refer to excellent previous review articles for more details.[11-19]

Biochemical signals generated in response to hormones or other soluble factors have been investigated extensively, whereas much less is known about mechanical signaling, which refers to intracellular signaling events triggered by a mechanical force, in the female reproductive system.[4] Living cells generate and sense forces.[1-3] Intrinsic or intracellularly generated forces, including elasticity, stiffness, viscoelasticity, and adhesion, are transmitted to other cells either directly via cell-cell junctions, such as cadherin-based adhesions, or indirectly via cell-extracellular matrix (ECM) interactions, such as integrin-based adhesions.[13-19] Extrinsic forces comprise solid forces (substrate mechanics, strain, and compression) and fluid forces (luminal and interstitial) applied externally on cells.[13-19]

Mechanical signaling differs from biochemical signaling in signaling characteristics, mode of transmission, and directionality.[11,12] Mechanical signaling can transmit information over long distances more rapidly and efficiently compared with biochemical signaling.[11,12] Biochemical signaling is diffusion-based, and the strength of the signal decreases with distance, whereas mechanical forces can act at a distance to induce mechanochemical conversion in the nucleus and alter gene activities, and the strength of the signal does not decrease with increasing distance or time.[11,12]

Mechanotransduction is the overall process by which cells sense and respond to externally applied or internally generated mechanical forces and convert them into an intracellular response. Mechanoreceptors such as integrins and cadherins, together with various signal transduction pathways, are involved in the mechanotransduction process that ultimately regulates critical nuclear events.[13-19] Forces are transmitted from the cell surface and cytoskeleton across the nuclear envelope to the interior of the nucleus, triggering changes in chromatin organization and gene expression.[14-17] Furthermore, findings suggest that the nucleus can act as a cellular mechanosensor and respond directly to mechanical forces.[13,18,19]

Biomechanical and biochemical morphogenetic processes in embryonic development were long considered to be disconnected.[20-22] However, recent data indicate robust coordination between biochemical and biomechanical morphogenesis during embryonic development.[20-22] There are feedback loops between mechanical and biochemical signals, also termed mechanochemical feedback loops.[20-22] These bidirectional interactions between mechanical and biochemical signals play key roles in morphogenesis and pattern formation in both development and disease.[20-22] To determine how mechanical cues are translated into function, we need to understand the interplay between mechanical and biochemical signals in the complex environment of a living organism.

3 | CURRENT KNOWLEDGE ON MECHANOBIOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM

3.1 | Ovary

3.1.1 | Mechanical properties of the two major ovarian compartments: cortex and medulla

The ovary has two major compartments, the cortex and medulla, which differ in their ECM composition and structure (Tables 1 and 2).[23] Decellularized human and bovine ovarian tissues exhibit radially aligned collagen fibers in the cortex, whereas the medulla is composed of a network of pores with anisotropic collagen fibers, suggesting differences between cortical and medullary ECM-producing

TABLE 1 Summary of mechanical properties reported in the literature

Type of cells, tissue or organ	Species	In vitro or in vivo	Mechanical properties	Technique	Main findings	Ref. year
Bisected ovary	Mouse	In vitro	Spatial profile of stiffness	Colloidal probe atomic force microscope	Ovarian edge and center (2–3 kPa) < intermediate zone (~7 kPa)	27 2021
Whole ovary	Bovine	Ex in vivo	SW velocity	Shear Wave (SW) ultrasound elastography	SW velocity was higher in the medulla than the cortex	30 2020
Antral follicle	Rabbit	In vivo	Intrafollicular pressure	Direct cannulation with micropipettes	No significant difference: just before ovulation Artificial increasing intrafollicular pressure by the injection of fluid: Not induce follicle rapture	33 1964
Antral follicle	Rabbit	In vivo	Intrafollicular pressure	Direct cannulation with micropipettes	The average pressure: ~17 mm Hg No difference: pre- versus post-coital follicles	34 1963
Antral follicle	Pig	In vivo	Intrafollicular pressure	Direct cannulation with micropipettes	Intrafollicular pressure >30 cm H_2O (~22 mm Hg) Artificial increasing intrafollicular pressure (>400 cm H_2O [~294 mm Hg]): Not induce follicle rapture	1979
Antral follicle	Rat	In vivo	Intrafollicular pressure after hCG stimulation.	Servo null micropipette system	Increase: from the preovulatory phase (16.6 ± 1.0 mm Hg, mean ± SD) to the late ovulatory phase (23.9 ± 1.9 mm Hg)	36 2001
Zona pellucida	Mouse	In vitro	Stiffness	Micro tactile sensor	Softened during oocyte maturation from the GV oocyte (22.8 ± 10.4 kPa, mean ± SD) to MII stage (8.26 ± 5.22 kPa), and hardened at the PN stage (22.3 ± 10.5 kPa). Then gradually softened as the embryo developed from 2-cell (13.8 ± 3.54 kPa) to morulae (1.88 ± 1.34 kPa) and early blastocyst (3.39 ± 1.86 kPa)	44 2006
Oocyte	Mouse	In vitro	Stiffness	Automated micropipette aspiration	Softened from GV to MI and from MI to MII, As oocytes mature	45 2016
Oocyte about 3–4 h after retrieval	Human	In vitro	Stiffness	Indentation measurements with atomic force spectroscopy	ZP outer layer of the immature MI is stiffer than that of mature MII ZP outer layer of "rejected" MII was softer than that of "suitable'' MII Note: MII oocytes were classified as ''suitable'' and ''rejected'' according to their morphological characteristics	46 2016
Zona pellucida of fresh oocyte	Human	In vitro	Zona pellucida shear modulus (ZPSM)	Computational methodology to calculate the mechanical parameters that govern ZP deformation during a routine ICSI procedure	C10 value (ZPSM): in the ranges of 0.20–0.30 kPa and 0.30–0.40 kPa: the highest implantation rates (6/12: 50% and 8/9: 88.8%, respectively) Outside this range: Average implantation rate: 6.70%	47 2020

(Continues)

TABLE 1 (Continued)

Type of cells, tissue or organ	Species	In vitro or in vivo	Mechanical properties	Technique	Main findings	Ref. year
Thawed 2PN stage zygotes 2PN stage zygotes	Human Mouse	In vitro	Viscoelastic properties	Automated micropipette aspiration system The mechanical model (the modified Zener model): to extract mechanical parameters	Prediction of blastocyst formation: In humans within hours after fertilization: >90% precision, 95% specificity and 75% sensitivity In mouse, 79% specificity and 76% sensitivity Prediction of live birth in mouse	45 2016
Blastocyst-stage embryo	Mouse	In vitro	Lumenal pressure	The **microelectrode** is connected to the 900A **micropressure system**	Mid-stage blastocysts (E4.0): ~650 Pa Mature blastocyst stage (E4.5): ~1500 Pa	97 2018
Blastocyst-stage embryo	Mouse	In vitro	Inner pressure Stiffness: trophoblasts and ICM	Laser-assisted magnetic tweezer	Inner pressure at E4.5 is ~3.7 times that of E3.0 Stiffness: Trophoblast at E4.5 is ~1.6 times that of E3.0 ICM at E4.5 is ~3.6 times that of E3.0	98 2019
Uterus during pregnancy from E3.5 to E6.5	Mouse	In vivo	Intrauterine pressure	1.2 F microtransducer-mounted pressure catheter	Amplitude: the highest and most frequent at E5.5 (just after implantation)	87 2020
Decidual tissue surrounding the embryo at 5.5 dpc	Mouse	In vitro	Stiffness	Atomic force microscopy	23.26 ± 9.8 kPa (mean ± SD)	87 2020
Endometrium	Human	In vivo	Stiffness	Three-dimensional multifrequency magnetic resonance elastography	Secretory phase (1.97 ± 0.34 kPa, mean ± SD) < proliferative phase (3.34 ± 0.42 kPa)	75 2014
Endometrial stromal cells	Human	In vitro	Stiffness	Colloidal probe atomic force microscope	Decreased during in vitro decidualization	77 2018
Fresh tissue samples: first-trimester placental and decidual tissues (6–12 weeks) and the secretory-phase endometrium	Human	In vitro	Stiffness	Atomic force microscopy	Secreatory phase endometrium, decidua parietalis, and placenta (at a magnitude of 10^2 Pa) < decidua basalis (at a magnitude of 10^3 Pa)	118 2019

Abbreviation: 2PN, two-pronuclear; dpc, days post coitum; E, embryonic day; GV oocyte, germinal vesicle oocyte; ICM, inner cell mass; kPa, kilopascals; MI, metaphase I; MII, Metaphase II; ZP, Zona pellucida;

TABLE 2 Summary of manipulation of mechanical properties and/or mechanical stimulation reported in the literature

Type pf cells, tissue or organ	Species	In vitro or in vivo	Manipulation of mechanical properties/mechanical stimulation	Methods/Models	Main findings	Ref. year
Whole ovary	Mouse	In vitro	Reduced (Extra cellular matrix) ECM stiffness	Incubation with a collagenase containing solution	Translocation of foxo 3 into cytoplasm in the oocytes at the edge of the cortical region Some of the oocytes started follicular growth to secondary follicles	26 2019
Two-layered and multilayered secondary follicles	Mouse	In vitro	Substrate stiffness: mimicking the mechanical cues from the ECM	Cultured on alginate hydrogels with different concentrations: 0.7%, 1.5%, or 3% (w/v)	Follicles cultured in low shear elastic modulus (alginate: 0.7%): increased follicle growth, higher rates of antrum and theca layer formation and higher quality of oocytes The high level of androstenedione and progesterone: in the immature, two-layered follicles cultured under increased matrix stiffness of alginate hydrogels (3%)	42 2007
Frozen 2-cell-stage embryos	Mouse	In vitro	Shear stress: mimicking the mechanical effects in the fallopian tube	The tilting embryo culture system (TECS): To move embryos along the bottom of the dish at around 1 mm/min fluid motion of the microdroplet for 3 days	Blastocyst development rates (59%, $n = 145$) using TECS significantly improved over those of static controls (46%, $n = 151$)	50 2006
Oocytes during preincubation before IVF/ICSI A total of 450 retrieved oocytes from 32 IVF/ICSI cycles of 32 women A randomized controlled trial	Human	In vitro	Shear stress: mimicking the mechanical effects in the fallopian tube	he TECS: at around 1 mm/min fluid motion for 4 h	The rates of fertilization per mature oocyte and high-grade cleavage-stage embryo formation: no difference versus the control group The rates of blastocyst formation and of blastocysts graded 3BB or higher at day-5: significantly higher in the TECS group than those in the control group (45.3% (67/148) versus 32.1% (51/159), (29.1% (43/148) versus 17.6% (28/159), respectively)	51 2013
Pronuclear embryo Group 1 (74 patients, $n = 148$), without mechanical agitation) Group 2 (74 patients, $n = 148$), with mechanical agitation	Human	In vitro	Mechanical micro-vibration: mimicking the mechanical effects in the fallopian tube	With 5 s intervals of 44 Hz/h	Significantly higher percentage of excellent (grade A) and good (grade B) quality embryos at the 4–6 blastomere stage was observed in Group 2 compared with Group 1 ($90.1 \pm 1.7\%$ versus $77.9 \pm 4.4\%$, mean $\pm$ SD) The percentage of embryos at different blastocyst stages was 10% higher in Group 2 than that in Group 1 (14.1 ± 2.8 versus 4.5 ± 1.7). Significantly higher pregnancy rate in Group 2 versus Group 1 ($78.4 \pm 3.2\%$ versus $50.1 \pm 4.9\%$: transfer of day-3 embryos, $72.2 \pm 1.5\%$ versus $33.2 \pm 2.4\%$: transfer of day-5 embryos)	52 2011

(Continues)

TABLE 2 (Continued)

Type pf cells, tissue or organ	Species	In vitro or in vivo	Manipulation of mechanical properties/mechanical stimulation	Methods/Models	Main findings	Ref. year
Fertilized oocytes after 22 h incubation with sperm	Bovine	In vitro	Constriction: mimicking peristaltic muscle contractions of the inner muscle of the oviduct Shear stress	Microfluidic platform: Embryos: cultivated in constricted channels, with different constriction widths of the channel (150 and 160 μm) versus in a straight channel (control) Incubated on a tilting machine, which made a cyclic ± 10° tilt over 1 min to provide embryo movement via gravity for 44 h	Significantly more 2-cell embryos developing into eight-cell embryos cultivated in constricted channels with 150 (37.8 ± 11.0%, mean ± SD) and 160 μm (56.7 ± 13.7%) constriction widths than that in the straight channel (23.9 ± 11.0)	53 2009
Fertilized oocytes after 22h incubation with sperm	Bovine	In vitro	Compression: mimicking the mechanical effects in the fallopian tube	Microfluidic platform: Stimulate embryos using a maximum pressure of 150 Pa and a duration time of 12 s or 2 s for 3 days	Successful cleavage from the non-stimulated area and from mechanically stimulated area for 2 s Squeezed embryos were underdeveloped in the mechanically stimulated area for 12 s	54 2011
wo-cell embryos	Mouse	In vitro	Shear stress: mimicking the mechanical effects in the fallopian tube	Microelectromechanical systems (MEMS): Four groups: (1) microdrop (control), (2) microdevice under static medium conditions, (3) microdevice under dynamic medium conditions at flow rate 0.1 μl/h and 4) at flow rate 0.5 μl/h, for 72 h	The static environment microdevice treatment group (group 2): significantly higher proportions of blastocysts and morulas and lower proportions of abnormal and eight-cell embryos, compared with the dynamic environment microdevice treatment groups (Groups 3 and 4)	55 2002
E3.5 or E2.5	Mouse	In vitro	Shear stress: mimicking the mechanical effects in the fallopian tube	1.2 dynes/cm^2 shear stress using a rotating wall vessel	Caused lethality within 12 h for E3.5 embryo The E2.5 embryo was more sensitive to shear stress than the E3.5 embryo	49 2006
Zygotes	Mouse	In vitro	Shear stress: mimicking the mechanical effects in the fallopian tube	Computer-controlled microfluidic culture platform Periodic fluid pulses of media (0.135 Hz) for 96 h	Increased percentage of hatching or hatched blastocysts (Microdrop-control 31%; Microfunnel-control 23%; Microfunnel-pulsatile 71%) and significantly higher average number of cells per blastocyst (Microdrop-control 67 ± 3; Microfunnel-control 60 ± 3; Microfunnel-pulsatile 109 ± 5)	57 2010
Embryos at 3 dpc (Morula)	Mouse	In vitro	Shear stress: mimicking the mechanical effects in the fallopian tube	Pulsatile delivery of medium once (1–2 min, flow (4–50 μl/h; maximum shear stress: 0.17 dyne cm^{-2}) in a nanoliter chamber	Much higher flow (4–50 μl/h) than that applied by Hickman et al. (0.1 μl/h or 0.5 μl/h) had no influence on embryo preimplantation rates, 1 time medium refreshment increased birth rates (28.8%) compared with that without medium refreshment (16.9%)	58 2013

(Continues)

TABLE 2 (Continued)

Type pf cells, tissue or organ	Species	In vitro or in vivo	Manipulation of mechanical properties/mechanical stimulation	Methods/Models	Main findings	Ref. year
Two-cell stage embryo	Mouse	In vitro	Mimicking the movement of a cleavage embryo in an oviduct	EWOD (electrowetting-on-a-dielectric) platform Controlled droplet velocity in dynamic (1 time (15) s/0.5 h, 60 voltage or 1 time (15 s)/2.0 h) EWOD chip or in a static EWOD chip (control)	The embryo development in dynamic culture with the droplet velocity (1 time 15 s /0.5 h): hatching earlier compared with the control	59 2015
IVM oocytes IVF was performed either in a 4-well dish (control) or in the oviduct-on-a-chip device	Bovine	In vitro	Uniform shear stress across the entire bovine oviductal epithelial layer under perfusion: mimicking the mechanical effects in the fallopian tube	A microfluidic "oviduct-on-a-chip platform": perfusion: 5 μl h^{-1} The average shear stress exerted on the embryos: 0.70 ± 0.46 dyne cm^{-2}	Both cleavage (56.0% vs. 84.4%) and 8–16 cells formation rates (36.7% vs. 53.7%) on chip were lower in a four-well dish	60 2018
Zygotes	Mouse		Substrate stiffness	Cultured on 3D type I collagen gels (1 kPa stiffness) or on the polystyrene petri dish (control)	Embryos cultured on 3D type I collagen gels increased in 2-cell, blastocyst, and hatching frequency and TE cell number Fetuses derived from embryos cultured on 3D type I collagen gels had a greater placental weight at E12.5	157 2012
Embryos from E5.0 to E5.75.	Mouse	In vivo	Relaxation of uterine smooth muscle contractions	Salbutamol (an activator of the β2-adrenoreceptor): three times every 6 h from E5.0 to E5.5	Little adverse effect on implantation and the early pregnancy phase of the uterus DVE-related markers, Cer1 and Hex: not expressed at E5.75. At E6.5, salbutamol-treated embryos expressed Cer1, an AVE marker, and T, a primitive streak marker, at a normal position	87 2020
Embryos 5.0 dpc	Mouse	In vitro	Mimicking mechanical forces from maternal tissues	Embryos cultured into the microcavities formed inside agarose gels of different stiffness	Agarose gels over 22.94 kPa stiffness: promote the elongated shape of the egg cylinder as well as DVE formation. Agarose gels under 7.29 kPa stiffness: cannot induce either an elongated shape of the egg cylinder or DVE formation	90 2013
			Mechanical forces due to the proper embryonic growth	Impairment of cell proliferation of embryos, treated with SB431542 (an inhibitor of activin receptor-like kinase receptor-dependent signaling)	Failure in DVE formation	
2/16-doublets of 16-cell-stage blastomeres	Mouse	In vitro	pMyosin-II-mediated contractility	Blocked by blebbistatin treatment (a selective myosin II ATPase inhibitor)	Both blastomeres to become inner-cell-like with respect to phosphorylated Yap localization and Cdx2 levels, despite their external position	96 2016

(Continues)

TABLE 2 (Continued)

Type pf cells, tissue or organ	Species	In vitro or in vivo	Manipulation of mechanical properties/mechanical stimulation	Methods/Models	Main findings	Ref. year
Embryos from E3.5 to E4.5.	Mouse	In vitro	Reduced luminal pressure	By leaky cell junctions Bb(−): a selective inhibitor of myosin II ATPase activity) By reduced cortical tension (heterozygous [Myh9+/−] embryos derived from m−/− Myh9+/− or m+/−Myh9+/−)	Reduced luminal expansion rate and blastocyst size	97 2019
			Softening the trophectoderm shell	Weakens cell–cell adhesion and tissue stiffness by using ECCD1 (an E-cadherin blocking antibody)	Increased cavity growth Decreased ratio of ICM to trophectoderm cells	
			Stiffening the trophectoderm shell	Activate cortical contractility: lysophosphatidic acid and calyculin A	Reduced luminal expansion rate and size	
			Reduced luminal expansion	Reduced actomyosin contractility: Bb(−) Heterozygous (Myh9+/−) embryos that have reduced cortical tension Reduced fluid influx: recombinant GST-C-CPE fusion protein (an inhibitor of claudin4 and claudin6, Tight-junction inhibition) Ouabain (a inhibitor of Atp1 Hypertonic)	A higher ratio of ICM to trophectoderm cells Occasionally did not spatially segregate into the inner and outer cells, as shown by Cdx2-positive cells in the ICM	
Embryos: E3.5–E4.0	Mouse	In vitro	Reduced lumen expansion	Ouabain	Significant reduction in the fluorescence levels of both EPI (Sox2) and PrE (Gata4) markers Significantly higher degree of overlap between EPI and PrE populations	99 2019
				Mechanically deflated through inserting a microneedle into the lumen at the junctions of mural TE cells and applying negative pressure to counteract expansion	PrE specification levels are significantly reduced, while EPI specification levels are maintained Significantly higher degree of overlap between EPI and PrE populations	
Embryos at E4.5	Mouse	In vitro	pMyosin-II-mediated contractility	Blocked by Blebbistatin at E4.5 for 20 h	Forced the mouse epiblast into a disk-like shape characteristic of human embryos.	104 2021
Endometrial stromal cells	Human	In vitro	Cyclic tensile stretch: mimicking the peristaltic motion of the uterine smooth muscle layer	Two cycles per minute (23-s stretch and 7-s release) for up to 24 h	Cyclic stretch stimulated IL–8 production	63 2005

(Continues)

TABLE 2 (Continued)

Type pf cells, tissue or organ	Species	In vitro or in vivo	Manipulation of mechanical properties/mechanical stimulation	Methods/Models	Main findings	Ref. year
Decidualized and non-decidualized endometrial stromal cells (ESs)	Human	In vitro	Cyclic tensile stretch: mimicking the peristaltic motion of the uterine smooth muscle layer	Two cycles per minute (23-s stretch and 7-s release) for up to 24 h	Cyclic stretch induced the secretion of IGFBP-1 from decidualized ESs No effect on non-decidualized ESs	64 2006
Endometrial stromal cells	Human	In vitro	Uniaxial cyclic strain: mimicking the peristaltic motion of the uterine	15% of uniaxial cyclic strain at 0.1 Hz for 7 days	Up-regulated alpha-smooth muscle actin expression and enabled the endometrial stromal cells to acquire contractility	62 2020
Co-culture Endometrial epithelial cell line (RL95-2) (EEs) and primary myometrial smooth muscle cells (MSMCs)	Human	In vitro	eristaltic wall shear stresses (PWSSs): mimicking the peristaltic motion of the uterine	omputed PWSSs (<0.05 kPa) for 0 (control), 60, and 120 min EEs: in direct contact with the shearing fluid.	Increased F-actin polymerization in EEs and MSMCs More pronounced in the EEs, than in the inner layer of MSMCs	65 2020
Co-culture primary human endometrial stromal and uterine microvascular endothelial cells	Human	In vitro	Continuous laminar shear stress (perfusion) of the endothelial compartment: mimicking hemodynamic forces derived from the blood flow	Microfluidic device: 1 μl/min for 14 days	Hemodynamic forces induced secretion of specific endothelial cell-derived prostanoids that enhanced endometrial perivascular decidualization via a paracrine mechanism	153 2019
Primary human endometrial epithelial cells (EEs)	Human	In vitro	Substrate stiffness: mimicking the mechanical cues from the ECM	Grown on polyacrylamide hydrogels (PGS) with different levels of stiffness (2, 4, 8, 16, or 30-kPa) or on plastic	EECs grown on 2-kPa PGS retained the epithelial-related phenotype, whereas those on 30-kPa PGS became elongated and showed F-actin + stress fiber-like structures. In EEs grown on plastic, only cells located in the center retained the epithelial-related phenotype. When EEs were stimulated with TGF-β1, cells began to undergo a partial epithelial to mesenchymal transition (EMT)-like process even on a soft matrix (2-kPa)	158 2017
Pluripotent stem cells	Human	In vitro	Substrate stiffness: mimicking the mechanical cues from the ECM	Cultured on polyacrylamide hydrogels with different levels of stiffness (3 or 165-kPa)	RNA sequencing analysis showed a substrate with a stiffness similar to the liver (3 kPa) triggered the expression of endoderm-specific genes (EOMES, SOX17, and FOXA2), whereas hard substrates (165 kPa) did not	159 2020

(Continues)

TABLE 2 (Continued)

Type pf cells, tissue or organ	Species	In vitro or in vivo	Manipulation of mechanical properties/mechanical stimulation	Methods/Models	Main findings	Ref. year
Primary isolated vCTBs from patients at the time of natural vaginal delivery or BeWo cells (human placental choriocarcinoma cells)	Human	In vitro	Substrate stiffness: mimicking the mechanical cues from the ECM	Cultured on substrates with different substrate stiffness: sub-physiological (0.1 kPa), normal (1.3 kPa), pathological (7 kPa; preeclamptic), and extreme pathological (17.4 kPa; preeclamptic)	Fusion ratio of vCTBs cultured on normal tissue stiffness (1.3 kPa): nearly twofold higher compared with those cultured on stiff substrates (7 kPa) BeWo cells grown on composite hydrogel substrate consisting of parallel soft (1.3 kPa) and stiff (17.4 kPa) strips: fusion ratio on both soft and stiff regions was significantly lower than on uniformly soft substrates	160 2020

Abbreviation: 3D, three dimensional; Atp1, a Na+/K+-ATPase activity; AVE, anterior visceral endoderm; DVE, distal visceral endoderm; E, embryonic day; ECM, extracellular matrix; Epi, Epiblast; GST-C-CPE, glutathione S-transferase (GST) tagged C-terminal fragment (C) of clostridium perfringens enterotoxin (CPE); ICSI, intracytoplasmic Sperm Injection; IGFBP-1, Insulin-like growth factor-binding protein 1; IL-8, interleukin 8; IVF, In vitro fertilization; IVM oocytes, In vitro matured oocytes.; kPa, kilopascals; PrE, primitive endoderm; TE, trophectoderm; vCTBs, villous cytotrophoblasts.

stromal cells.[23] The dominant paradigm is that the ovarian cortex, where primordial follicles reside during dormancy, is stiff versus the less dense medulla, where antral follicles vigorously remodel the ECM via proteolytic degradation as they reach the preovulatory stages.[24] The stiff cortex is considered to maintain quiescence of the immature primordial follicle pool.[24,25] Nagamatsu et al. showed that when whole mouse ovaries were incubated with a collagenase solution that digests ECM in the cortical region, translocation of FOXO3 into the cytoplasm of oocytes was observed at the edge of the cortical region, and some of the oocytes had started development into secondary follicles.[26] However, until recently, differences in the mechanical properties of the cortex and medulla had never been quantified. A very recent study mapped and quantified the mechanical microenvironment in mouse ovaries using colloidal probe atomic force microscopy.[27] The authors showed lower stiffness (Young's modulus, 2–3 kilopascals [kPa]) at the ovarian edge and center, which are dominated by extrafollicular ECM and higher collagen IV density, and the greatest stiffness (Young's modulus, ~7 kPa) in an intermediate zone dominated by large follicles with lower collagen IV density.[27] It has been postulated that the stiffest region of the ovarian microenvironment is the edge (cortex) of the ovary due to the high concentration of collagen.[24] However, there is an inverse relationship between collagen IV density and stiffness in mouse ovaries.[27] The prominence of ovarian cortical and medullary regionalization differs across species and is notably reduced in rodent ovaries compared with human ovaries.[28] The mouse is exceptional among mammals because its ovarian regionalization is much less relevant than that in other species.[28] Thus, findings in mouse ovaries may not be translated to human ovaries.[28] Large animal models, particularly bovine, equine, and ovine models, have been used to understand ovarian function in women.[29] Gargus et al.[30] assessed whole bovine ovaries ex vivo, using shear wave (SW) ultrasound elastography. They showed that the SW velocity was higher in the medulla than the cortex.[30] Although laboratory mice are not entirely suitable for investigation of mechanobiology in ovaries, recent studies in both murine and bovine ovaries have provided data contradicting the long-standing dominant paradigm of a stiff cortex versus Less stiff medulla.[27,30]

3.1.2 | Follicle rupture during ovulation

Numerous studies over time have shown that both mechanical and enzymatic factors are potentially involved in the mechanisms underlying follicle rupture during ovulation.[31,32] However, the precise underlying mechanisms remain to be clarified.[31,32] More than 50 years ago, the hydrostatic pressure within graafian follicles in rabbits was determined by direct cannulation using micropipettes.[33,34] Rondell[33] showed no significant measurable difference just before ovulation. Furthermore, artificially increasing intrafollicular pressure by fluid injection did not induce follicle rupture.[33] Tension-length diagrams for the elastic elements of the follicular wall showed that a large increase in extensibility precedes rupture.[33] He concluded that such

a change in the physical characteristics of the follicular wall may be involved in the mechanism of ovulation.[33] Similarly, Espey and Lipner[34] also hypothesized that structural changes in the thecal wall under the force of steady pressure in the antrum induce ballooning and follicle rupture. Later, another group also showed by direct cannulation using micropipettes no increase in intrafollicular pressure as ovulation approached in pigs.[35] Intrafollicular pressure in many follicles was greater than 30 cm H_2O (~22 mm Hg), and consistent with the findings by Rondell,[33] it was increased to over 400 cm H_2O (~294 mm Hg) by antral injection of mineral oil without follicle rupture.[35] Therefore, it had been considered that intrafollicular pressure might not contribute to follicle rupture but rather to a change in the physical characteristics of the follicular wall under the force of a steady pressure in the antrum.[33-35] However, the method used in those previous studies might not be capable of detecting subtle changes in intrafollicular pressure. In a later study, use of a servo-null micropipette system, which detects very rapid and subtle changes within very small fluid-filled spaces, showed a significant increase in intrafollicular pressure from the preovulatory phase (16.6 ± 1.0 mm Hg, diameter 1045 ± 84 μm) to the late ovulatory phase (23.9 ± 1.9 mm Hg, diameter 1071 ± 18 μm) in rat ovaries after hCG stimulation.[36] Those authors speculated that the increased intrafollicular pressure, along with enzymatic degradation of the ECM, may be important for follicle rupture before ovulation.[36] Further studies are required to investigate the role of mechanical and biochemical cues in follicle rupture during ovulation, using large animal models such as cows, horses, and sheep, which have been validated for their use in evaluating ovarian function in women.[29]

3.1.3 | Polycystic ovarian syndrome

The cortex of human ovaries in polycystic ovarian syndrome (PCOS) is harder than that of normal ovaries.[8,24,37] However, it remains inconclusive whether a difference in SW propagation exists in ovaries between women with PCOS and those with normal ovulation.[38-40] Previous studies using SW ultrasound elastography reported no significant differences in ovarian mechanical properties between PCOS and normally ovulating women.[39,40] However, those studies did not investigate the differences in the mechanical properties of the ovarian cortex separately from the medulla between women with PCOS and normally ovulating women.[38-40] In PCOS, intra-ovarian hyperandrogenism may promote early follicular growth, leading to follicles sized 2 ± 5 mm.[41] Three-dimensional (3D) culture of mouse follicles in alginate hydrogels resulted in high levels of androstenedione and progesterone in immature two-layered follicles, whereas androgen accumulation was not as pronounced in mature multilayered follicles cultured under increased matrix stiffness in alginate hydrogels (3%).[42] There is a marked but temporary reduction in ovarian androstenedione secretion in PCOS patients after wedge resection.[43] These findings suggest that the harder cortex in PCOS ovaries than normal ovaries may promote early follicular growth via excess ovarian androstenedione.[42,43] Ovarian wedge resection may temporarily promote ECM degradation, resulting in a marked but temporary reduction in ovarian androstenedione secretion.[43]

3.2 | Oocyte and embryo

Murayama et al.[44] measured the Young's modulus of the zona pellucida during oocyte maturation, fertilization, and early embryonic development in mice using a micro tactile sensor. The zona pellucida softened during oocyte maturation from the germinal vesicle (GV) oocyte (Young's modulus, 22.8 ± 10.4 kPa) to metaphase II (MII; 8.26 ± 5.22 kPa) stage.[44] Then, the zona pellucida hardened at the pronuclear (PN) stage (22.3 ± 10.5 kPa) to a similar level as that of the GV oocyte, followed by gradual softening as the embryo developed from the 2-cell (13.8 ± 3.54 kPa) to morula (1.88 ± 1.34 kPa) and early blastocyst (3.39 ± 1.86 kPa) stages.[44] Using an automated micropipette aspiration technique, Yanez et al.[45] also showed that oocytes soften as they mature from GV to metaphase I (MI) to MII. Other studies additionally demonstrated that human oocytes become softer during maturation, and an optimal stiffness range of the zona pellucida before fertilization is required for successful embryo implantation.[45-47] Using indentation measurements via atomic force spectroscopy, Andolfi et al.[46] investigated the mechanical properties of whole human oocytes approximately 3–4 h after retrieval under physiological conditions. Measurements were performed on immature (MI) and mature (MII) oocytes, and MII oocytes were further classified as ''suitable'' or ''rejected'' according to their morphological characteristics.[46] Those authors demonstrated that the zona pellucida outer layer is stiffer in immature (MI) than mature (MII) oocytes.[46] Furthermore, the zona pellucida outer layer of "rejected" MII oocytes was softer than that of ''suitable'' MII oocytes.[46] Another study measured the zona pellucida shear modulus of fresh human oocytes using computational methodology to calculate the mechanical parameters that govern zona pellucida deformation during a routine intracytoplasmic sperm injection (ICSI) procedure.[47] Then, those authors investigated the association between parameter C10, which represents the zona pellucida shear modulus, and implantation rate and showed that oocytes with C10 values of 0.20–0.30 and 0.30–0.40 kPa had the highest implantation rates (50% [6/12] and 88.8% [8/9], respectively).[47] Outside of these ranges, the average implantation rate was 6.70%.[46,47] After fertilization, optimal stiffness in the zygote is required for successful human embryonic development.[45-47] A study demonstrated that the viscoelastic properties of the zygote within hours after fertilization can predict blastocyst formation in humans and mice.[45] Appropriate changes in mechanical properties may be required to develop successfully, and too stiff or too soft zygotes may result in non-viable embryos.[45] RNA-seq data revealed that non-viable human embryos exhibit altered expression of several genes involved in fertilization, which could impair cortical granule release and zona pellucida hardening, thus affecting the mechanical phenotype of the embryo.[45] Non-viable mouse embryos had abnormalities in cortical granule release and speculated that abnormalities in oocyte softening and

maturation before fertilization and/or in cortical granule exocytosis at fertilization may result in different mechanics between viable and non-viable embryos.[45] A limitation of that study was that the mechanical properties were measured in the whole embryo.[45] The embryo is a mechanically heterogeneous structure consisting of relatively soft cells surrounded by a stiff zona pellucida. Thus, the contributions of separate structures (zona pellucida and cytoplasm) to the bulk mechanics and developmental potential of the embryo remain to be clarified.[45]

Blastocyst hatching, which is a crucial step in embryo implantation, was shown to be driven by mechanical forces in mouse embryos.[48] Using a hydrogel deformation-based method, the hydraulic pressure inside blastocysts was measured.[48] The blastocyst was mathematically modeled as a thin-walled pressure vessel.[48] The mouse blastocysts were cultured in polyacrylamide hydrogels with cylindrical cavities from the early cavity stage at ~3.5 days and during embryonic development; the hydraulic pressure and volume of the blastocysts increased, causing mechanical stretching and consequently decreased thickness of the zona pellucida.[48] This model demonstrates that volumetric expansion, rather than pressure generation, induces zona pellucida rupture and embryo hatching.[48] The mathematical model estimated that zona pellucida thickness decreases to one-third of its original thickness when embryo hatching occurs.[48]

3.3 | Oviduct and fallopian tube

Segmental muscular contractions in the oviduct and mechanical agitation of oviduct fluid by epithelial cilia result in mechanical effects on embryos, including shear stress by tubal fluid flow, compression by peristaltic tubal wall movement, and kinetic friction forces between the embryo and cilia.[49] Consequently, embryos are continually moving preimplantation, which provides a homogenous environment. Thus, dynamic culture platforms are required to provide an in vitro environment that recapitulates the in vivo growth environment more closely.[49]

To mimic mechanical stimuli similar to those in the fallopian tube (shear stress, compression, and friction forces), different culture systems have been developed. Matsuura et al. developed the tilting embryo culture system (TECS) to move embryos along the bottom of a dish at a rate of ~1 mm/min via the fluid motion of microdroplets.[50] They showed that the development rate of mouse blastocysts in the TECS was significantly better than that of static control blastocysts.[50] The TECS produced more high-grade blastocyst of fresh human embryos compared with a control static culture system.[51] Isachenko et al.[52] showed that mechanical micro-vibration at 44 Hz/h over 5 s intervals significantly increased the developmental competence and quality of human embryos in vitro. Mechanical micro-vibration of embryos in vitro resulted in a significantly higher pregnancy rate, compared with no micro-vibration, after transfer of day-3 embryos (78.4 ± 3.2% vs. 50.1 ± 4.9%) and day-5 embryos (72.2 ± 1.5% vs. 33.2 ± 2.4%) in women.[52] As both studies used conventional culture dishes placed on a moving platform inside an incubator,[51,52] clinical implementation of the techniques can be achieved easily. Further studies in human embryos may be required to investigate whether a significantly higher live birth rate can be achieved using this approach.

Kim et al.[53] compared the development rates of embryos cultivated in a straight channel versus constricted channels with different constriction widths (150 and 160 μm) to mimic peristaltic muscle contraction. Bovine embryos were incubated on a tilting machine, with a cyclic ±10° tilt over 1 min, to move the embryo via gravity.[53] They showed significantly more two-cell embryos developing into eight-cell embryos when cultivated in constricted channels with widths of 150 (37.8 ± 11.0%, mean ± SD) and 160 μm (56.7 ± 13.7%) compared with straight channels (23.9 ± 11.0%).[53] However, in their preliminary experiments, only ~10% of embryos cultivated in the constricted channels with a 140 μm width developed until the eight-cell stage, and many did not exhibit a healthy morphology.[53] Then, the same group evaluated the amplitude of the pressure and duration of the stimulus on the development of bovine embryos, to investigate the effect of defined embryo culture conditions on stimulation of the embryo in the fallopian tube.[54] They developed a microfluidic platform with varying patterns of compressive force via Polydimethylsiloxane membrane deformation independently controlled by a micro-modulated syringe pump. Bovine embryos were stimulated using a maximum pressure of 150 Pa over a duration of 12 s or 2 s.[54] After culturing in the device for 3 days, successful cleavage of non-stimulated embryos and of embryos mechanically stimulated for 2 s was observed.[54] On the other hand, the embryos mechanically stimulated for 12 s were underdeveloped.[54] These findings clearly showed that both the amplitude of the pressure and duration of the stimulus are significant factors affecting bovine embryonic development in vitro.[53,54]

Advances in microfluidic systems have enabled the development of automated approaches to achieve precise fluid flow.[49,53-60] In the early 2000s, studies showed that excess shear force may be harmful to mouse embryonic development.[49,53-55] Hickman et al.[55] developed a silicon-glass microdevice for in vitro manipulation of preimplantation embryos using microelectromechanical system technology. They evaluated the effects of dynamic medium conditions on the development of mouse embryos cultivated under four different conditions: microdrop (control), microdevice under static medium conditions, and microdevice under dynamic medium conditions at flow rates of 0.1 and 0.5 μl/h. Two-cell mouse embryos were cultured for 72 h.[55] Significantly higher proportions of blastocysts and morulae and lower proportions of abnormal and eight-cell embryos were observed in embryos cultivated in the microdevice under static medium conditions compared with dynamic medium conditions.[55] The authors proposed two potential explanations for this result: (1) both flow rates used were too high for mouse preimplantation embryos, and (2) necessary compounds in addition to waste products might have been washed away.[55]

Xie et al.[49] showed that shear stress at 1.2 dynes/cm^2 using a rotating wall vessel caused lethality in embryonic day (E) 3.5 (early

blastocyst stage) mouse embryos within 12 h. E2.5 (compacted 8-cell/early morula stage) embryos were more sensitive to shear stress than E3.5 embryos.[49] They speculated that the experimental shear stress at 1.2 dynes/cm^2 is presumably 10-fold greater than that of embryos in the oviduct.[49] Furthermore, the maximum velocity duration of embryos in the oviduct was shorter than 12 h.[49] It is presumed that the shear stress acting on embryos in the oviduct in vivo has low velocities or high velocities but short durations that prevent harm to the embryo.[55]

After these earlier studies showing the harmful effects of excess shear force on mouse embryonic development,[49,53-55] Heo et al. evaluated periodic fluid pulses in medium at physiological frequencies (0.135 Hz in rabbit oviduct[56]) using a computer-controlled microfluidic culture platform in mouse embryos.[57] The blastocyst developmental stage as well as the rates of embryo implantation and ongoing pregnancy was significantly higher under dynamic microfunnel culture conditions than static conditions (microdrop control and microfunnel static control). Embryo implantation and ongoing pregnancy rates under dynamic microfunnel culture conditions more closely resembled the rates obtained from in vivo blastocysts.[57]

Esteves et al. further showed that one-time pulsatile delivery of medium in a nanoliter chamber at 3 days postcoitum (dpc; morula stage) may benefit mouse embryonic development.[58] They showed that a transient (1–2 min) but much higher flow rate (4–50 μl/h; maximum shear stress: 0.17 dynes/cm^2) than that applied by Hickman et al.[55] (0.1 μl/h or 0.5 μl/h) had no influence on embryo preimplantation rates, and more importantly, this one-time medium refreshment increased birth rate (28.8%) compared with that without medium refreshment (16.9%).[58] Another group evaluated mouse embryonic development in an electrowetting-on-a-dielectric substrate.[59] The droplet velocity was controlled in the range of 0.3–1 mm/s to mimic the movement of a cleaved embryo in the oviduct.[59] Embryonic development from the 4- or 8-cell blastocyst stage in dynamic culture with a droplet velocity (15s/0.5 h, 60 V or 15 s/2.0 h, 60 V) was compared with the development of embryos in static culture.[59]

Ferraz et al. designed a microfluidic oviduct-on-a-chip device with uniform shear stress across the entire epithelial layer under perfusion (5 μl/h); bovine oviduct epithelial cells cultured in this device maintained their morphology and function, similar to in vivo oviduct epithelial cells. Using in vitro matured oocytes, in vitro fertilization (IVF) was performed either in a four-well dish or inside the microfluidic device.[60] The average shear stress exerted on the embryos was 0.70 ± 0.46 dynes/cm^2. Both cleavage (56.0% vs. 84.4%) and 8–16-cell embryo formation (36.7% vs. 53.7%) rates were lower on the microfluidic chip than on the four-well dish.[60] Nearly half of the mature oocytes/embryos had escaped through the pillars and subsequently either were lost during perfusion or became trapped between the pillars, which resulted in developmental arrest.[60] Furthermore, embryos trapped between the pillars were exposed to a maximum shear stress of 2.06 dynes/cm^2, greater than the stress (1.2 dynes/cm^2) that caused lethality within 12 h in E3.5 (early blastocyst stage) mouse embryos.[60] Thus, this oviduct-on-a-chip system did not mimic the in vivo oviduct environment.[60]

3.4 | Uterus

3.4.1 | Interaction between uterine peristaltic contractions and the endometrium

Three types of uterine peristaltic contractions can be distinguished during the menstrual cycle: cervico-fundal contractions, fundo-cervical contractions, and isthmical contractions.[61] Cervico-fundal contraction waves are seen during the follicular and luteal phases of the cycle, with a maximum frequency occurring during the preovulatory phase.[61] Maximum-frequency fundo-cervical contraction waves (fundo-cervical peristalsis) are seen during the late menstrual period, after which they decrease progressively and disappear nearly completely following ovulation.[61]

In vitro studies showed that mechanical strain is transduced into biochemical signals in the endometrium.[62-65] Kim et al.[62] showed that mechanical strain mimicking the peristaltic motion of the uterine smooth muscle layer via cyclic tensile stretch in vitro upregulated alpha-smooth muscle actin expression and enabled the endometrial stromal cells to acquire contractility. These findings suggest that the endometrium passively acquires new contractile functions in vivo under mechanical stimulation by the myometrium.[62] Myometrial contractions deform the endometrial layer and induce peristaltic intrauterine fluid flow.[65] Another study investigated the effects of peristaltic intrauterine fluid flow on the polymerization of F-actin filaments in endometrial epithelial cells co-cultured on top of myometrial smooth muscle cells, using a commercially available human endometrial epithelial cell line, RL95-2, and uterine muscle cells.[65] In contrast to the study by Kim et al.,[61] the endometrial epithelial cells were in direct contact with the shearing fluid.[65] The cyclic shear stress reached values as high as 0.045 Pa.[65] F-actin polymerization showed a more pronounced increased in the endometrial epithelial cells but was also increased in the inner layer of myometrial smooth muscle cells that were not in direct contact with the shearing fluid.[65] That study clearly showed that mechanical force can be applied at a distance and transmitted through another structure, which is a typical example of mechanical signaling.[65]

Clinical studies suggested that optimal cervico-fundal contractions are required to guide spermatozoa to the ovum during ovulation, and an overall dampening of endometrial wave activity during the secretory phase is necessary for successful embryo implantation.[66-69] In infertile patients with endometriosis, the mean overall peristaltic activity was significantly increased compared with healthy controls during the late menstrual, early and mid-follicular, and mid-luteal phases.[61] IL-8 may be involved in the pathophysiology of endometriosis.[70] IL-8 protein expression was highest during the late secretory and early proliferative phase in the endometrium of women without endometrial disease.[70] IL-8 stimulates endometrial cell proliferation and attachment to the ECM.[70] An in vitro study showed that cyclic stretch stimulated IL-8 production in human endometrial stromal cells (ESs).[63] These findings suggest that the greater peristaltic activity in patients with endometriosis may induce greater IL-8 production in the ESs of infertile patients

with endometriosis.[63] Another study by the same group also showed that mechanical stretch induced the secretion of IGFBP-1, a marker of decidualization, from decidualized ESs, but not from non-decidualized ESs.[64] Those two in vitro studies suggest that optimal, well-controlled uterine contractility throughout the menstrual cycle is required for normal endometrial physiology.[63,64]

Submucosal and intramural leiomyoma cause pregnancy loss or infertility.[71] Cine magnetic resonance imaging (MRI) detected abnormal peristaltic patterns in patients with uterine leiomyoma (submucosal $n = 6/7$, intramural and subserosal: $n = 3/4$) in the mid-luteal phase of the cycle.[72,73]A high frequency of uterine peristalsis (≥2 times/3 min) during the mid-luteal phase in patients with intramural leiomyoma significantly decreased pregnancy rate ($n = 0/22$) compared with that of the low-frequency group ($n = 10/29$).[74]The frequency of uterine peristalsis was normalized (0 or 1 time/3 min) after myomectomy in 14 out of 15 patients with intramural leiomyoma.[75]Abnormal uterine peristalsis during the mid-luteal phase might be one of the causes of infertility in patients with submucosal and/or intramural leiomyoma.

Previous in vitro studies suggested that mechanical uterine movements are transduced into biochemical signals in the endometrium.[62-65] However, comparison with precise in vivo measurements of mechanical stress by uterine peristaltic movements is ultimately required to validate these previous in vitro study findings.

3.4.2 | Endometrial stiffness and embryo implantation

Using 3D multifrequency magnetic resonance elastography, a study showed lower elasticity of the endometrium during the secretory phase than proliferative phase (1.97 ± 0.34 vs. 3.34 ± 0.42 kPa, mean ± SD).[76] Because the ECM composition generally determines the stiffness of a tissue,[77] that finding suggests that endometrial ECM stiffness is lower during the secretory phase than proliferative phase. An in vitro study showed that the stiffness of human ESs decreased during in vitro decidualization.[78] They speculated that the reduced stiffness of ESs was most likely attributed to the cytoskeletal changes promoted by mesenchymal-epithelial transition, including downregulated expression of vimentin, alpha-smooth muscle actin and myosin light chain kinase, and F-actin destabilization.[78] Myosin light chain kinase downregulation has also been shown to result in a decrease in MLC20 phosphorylation, thereby reducing the cell stiffness contributing to decidualized ES plasticity.[79,80] Thus, decidualized human ESs might be softer than non-decidualized ESs. Furthermore, studies in both mice and non-human primates suggested that changes in the surrounding ECM may differ between implantation and non-implantation sites.[81-83] A mouse study showed specific localization of lysyl oxidase (LOX), an ECM-shaping enzyme, in the stromal cells surrounding the implanting embryos.[83] LOX is a copper-containing amine oxidase that catalyzes the covalent cross-linking of collagen and elastin in the ECM.[84] The ECM, especially its interstitial collagens, exerts mechanical forces on surrounding cells, leading to the transduction of mechanical signals on the cell surface and the initiation of chemical cascades.[5,14,15] Thus, the ECM surrounding the implanted mouse embryos may increase matrix stiffness. In "fertile women" undergoing infertility treatment due to tubal ligation or male factor infertility, LOX expression in stromal cells was significantly higher during the mid-secretory phase (7–10 days post-ovulation) that is during the window of embryo implantation, than during the proliferative phase.[85] These findings suggest that the stiffness of human endometrial ECM during the implantation window may be modified. An in vitro study showed that the motility of human endometrial stromal cells is increased at the embryo implantation site.[86] The stiffness of cancer cells is inversely correlated with their migration and invasion through 3D basement membranes.[87] The decreased stiffness of decidualized human ESs may increase their migration capacity. In mice, blastocyst invasion and stromal cell migration were impeded by an inhibitor of Lox activity, β-aminopropionitrile.[83] Further studies are required to investigate whether spatial changes occur in the stiffness of the human endometrial ECM at the implantation site, and whether such changes, as well as decreased stiffness of decidualized ESs, are required during the initial phase of human embryo implantation.

3.4.3 | Effects of external mechanical cues from the uterine environment on mouse embryonic development

There is very limited information about how intrauterine pressure produced by uterine smooth muscle contractions is produced and accurately adjusted, and how it contributes to mammalian embryogenesis. Ueda et al. investigated the effects of intrauterine pressure on early mouse embryonic development.[88] In mouse embryos, after fertilization from E3.5 to E6.5, the amplitude of intrauterine pressure was the highest and most frequent at E5.5, just after implantation.[88] Mouse anterior-posterior axis polarization is preceded by formation of the distal visceral endoderm (DVE).[89,90] The mouse anterior-posterior axis is initially generated in a proximal-distal (P-D) direction at around 5.5 days post-coitum (dpc).[89,90] Those authors[88] showed that adjusting the intrauterine pressure within the sealed space between the embryo and uterus contributes to early egg cylinder morphogenesis in a P-D orientation. Then, they investigated the effects of relaxation of uterine smooth muscle contractions on mouse embryonic development.[88] Salbutamol, an activator of β2-adrenoreceptor, was administered three times every 6 h from E5.0 to E5.5, and the embryos were fixed at E5.75.[88] Salbutamol had little adverse effect on implantation and the early pregnancy phase in the uterus.[88] However, in salbutamol-treated embryos, the DVE-related markers Cer1 and Hex were not expressed, and Lhx1 and Lefty1/2 were not properly induced, at E5.75, whereas at E6.5, the anterior visceral endoderm (AVE) marker Cer1 and primitive streak marker T were expressed at the normal sites.[88] Salbutamol-treated embryos proceeded to undergo normal fetal development and delivery.[88] These findings suggest that intrauterine pressure is

necessary for timely specification of the DVE.[88] By culturing mouse embryos immediately after implantation in microfabricated cavities in vitro, the same group showed that the mechanical environment outside of the embryo can direct selective elongation of embryos in the P-D direction.[91] Furthermore, they showed that the mechanical forces induce a breach in the basement membrane barrier at the distal portion locally and induce transmigration of epiblast cells directly into the visceral endoderm.[91] By in vitro culturing of mouse embryos, they showed that confinement by a sufficiently stiff external environment is required for proper formation of the elongated egg cylinder shape and the DVE.[91] Stage 1 embryos (5.0 dpc) inserted into the cavities formed inside agarose gels of different stiffness levels.[91] Agarose gels with a stiffness over 22.94 kPa can promote the elongated shape of the egg cylinder as well as DVE formation, whereas agarose gels with a stiffness below 7.29 kPa cannot induce either an elongated egg cylinder shape or DVE formation.[91] Using atomic force microscopy, they validated that the in vivo stiffness of the decidual tissue surrounding the embryo, isolated at 5.5 dpc (23.26 ± 9.8 kPa, mean ± SD), is comparable with the gel stiffness sufficient for DVE formation.[91] Furthermore, impairment of cell proliferation in embryos treated with SB431542, an inhibitor of activin receptor-like kinase receptor-dependent signaling, resulted in failure of DVE formation.[91] These findings suggest that the mechanical cues arising from the appropriate physical growth of embryos in terms of size and the external geometrical constraints imposed by the uterine environment directly regulate the local breach in the basement membrane and transmigration of epiblast cells into the visceral endoderm at the distal tip, thereby establishing the mammalian primary body axis in the mouse embryo.[91] However, it remains to be clarified whether stimulated contractions and/or increased ECM stiffness at the site of embryo implantation in the human uterus occur after embryo implantation, and whether such external mechanical cues from the uterine environment are involved in human embryonic development, as shown in mouse embryos.

3.5 | Embryonic development: lineage specification and epiblast shape

The mammalian preimplantation embryo contains three cell lineages: pluripotent embryonic epiblast cells that generate embryonic tissues (EPI cells) and two extra-embryonic lineages, the trophectoderm, which contributes to the placenta, and the primitive endoderm, which contributes mostly to the extra-embryonic yolk sac.[89,90,92] The molecular mechanisms underlying the specification of these distinct lineages have been extensively studied in the mouse.[89,90] In the mouse, the first cell fate decision, in which outer cells are specified to differentiate into the trophectoderm and inner cells into the inner cell mass (ICM), involves differential Hippo signaling during compaction.[89,90] Differential FGF signaling during the blastocyst stage leads to the second cell fate decision, involving segregation of the EPI and primitive endodermal lineages within the ICM.[89,90,92] Comparatively little is known about the mechanisms of lineage specification in human embryogenesis.[90,92]

3.5.1 | Mechanical cues in the first cell fate specification in the mouse embryo

Fertilized mouse eggs undergo three rounds of cleavage, followed by polarization concomitant with compaction at the eight-cell stage.[89] From the eight-cell stage onward, asymmetric division rounds result in generation of two distinct cell types: those located inside versus outside of the embryo.[89] Cells that inherit the apical domain differentiate into the trophectoderm, whereas cells that do not inherit the apical domain and are internalized become the ICM (precursors of the embryonic epiblast and extra-embryonic primitive endoderm in mouse embryos).[89] Transcriptionally active Tead4 can induce the expression of Cdx2 and other trophoblast genes.[93] Nishioka et al.[93] showed that two components of the Hippo signaling pathway, Lats and Yap, are involved in the establishment of position-dependent Tead4 activity and cell fate specification. In mouse embryos, Yap, the coactivator of Tead4, localizes to the nuclei of the outer cells, whereas in the inner cells, Yap is phosphorylated in the cytoplasm by Lats-mediated inhibition of nuclear Yap localization.[93] It was shown that a combination of cell polarity and cell-cell adhesion establishes position-dependent Hippo signaling.[94] In the nonpolar inner cells, which have no contact-free surface, the junction-associated protein angiomotin localizes to adherens junctions (AJs), and cell-cell adhesion activates the Hippo pathway.[94] In the outer cells, cell polarity disconnects the Hippo pathway from cell-cell adhesion by sequestering angiomotin from adherens junctions, thereby suppressing Hippo signaling.[94] However, Anani et al. showed that cell-cell contact is involved in, but not essential for, the initiation of differential Yap localization in polar/apolar cells.[95] They showed that phosphorylated YAP (p-YAP) is differentially regulated between polar and apolar cells prior to localization to the outer/inner positions as a result of higher cortical tension in apolar cells than polar cells.[95] Later, another group showed that the apical domain is required and sufficient to control the first cell fate decision.[96] Their findings suggested that the difference in cell-surface contact is the crucial signal distinguishing the outer and inner positions, independent of Cdh1.[96] The apical domain emerges at the center of the contact-free surface, and the apical domain induces asymmetric division: The apical domain is inherited differentially by two daughter cells, one with and one without polarity.[96] Apolar cells compete for a position inside the embryo, and those that are pushed out toward the surface assume a position outside of the embryo.[96] They showed that Cdx2, a master regulator of the trophectoderm in mice, is specifically upregulated only in polar daughter cells.[96] Their previous study also showed that the apical domain exhibits reduced cortical contractility and distinct actomyosin contractility, and tension between polar and apolar cells triggers their sorting into the inner and outer positions and regulates Yap localization.[97] Without contractile forces, blastomeres adopt an inner-cell-like fate, regardless of their position.[97] When contractile

forces are blocked by treatment with blebbistatin, a selective myosin II ATPase inhibitor, phosphorylated Yap localization, and Cdx2 levels become homogeneous within doublets of 16-cell-stage blastomeres, regardless of their position.[97] These findings indicate that contractile forces are required for fate specification.[97] Altogether, these findings suggest that interactions among cell contact, polarity, and mechanics may transduce the signaling necessary for cell fate specification in mouse embryos.[93-97]

Furthermore, the fluid-filled lumen of blastocysts plays a key role in the control of embryo size and spatial allocation of the trophectoderm and ICM within cells, as demonstrated in mouse embryos.[98] In mice, mid-stage blastocysts (E4.0) have a lumenal pressure of approximately 650 Pa, which increases to ~1500 Pa by the mature blastocyst stage (E4.5), which translates into a concomitant increase in cell cortical tension and tissue stiffness in the trophectoderm lining the lumen.[98] Wang et al. also measured the inner pressure and Young's modules of the trophoblasts and ICM of blastocyst-stage mouse embryos using a laser-assisted magnetic tweezer technique.[99] The inner pressure levels of the embryo and Young's modulus of the trophoblast were ~3.7 and ~1.6 times higher, respectively, at E4.5 than at E3.0. The Young's modulus of the ICM increased 3.6 fold from E3.0 to E4.5.[99] Increased cortical tension leads to vinculin mechanosensing and maturation of functional tight junctions, which establish a positive feedback loop to promote lumen growth.[98] The presence of a cortical tension threshold leads to trophectoderm rupture and blastocyst collapse.[98] Tight junctions then reseal, and the blastocyst expands again, and the whole process repeats itself to enable size oscillations and size control.[98] Leaky cell junctions also lead to a reduced expansion rate and blastocyst size, and softening (or stiffening) of the trophectoderm shell leads to an increase (or decrease) in the expansion rate and size.[98] The blastocyst cavity shows an initial steady increase in volume.[98] Collapse events are rarely observed in the early- and mid-blastocyst stages, suggesting that high cortical tension may be required to "prime" junctional rupture during mitosis.[98] Inter-collapse intervals become more frequent toward the late blastocyst stage (E4.5).[98] Changes in cavity size or trophectoderm stiffness can independently affect lineage composition.[98] Embryos with a reduced cavity size occasionally do not spatially segregate into the inner and outer cells.[98] Furthermore, embryos under hypertonic conditions showed an increased frequency of an outer cell undergoing asymmetric division to generate one trophectoderm-forming and one ICM-forming cell.[98]

3.5.2 | Mechanical cues in the second cell fate specification in the mouse embryo

Once the mouse embryo reaches the blastocyst stage at E3.5, mouse embryos undergo a second cell fate specification event, in which the ICM segregates into pluripotent epiblast and primitive endoderm (in humans, extra-embryonic hypoblast).[89] Formation of a hollow cavity, the blastocoel, denotes the formation of the blastocyst, which by E4 (mouse) and E6 (human) is composed of three main tissues: epiblast, hypoblast, and trophectoderm.[89,90,92] Epiblast (Epi)-primitive endoderm (PrE) specification and spatial segregation within the ICM are dependent on luminal expansion in mouse embryos.[100] Luminal deposition of FGF4 can partially rescue EPI-PrE specification in embryos with reduced lumen expansion.[100] A potential explanation is that luminal expansion may increase the luminal FGF4 concentration to guide ICM differentiation.[100] These findings imply that chemical and physical cues are integrated to guide ICM self-organization in the mouse blastocyst.[100]

3.5.3 | Mechanical cues in human embryonic development

The molecular mechanisms that regulate the first cell fate decisions in the human embryo are not well understood.[90,92] However, studies showed significant differences in both the timing and expression pattern of transcriptional factors between mouse and human embryos.[89,90,92] In human embryos, lineage segregation between the ICM and trophectoderm occurs later than in mouse embryos.[89,90,92] At ~5 days post-fertilization, the blastocyst segregates into two lineages.[90,92] In contrast to mice, strong nuclear expression of YAP is also detected in ICM cells during the early stage of blastocyst formation (E5), whereas during the late blastocyst stage, YAP nuclear localization is restricted to the trophectoderm, similar to mice.[101] In mouse embryos, polarization determines p-YAP nuclear localization partly via mechanical control, as described above,[93-97] and thus controls the first lineage segregation. It is very likely that YAP is also required to mediate trophectoderm lineage segregation in human embryos, as dual suppression of YAP and TAZ strongly suppressed trophectoderm induction in human embryonic stem cells (ESCs).[102] However, in humans, pYAP is localized in the nucleus in both the ICM and trophectoderm during the early blastocyst stage (E5), and the trophectoderm at E5 can regenerate an ICM population.[103] These findings suggest that polarization alone cannot determine p-YAP nuclear localization and, thus, the first lineage segregation in human embryos. OCT4, an ICM-specific pluripotency marker, is initially expressed at the eight-cell stage, similar to mouse embryos, following genome activation and is expressed in all cells until the blastocyst stage at day 6, when OCT4 is restricted to EPI cells.[104] CDX2, a master regulator of the trophectoderm in mouse embryos, is first detected in human embryos at E5, in the trophectoderm of blastocysts following cavitation.[104] In contrast, in mice, Cdx2 initially exhibits mosaic expression at the 16-cell stage and is eventually uniformly expressed in all trophectoderm cells.[104] Thus, in human embryos at the early stage of blastocyst formation (E5), in contrast to mouse embryos, nuclear expression of YAP is detected in both the ICM and trophectoderm, whereas CDX2 expression overlaps the expression of OCT4 in nearly all trophectoderm cells, similar to the pattern in mouse embryos.[104] In human embryos, the first lineage segregation occurs at ~E5 following cavitation of blastocysts, when CDX2 is first detected.[90,92,104] Thus, the fluid-filled lumen of blastocysts, rather than cell polarization, may play a key role in the spatial allocation of

trophectoderm and ICM cells in human embryos. Further studies are required to clarify the role of the forces and changes in mechanical properties in fate determination in human embryos.

3.5.4 | Mechanical cues in the polar trophectoderm

Because of ethical and technical constraints limiting research, little is known about the early human embryo after the polar trophectoderm of day (D) 6 to D7 blastocysts adheres to the uterine wall.[90,92] Our knowledge has been limited mainly to the analysis of human embryo specimens from the Carnegie collection, which has been unable to provide insights into the dynamic process of human embryo implantation and what can go wrong.[90,105]

A very recent study demonstrated that the polar trophectoderm is an evolutionarily conserved regulator of epiblast shape after implantation.[105] Species-specific remodeling after implantation is likely due to differences in the behavior of the trophectoderm.[105] During the transition from pre- to post-implantation (E4.5 to E5.0), the mouse epiblast transforms from an oval to a cup shape.[89,90,105] In mouse embryos, implantation and invasion into the maternal endometrium are mediated by the mural trophectoderm.[89,90,105] Increasing contractility and tension in the polar trophectoderm generate a physical force to push the epiblast into its post-implantation configuration, which leads to the acquisition of the cup shape.[105] Further apical constriction of the polar trophectoderm results in formation of the post-implantation egg cylinder.[105] In contrast, implantation and invasion of human embryos into the maternal endometrium are mediated by the polar trophectoderm,[90,92] resulting in pulling and stretching of the embryo.[105] The human embryo does not exhibit any sign of horizontal constraint, and polar trophectoderm stretching alone could shape the epiblast into a disk structure.[105] Epiblast shape was analyzed after implantation of human embryos from the Carnegie collection.[105] It was confirmed that human epiblasts initially form an oval shape but then become disk-like, growing horizontally but not vertically.[105] Rock inhibition of mouse embryos by blebbistatin treatment for 20 h at E4.5 prevented pMyosin-II-mediated contractility and forced the epiblasts into a disk-like shape characteristic of human embryos.[105]

Altogether, these findings suggest that physical cues play an essential role in controlling embryonic development in humans but potentially via different mechanisms compared with those in mice.[89-105] Greater insight into early human embryonic development will be achieved using recent technological advancements, including CRISPR-Cas9-mediated genome editing,[106] novel imaging techniques with new computational methods that enable high-resolution fate maps and maps of tissue morphogenesis at the single-cell level,[107] a new 3D blastocyst culture system enabling human blastocyst development up to the primitive streak stage (D14),[108] human ESCs that mimic different aspects of embryonic development in vitro,[109,110] and development of self-renewing human trophoblast stem cells[111] and trophoblast organoids[112,113] as well as endometrial epithelial organoids (Figure 1).[114,115]

3.6 | Early placental development

The placenta plays a critical role in reproductive success, and it may also be associated with susceptibility to chronic diseases in adults.[116,117] Despite the importance of the placenta, little is known about early placental development in humans.[118] The ethical and technical constraints on research, the high divergence of the placenta across different species, and the lack of physiologically relevant *in vitro* models complicate animal and in vitro experiments.[118]

The stiffness of fresh human samples of first-trimester placental and decidual tissues (6–12 weeks of gestation) and secretory-phase endometrial tissues (7–10 days after the preovulatory luteinizing hormone surge) was measured using atomic force microscopy.[119] Nonpregnant secretory-phase endometrial, decidua parietalis, and placental tissues had a stiffness of 102 Pa, whereas the decidua basalis was an order of magnitude stiffer at 103 Pa.[119] They speculated that the increase in stiffness in the decidua basalis is likely due to changes induced by extravillous trophoblast (EVT) invasion; this is because the pre-decidualized endometrium and fully decidualized decidua parietalis have similar stiffness values, and thus changes in the ECM during decidualization before embryo implantation do not drive the change in tissue stiffness.[119] As EVTs invade, they degrade stromal ECM proteins while simultaneously producing their own distinct ECM networks.[119-121] Implantation involves invasion of the embryonic trophoblast through the endometrial stroma.[86] Decidualization is accompanied by metalloproteinase (MMP)-dependent ECM degradation.[122] A potential underlying mechanism of the increase in stiffness in the decidua basalis as a result of EVT invasion may be partly explained by epithelial to mesenchymal transition (EMT), which occurs during physiological EVT differentiation.[123,124] Mechanical cues, including matrix stiffness, tissue geometry, and intracellular forces, could act in concert with biochemical signals to regulate EMT.[125-128] EMT has been extensively studied in numerous cancer cells, and increased matrix stiffness in the tumor microenvironment induces tumor cell migration and invasion through the EMT.[125-128] It remains to be clarified when and how the tumor ECM increases matrix stiffness. However, studies have suggested that tumor stiffening is mediated by tumor cells themselves.[125] Cells undergoing EMT feed forward to enhance matrix remodeling via a positive feedback loop.[125] Tumor cells undergoing EMT can in turn modulate the mechanical properties of the ECM by increasing cell contractile forces, inducing extensive deposition of ECM proteins, and inducing cancer-associated fibroblasts (CAF)-mediated ECM remodeling to further stiffen the ECM.[125] These findings suggest that EVT invasion via EMT itself may increase the stiffness of the decidua basalis. YAP/TAZ, the key transcriptional coactivator in the Hippo pathway, is regulated by various mechanical cues, including matrix stiffness, cell-cell contact, and cell shape.[129] TAZ was found to be weakly expressed in the villous cytotrophoblasts (vCTBs) and cell column trophoblasts (CCTs) of early placental tissues.[130] TAZ promotes EMT in various cancer cells,[131-133] in turn inducing EVT invasion, which may increase the stiffness of the

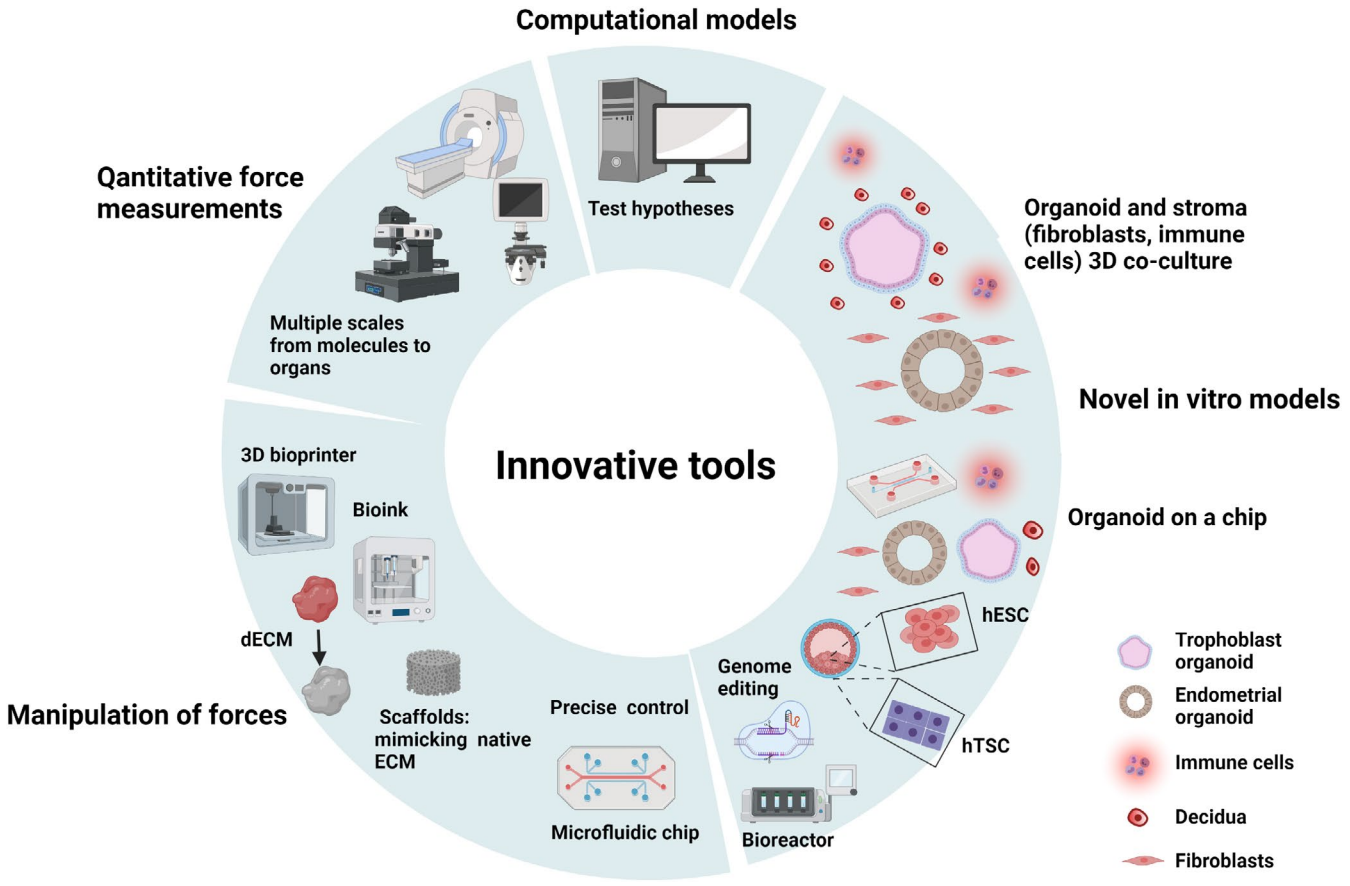

FIGURE 1 Recent and future developments of innovative tools for mechanobiology. The recent and future developments of novel tools (quantitative force measurements, manipulation of forces, novel in vitro models, computational models) will help us broaden our understanding of the mechanobiology of the female reproductive system. 3D, three dimensional; ECM, extracellular matrix; dECM, decellularized ECM; hESC, human embryonic stem cell; hTSC, human trophoblast stem cell [Colour figure can be viewed at wileyonlinelibrary.com]

decidua basalis. In contrast to TAZ, YAP was strongly expressed in the nuclei and cytoplasm of vCTBs and CCTs; however, low levels were detected in EVTs.[130] YAP expression is primarily associated with trophoblast stemness and proliferation.[130] YAP is a cofactor for TEAD4, and both are specifically expressed in CTB progenitors in the first-trimester human placenta.[134] The TEAD4/YAP1 complex is essential for trophoblast progenitor self-renewal.[134] TEAD4 expression in vCTBs and CCTs is strongly reduced in patients experiencing idiopathic recurrent pregnancy loss (RPL).[134] Human trophoblast stem cells from RPL patients showed a strong reduction in proliferation and a prominent defect in placental villus formation.[134] Rescue of TEAD4 expression restored the self-renewal ability of trophoblast stem cells from RPL patients.[134] Impairment of the Hippo signaling pathway could be a molecular cause of early human pregnancy loss. YAP/TAZ is regulated by various mechanical cues.[16] Further studies are required to determine whether and how mechanical cues play a role in early human placental development, and whether mechanical perturbation results in early pregnancy loss. The recent development of novel tools, such as self-renewing human trophoblast stem cells[111] and trophoblast organoids,[112,113] may further improve our understanding of early human placental development (Figure 1).

4 | EXPERIMENTAL TOOLS

A major challenge in mechanobiology is investigation of the cellular and molecular mechanisms linking mechanical forces to biological responses and understanding how these cellular and molecular events contribute to development, physiology, and disease.[135] Progress in the field of mechanobiology has been limited by the lack of appropriate experimental tools for (1) quantitative force measurement and (2) force manipulation.[135]

Traditional animal models and standard two-dimensional (2D) in vitro cell culture are not appropriate due to poor control of applied force and highly artificial cell culture environments, which do not recapitulate the in vivo situation.[134] Recent technological advancements have contributed to our understanding of the role of mechanical cues in both physiology and diseases.[135-140]

4.1 | Force measurements

Measurements of forces have been critical in enabling the growing field of mechanobiology (Table 1).[134-139] There are two types of tools for force measurements: one measures the forces actively generated

by cells without applying any external force, and the other measures cellular responses to mechanical forces or mechanical quantities such as cell stiffness.[135-140] Over the past 20 years, a wide repertoire of techniques has been developed to measure forces.[135-140] These techniques, based mostly on ex vivo and in vitro conditions, have been extensively reviewed in.[135-140]

However, these techniques are still far from becoming routine biological laboratory tools because multidisciplinary expertise is required to conduct and interpret the measurements.[135-140] Furthermore, quantitative comparisons of the mechanical properties measured by different methods are limited.[140] Wu et al. evaluated and compared the mechanical properties of MCF-7 human breast cancer cells cultured under the same environmental conditions in vitro using well-established techniques (atomic force microscopy, magnetic twisting cytometry, particle-tracking microrheology, parallel-plate rheometry, cell monolayer rheology, and optical stretching).[141] They showed that the mechanical properties of MCF-7 human breast cancer cells can vary by orders of magnitude, depending on the length scale at which cell viscoelasticity is probed (eg, from the diameter of an actin fiber to the size of a whole cell).[141] Those authors clearly showed that cell mechanics depend on the level of mechanical stress and rate of deformation to which the cell is subjected, the geometry of the mechanical probe used in the experiments, the probe-cell contact area, the probed location in the cell, and the extracellular context.[141] Janmey et al.[142] speculated that these differences in mechanical properties probably reflect the heterogeneous structure of the cell and noncontinuous nature of the cytoskeleton more than the inherent uncertainty in measurements by any single method. Thus, it may also be true that quantitative comparison among different cells even when using the same methods may be limited. Measurement of the stiffness of a tissue includes the cellular, nuclear, and ECM stiffnesses as a whole. However, in general, the composition of the ECM determines the stiffness of a tissue.[143,144] Dynamic cellular interactions and remodeling processes in the ECM result in heterogeneity in ECM mechanical properties.[145] Thus, when measuring the stiffness of different tissues (eg, diseased versus their counterpart normal tissues) using the same methods, caution in interpreting the results may be required. Furthermore, the measurement of cell and tissue mechanical properties in vivo is not completely feasible and extremely challenging.[135] In vivo, cells are connected to a host of materials and other cells, all of which contribute to the generation and propagation of cellular forces.[135,137] Very recently, a 3D magnetic device was developed to quantify the spatial stiffness distribution in living mouse embryos; it can map the absolute stiffness and viscosity of bulk tissues that are not influenced by morphogenetic cell behaviors.[146]

Currently, in the clinical setting, elastography techniques based on ultrasonography, optical coherence tomography, and MRI are widely used for noninvasive measurement of in vivo mechanical properties in patients.[147,148] Elastography techniques are very useful to measure the in vivo mechanical properties of bulk tissue and provide valuable information for diagnosis and therapy.[147,148] However, they lack the resolution to dissect dynamic cell-cell and cell-ECM interactions, spatiotemporal changes in the ECM, or internal or external mechanical forces.[136]

4.2 | Manipulation of forces

4.2.1 | Microfluidics

Microfluidics represents the engineered manipulation of fluids, usually in the range of microliters (10^{-6}) to picoliters (10^{-12}), in networks of channels with dimensions of tens to hundreds of micrometers to provide precise control of fluids at microliter volumes (Table 2).[149,150] In the human reproductive system, microfluidics was proposed in the 1990s as a new approach to minimize the specialized requirements required for successful IVF.[151-153] Several groups have developed microfluidic technology platforms to evaluate the female reproductive system.[151-153]

Gnecco et al.[154] developed an organ-on-a-chip microfluidic model of the human endometrial perivascular stroma to examine the crosstalk between stromal and endothelial cells. Primary human uterine microvascular endothelial cells were seeded on the top chamber and primary human ESs on the bottom chamber of the chip.[154] The microfluidic device provided continuous laminar shear stress (perfusion) to the endothelial cells to mimic hemodynamic forces derived from blood flow.[154] The work of Gnecco et al.[154] demonstrated that hemodynamic forces induce secretion of specific endothelial cell-derived prostanoids that enhance endometrial perivascular decidualization via a paracrine mechanism.

Several groups have developed microfluidic technology platforms that mimic mechanical stimuli similar to those in the oviduct in vivo to investigate the effects of mechanical cues on embryonic development.[57-59,151-153] Furthermore, a microfluidic oviduct-on-a-chip platform was developed to maintain the morphological and functional structure of oviduct epithelial cells, similar to the in vivo oviduct.[60] For more details, please see the above section regarding the effect of mechanical cues in the oviduct on embryonic development.

4.2.2 | Cell-ECM interactions

A hydrogel is a water-swollen, physically or chemically crosslinked 3D network of hydrophilic polymer chains.[155,156] As naïve-ECM mimics, both natural hydrogels, formed from natural polymers, and synthetic hydrogels, formed from synthetic polymers, have been widely used to investigate the effects of ECM mechanical properties on cell function.[155-157]

In the field of female reproduction, alginate hydrogels have been used to investigate secondary follicle growth in mice.[42] The growth of mouse secondary follicles was investigated on 3D alginate hydrogels with varying shear elastic modulus values: high (3% alginate), intermediate (1.5% alginate), and low (0.7%, oxidized and irradiated alginate).[42] Follicles cultured on the 0.7% (low) alginate hydrogels

showed increased follicle growth, higher rates of antral and thecal layer formation, and higher oocyte quality.[42] In another study, mouse embryos cultured on 3D type I collagen gels (1 kPa stiffness) had increased two-cell, blastocyst, and hatching frequencies and TE cell numbers compared with embryos cultured on polystyrene petri dishes.[158] Furthermore, fetuses derived from embryos cultured on collagen in vitro had a greater placental weight at E12.5.[158] There are many advantages to using natural hydrogels, such as they are biocompatible and bioactive, and the abundant endogenous factors promote the functions of many cell types.[155-157] However, there are some disadvantages, including low stiffness, limited long-term stability, and batch-to-batch variability.[155-157] Particularly, for mechanobiology studies, fine tuning the mechanical properties of natural hydrogels is difficult.[155-157] Tunable synthetic hydrogels may be more suitable as an experimental tool to finely control hydrogel stiffness.[155-157] The most common tool used for culturing cells on substrates of variable stiffness is polyacrylamide hydrogels, which can be easily fabricated using standard chemical techniques, and stiffness can be precisely adjusted by mixing various acrylamide (monomer) and bis-acrylamide (cross-linker) concentrations.[155-157] One major limitation is that polyacrylamide hydrogels cannot be used to encapsulate cells three-dimensionally due to toxicity of the hydrogel precursors.[154-156]

Our group used polyacrylamide hydrogels to investigate the effects of matrix stiffness on human endometrial epithelial cells (EEs).[159] Endometrial epithelial cells were grown on polyacrylamide hydrogels with varying levels of stiffness (Young's modulus, 2, 4, 8, 16, or 30 kPa) or on plastic.[159] The cells grown under 2 kPa retained their epithelial-related phenotype, whereas those grown under 30 kPa became elongated and showed F-actin-positive stress-fiber-like structures.[159] Among the cells grown on plastic, only those located in the center retained their epithelial phenotype.[159] Endometrial epithelial cells stimulated with TGF-β1 underwent a partial EMT-like process even on a soft matrix (2 kPa Young's modulus).[159] A recent study investigated the effects of controlled substrate stiffness, mimicking human tissue mechanics, using polyacrylamide hydrogels, on human pluripotent stem cells (hPSC) lineage specification.[160] RNA sequencing analysis showed that a substrate with a stiffness similar to that of the liver (3 kPa) triggered the expression of endoderm-specific genes (*EOMES*, *SOX17*, and *FOXA2*), whereas harder substrates (similar to bone or tissue culture polystyrene) did not.[160] The lncRNA *LINC00458* was identified as a critical regulator of endodermal lineage commitment in response to ECM stiffness.[158] Primary vCTBs isolated from patients at the time of natural vaginal delivery or BeWo cells (human placental choriocarcinoma cells) were cultured on substrates with different substrate stiffness levels: sub-physiological (shear elastic modulus, 0.1 kPa), normal (1.3 kPa), pathological (7 kPa; preeclamptic), and extreme pathological (17.4 kPa; preeclamptic).[161] The fusion ratio was nearly two-fold higher for vCTBs cultured on a substrate of normal stiffness (1.3 kPa) compared with pathological stiffness (7 kPa).[159] Furthermore, that study showed that when BeWo cells were grown on a composite hydrogel substrate consisting of parallel soft (1.3 kPa) and stiff (17.4 kPa) strips, the fusion ratio was significantly lower for both the soft and stiff regions compared with uniformly soft substrates, suggesting that fusion of trophoblasts grown on tissues of normal stiffness could be affected by adjacent pathologically stiff regions.[161] Matrix stiffness may have an indirect effect from a distance by being transmitted through another structure.[161] This is a good example of how mechanical cues can be transmitted through another structure.[12]

4.3 | Computational mechanobiology models

Although computational models of the female reproductive system are not very new, until now, very limited studies have developed computational mechanobiology models to investigate the female reproductive system,[162] partly because of the lack of high-quality experimental data on mechanobiology.[163] More than 50 years ago, computational models were used to reveal theoretical ovum transport mechanisms in the oviduct.[164,165] In 1980, Verdugo et al.[164] used a stochastic model to show that both ciliary forces and random tubal contractions are involved in ovum transport in the ampulla of the rabbit oviduct. Later in 1983, Blake et al.[165] demonstrated steady transport of the ovum, whereas muscular activity resulted in highly oscillatory motion, using a simple fluid dynamic model of ovum transport in the ampulla and isthmus of the human oviduct. A sequence of contractions in the uterine direction is required for ovum transport.[165] Eytan et al.[166] developed computational models to investigate the effects of uterine contractions on the site of embryo implantation. Their computational model of uterine fluid motion simulated by a finite, uniform 2D channel indicated that the magnitude of uterine contractions may be key in determining the success and site of implantation.[166] Then, using the same model, they showed that an increased pressure gradient within the fallopian tube in hydrosalpinx generates reflux currents that can push the embryo away from the implantation site.[167] To simulate the sagittal cross section of the uterus more accurately, those authors expanded their model to a 2D finite nonuniform channel and showed that transport phenomena were maximal when wall displacements were symmetric and the inclination angle small.[167] The intrauterine flow induced by relatively symmetric uterine contractions and the small angle between uterine walls may accelerate transport of the embryo away the implantation site.[167] Normal placental development is critical for reproductive success.[118] However, how the human placenta develops has not been clarified.[118] In vivo experiments of early human pregnancy are prohibited for ethical reasons.[118] Furthermore, there is great variation in placental types across mammals.[118] A computational model may be promising for investigating the development of the human placenta. Rejniaka et al.[168] developed a computational model of the growth mechanics of the trophoblast bilayer (inner CTBs and the outer syncytiotrophoblasts) in chorionic villi. This model revealed different viscoelastic properties of the three main components of the trophoblast tissue (basement membrane, CTBs, and syncytium), of which the syncytium layer is the most flexible and the basement

membrane most rigid.[168] The model also captured the mechanics of the viscous, incompressible fluid in which the tissue is immersed.[168] Three critical cell processes for whole placental development, division, growth, and fusion, were incorporated.[168] The model showed that the stiffness of these two layers of trophoblasts affects the shape of CTBs and the whole trophoblast.[168]

5 | DISCUSSION—PERSPECTIVES AND CHALLENGES

5.1 | Clinical implications

Any changes in the extracellular environment, cell structure, the cellular mechanosensing process itself, or the involved downstream signaling pathways can result in several disease conditions.[12,169,170] To understand how mechanical factors are involved in disorders of the female reproductive system, it is first critical to determine how they are involved in the physiology of the female reproductive system. Studies have shown that well-controlled mechanical cues are important in the early development of the embryo in mouse models.[88,91,93,102,105] New insights gained from previous studies may have translational applications.

5.1.1 | Uterine contractions

Recent mouse experiments showed that the external geometrical constraints imposed by the uterine environment are an important determinant of early mouse embryonic development.[88,91] It is speculated that mechanical imbalances among the embryo, decidua, and uterine muscle contractions may adversely affect early embryogenesis in utero and thereby result in spontaneous abortion at an early stage of mammalian pregnancy.[88,91] Peristaltic contractions of the human uterus throughout the menstrual cycle have been investigated extensively.[61,66-69] However, to date, no clinical study has focused on the role of uterine peristaltic contractions in human embryonic development after embryo implantation. Early pregnancy loss is a major clinical problem in reproductive health.[171] Furthermore, diseases associated with disturbed mechanotransduction signaling include developmental defects, such as congenital heart diseases.[170] Although extremely challenging, further investigation into whether and how mechanical imbalances among the embryo, decidua, and uterine muscle contractions affect early human embryonic development and induce early human pregnancy loss and/or developmental defects will provide insights on early pregnancy loss and developmental defects. To address these questions, mouse models are very useful, and eventually, we may be able to address these questions in women. Today, we can investigate whether and how uterine contractions occur after embryo implantation in women by MRI-based motion tracking, as used in one study to evaluate uterine contractions in 46 healthy pregnant women during the second trimester.[172] In that study, uterine contractions were detected on 18.6% of the MRI scans (11/59) at 14–18 weeks of gestation, increasing to 26.5% (14/53) at 19–24 weeks of gestation.[172]

5.1.2 | Improvements in IVF/ICSI outcomes

Oocyte/embryo selection

Previous studies have suggested that the biomechanical properties of oocytes/embryos are potentially novel biomarkers for selecting high-quality oocytes/embryos, leading to better IVF/ICSI outcomes.[45-47,152,153] However, before use of these biomarkers can become routine in clinical practice, there are many technical challenges that we must overcome.[152,153] First, we need to validate whether the biomechanical properties of bulk embryos are appropriate biomarkers.[45] It is very likely that a stiff zona pellucida and soft cytoplasm have distinct impacts on embryonic developmental, and thus a new method may be required to measure the mechanical properties of each structure individually.[45] Consequently, no invasive automated assessment of oocyte/embryo mechanics would be required.[152,153] Microfluidic technology may have great potential application in such assessments, although it is still far from routine use even in basic research.[152,153] Second, well-controlled prospective clinical studies are required to determine whether noninvasive, automated assessment of oocyte/embryo mechanics in terms of morphology is superior to conventional selection.[45-47,152,153] It is important to determine whether the assessment of mechanical properties can provide a clinical benefit for IVF patients.[152,153] Recent studies clearly showed high regulative flexibility of human preimplantation embryos, which may partly explain why successful live births can result from transfer of embryos of poor quality in terms of morphology.[102,103] It is clear that new biomarkers are required to select high-quality oocytes/embryos[173]; however, it remains unclear whether the biomechanical properties of oocytes/embryos are potential biomarkers.[152,153]

Mechanical stimuli similar to those in the fallopian tube

To simulate mechanical stimuli (shear stress, compression, and friction force) similar to those in the fallopian tube,[49] different culture systems have been developed.[49-55,57-60] In the early 2000s, studies showed that excess shear forces were harmful to mouse embryonic developments.[49,53-55] Subsequently, several groups showed that transient mechanical stimuli may benefit embryonic development.[57-59] Hydrosalpinges have a deleterious effect on embryonic development, and hydrosalpinx induces an increased pressure gradient within the fallopian tube.[174] Higher and continuous shear stress in hydrosalpinx may partly explain the deleterious effect of hydrosalpinges on embryonic development.[49,53-55,60,174] However, to date, there is no robust evidence that integration of physiological, mechanical stimuli mimicking those of the Fallopian tube has any clinical benefit on human oocytes/embryos during IVF procedures.

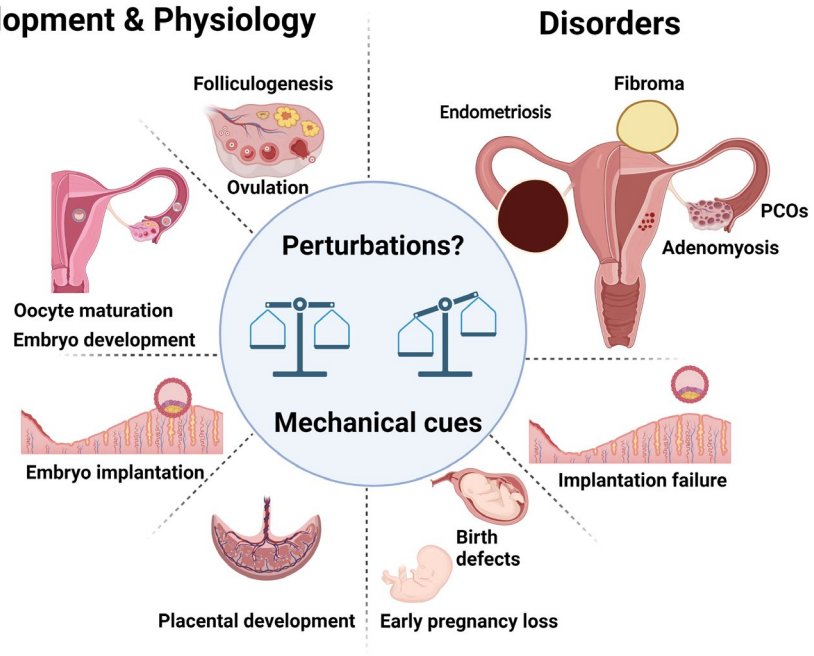

FIGURE 2 Potential involvement of perturbations in mechanical cues in disorders/diseases of the female reproductive system. Little is known about how mechanical perturbations result in the related disorders/diseases of the female reproductive system. Efforts to gain new insights into the mechanobiology of the female reproductive system will help us broaden our understanding of, and develop new strategies for, the treatment and/or prevention of these clinically important disorders/diseases in women [Colour figure can be viewed at wileyonlinelibrary.com]

5.1.3 | Disorders/diseases of the female reproductive tract

In this review, I focused on the early phases of reproduction from oocyte development to early embryonic development. However, perturbations in mechanical cues could be involved in many disorders/diseases of the female reproductive tract, such as preterm birth, cervical incompetence, intrauterine growth restriction, endometriosis, adenomyosis, uterine fibroma, and genital prolapse.[5-7,9,10,169,170] Efforts to gain new insights into the mechanobiology of the female reproductive system will help us broaden our understanding of, and develop new strategies for, the treatment and/or prevention of these clinically important disorders/diseases in women (Figure 2).[5-7,9,10,168,169]

5.2 | Immune system: mechanoimmunology

Immune cells migrate within varying environments via the blood and lymphatic circulation to perform immunosurveillance and effector functions.[175] Therefore, it is no surprise that immune cells experience a wide range of mechanical forces, from piconewtons at the nanoscale level to several orders of magnitude higher at the tissue level.[175-179] Thanks to recent technical advances in imaging modalities and biophysical tools, growing evidence indicates critical roles of mechanical forces in immune cell functions.[175-179] Endogenous mechanical forces from the ECM or neighboring cells, as well as exogenous mechanical forces, act at the cellular level in immune cells, similar to other cells described above.[175-179] Furthermore, immune receptors themselves respond to mechanical stimuli during antigen recognition, and thus lymphocyte activation is a mechanosensitive process.[175-179]

The immune system drives a range of reproductive processes and normal physiological interactions between the female reproductive and immune systems, which are critical in female reproductive health.[180-183] The field of mechanoimmunology is still in its infancy.[175-179] Furthermore, reproductive immunology has been incredibly difficult to understand for both clinicians and scientists.[180-183] However, because of the important roles of the immune system and mechanical cues in the female reproductive system, the field of "reproductive" mechanoimmunology will contribute to the future directions of basic research and clinical applications. This will be an immense challenge. At the very least, as Jain et al. clearly stated "We should keep in mind that any experimental data, without carefully consideration of the importance of physical forces and factors, may lead to the wrong assumptions[176].

5.3 | Mechanobiology of the ovary

The most essential and fundamental questions to be addressed first are whether mechanical properties differ between the two major compartments of the ovary (cortex and medulla) in normally ovulating human ovaries, and whether the difference is involved in dormancy and activation of primordial follicles, followed by folliculogenesis. Without this knowledge, we cannot understand the true mechanisms of human folliculogenesis and related disorders such as PCOS and primary ovarian insufficiency. In such disorders, the mechanical properties of the different compartments may differ from those of normally ovulating ovaries.[8,24] Because of ethical limitations in human investigations, and because the laboratory mouse is not entirely suitable for investigating the mechanobiology of human ovaries,[28] large animal models, particularly cows, horses, and sheep, which have been validated for evaluation of ovarian function

in humans, will be appropriate for addressing these questions.[29] Colloidal probe atomic force microscopy will be useful to map and quantify the mechanical microenvironment across different compartments as well as that surrounding follicles at different stages, in large animal models, as a very recent study demonstrated in mouse ovaries.[27] A new multipole magnetic device was recently developed for 3D mapping of the absolute stiffness and viscosity of tissue in a nontoxic manner.[146] This device will also be useful, although some modifications may be required, for quantification of the spatial stiffness distribution in the ovaries of large animals.

The mechanisms underlying follicle rupture during ovulation remain to be clarified. Human preovulatory follicles grow to approximately 20 mm, approximately 600 times larger than the initial activated primordial follicle.[184] Thus, it is very likely that volumetric expansion of pre-ovulatory follicles is a potential mechanism of follicle rupture before ovulation. A recent mathematical model of the blastocyst as a thin-walled pressure vessel demonstrated that volumetric expansion, rather than hydraulic pressure generated inside blastocysts, induces zona pellucida rupture and embryo hatching.[48] The model showed that embryos do not spontaneously hatch after reaching the peak pressure but rather require a further decrease in zona pellucida thickness to break, similar to a balloon bursting after being inflated beyond the critical pressure.[48] Very interestingly, more than 50 years ago, studies showed no significant increase in intrafollicular pressure before ovulation, and artificially increasing intrafollicular pressure by fluid or oil injection did not induce follicle rupture in rabbits[34,35] and pigs.[36] This mathematical model of a thin-walled pressure vessel[48] will be useful for investigating the roles of intrafollicular pressure and follicular wall thickness in follicle rupture during ovulation. Furthermore, this mathematical model[48] will be used to investigate the impact of mechanical forces from fluid-filled growing follicles on folliculogenesis.

Given the important roles of the hydraulic pressures inside blastocysts as well as volumetric expansion in the embryonic development,[98,100] cyclically well-controlled mechanical forces, such as stretching and compression, from fluid-filled growing follicles and a sudden decrease in mechanical forces after ovulation may play important roles in folliculogenesis in normally ovulating ovaries. Further studies are required to investigate whether and how such cyclical changes in follicular hydraulic pressure and/or volumetric expansion affect the surrounding cells, including somatic and germinal cells. Furthermore, using a similar mathematical model,[48] we may also investigate the impacts of mechanical forces induced by hydraulic intracystic pressure and volumetric expansion in endometriomas on ovarian function. Persistent stretching of adjacent ovarian tissue by endometriomas has been hypothesized to diminish the ovarian reserve.[185] However, no studies have investigated the mechanical forces in surrounding normal ovarian tissues/cells generated by endometrioma, the biochemical signaling pathways induced by mechanical forces, or the effects of mechanical, and biochemical cues generated by endometriomas on normal ovarian function.

3D models of in vitro ovarian folliculogenesis in large non-rodent animals will be very powerful tools to investigate ovarian mechanobiology.[186] However, there are many challenges to overcome before establishing such models.[186] The duration of folliculogenesis is much longer and the size of follicles much larger than those of mice, complicating the creation of 3D models.[186] To generate optimal microenvironments that can recapitulate in vivo folliculogenesis, precise spatial mapping of biochemical and mechanical properties in whole ovaries in vivo is required.[187] Next, we need to determine which signals are required to maintain dormancy versus activate primordial follicles.[187] New culture methods as well as 3D scaffolds will be required to establish long-term culture of 3D ovarian folliculogenesis models in large non-rodent animals.[187] The recent development of 3D printed bioprosthetic ovaries in mice showed 3D bio-printed scaffolds as promising tools for the development of 3D ovarian folliculogenesis models.[188]

5.4 | Durotaxis

Many cell types have been observed to migrate toward stiffer regions of mechanical gradients in a process termed durotaxis.[189-191] Durotaxis is the tendency of most cells to move toward stiffer substrates when migrating on a compliant gradient; this cellular behavior is based on the mechanical, rather than biochemical, properties of the microenvironment.[189-191] Cells spread and migrate more easily on stiff substrates than on compliant substrates.[189-191] Because many migratory cells express MMPs and ADAMs, which degrade and soften the ECM, the cells can degrade the ECM locally, thereby generating a stiffness gradient along the cells track.[191] Local softening can also be achieved independently of MMPs.[192,193] Durotaxis has been observed in various cell types in vitro and has been implicated in embryonic development, wound healing and fibrosis, and cancer me tastasis.[147,189-191,194,195] However, whether durotaxis occurs in vivo remains elusive, and addressing this will require the development of new tools.[190] First, we need appropriate tools to map tissue stiffness in bulk tissues three-dimensionally.[190] For this purpose, a multipole magnetic device was recently developed to map the absolute stiffness and viscosity of a living mouse embryo three-dimensionally.[146] Using this method, correlations between stiffness gradients and mesodermal cell migration patterns were found, raising the possibility that durotaxis guides cell movement in vivo.[146] However, other tools that precisely modify stiffness in vivo are required to functionally probe the role of stiffness gradients.[146,190] Because all directional migration cannot be explained solely by chemotaxis in living tissues, and mechanical cues play an essential role in biological control, it is very likely that durotaxis occurs in vivo.[190] In the female reproductive system, durotaxis may potentially be involved in several processes in addition to embryonic development.

The mechanisms underlying folliculogenesis remain to be clarified.[196] However, it has been suggested that after activation of primordial follicles, growing follicles migrate from the stiff cortex to soft medulla.[24] However, if the medulla is stiffer than the cortex in human ovaries, as recent studies have shown in bovine[30] and mouse ovaries,[27] durotaxis might be involved in human folliculogenesis. The

ovarian wound healing process after ovulation is not clear. However, during skin wound healing, MMP-1 expression (MMP-1 breaks down interstitial collagen types I, II, and III) increases rapidly and peaks in migrating basal keratinocytes at the wound edge on day, 1 followed by a gradual decrease.[197] Furthermore, fragmentation of the ovarian cortex facilitates the conversion of G-actin into F-actin, leading to disruption of the Hippo signaling pathway to allow secondary follicle growth.[198-200] These findings raise the hypothesis that in normally ovulating ovaries, ovarian tissue injuries from ovulation induce ECM degradation and matrix softening, which in turn trigger activation of dormant primordial follicles. Growing follicles may migrate into the medulla, which is stiffer than the cortex, via durotaxis. In PCOS ovaries, durotaxis may be impaired due to hardening of the cortex and/or loss of the stiffness gradient within the ovarian components, resulting in follicular arrest. Surgical treatment for PCOS may induce MMP-1, which degrades the ECM and induces matrix softening, resulting in a softer cortex before surgery. Consequently, follicles can migrate and grow in the medulla via durotaxis.

Durotaxis may also be involved in initial embryo implantation. During initial embryo implantation, embryos invade the underlying stromal cells.[86] In mice, Lox, which enhances ECM stiffness, is first detected in the subluminal stroma surrounding the implanted blastocyst on day-5 of pregnancy.[83] Then, from days 6 to 8, Lox expression is strongly detected in the decidual cells surrounding the embryos.[83] Blastocyst invasion and stromal cell migration are impeded by β-aminopropionitrile, an inhibitor of Lox activity.[83] Spatiotemporal changes in endometrial ECM stiffness may induce blastocyst invasion and stromal cell migration via durotaxis in mice. Further experiments will be required to confirm this in mouse models. An in vitro 2D co-culture model of human embryos with decidualized primary human ESCs showed that the motility of the stromal cells increases at implantation sites.[86] The migration of human ESCs away from the implantation site may facilitate trophoblast invasion and implantation of the embryo into the stromal compartment.[86] This increased motility of stromal cells at implantation sites was correlated with a localized increase in Rac1 activation and a reciprocal decrease in the level of RacGAP1, which inactivates Rac1.[86] cdGAP, an adhesion-localized Rac1 and Cdc42-specific GTPase-activating protein, is required for durotaxis in U2OS osteosarcoma cells.[201] The stiffness of human ESCs was decreased during in vitro decidualization.[78] The stiffness of patient-derived tumor cells and cancer cell lines is inversely correlated with migration and invasion through 3D basement membranes.[87] These findings raise the questions of whether durotaxis is involved in stromal cell migration and embryo invasion, and whether the decreased stiffness of decidualized ESCs facilitates stromal cell migration, during initial human embryo implantation.

Durotaxis may also play a role in EVT migration. EVTs migrate from softer endometrium to stiffer myometrium.[202] In normal pregnancies, the interstitial EVTs stop migrating once they reach the inner third of the myometrium.[202] It was shown that durotaxis requires individual focal adhesions to sense local ECM stiffness to guide durotaxis, and that FAK/phosphopaxillin/vinculin signaling defines the rigidity range over which this dynamic sensing process operates.[203,204] FAK is a key kinase involved in early trophoblast cell differentiation, and it plays a role in regulating cell proliferation and motility during early placental development.[205] Activated FAK is localized in proliferating EVTs between 4 and 8 weeks of human gestation and is decreased markedly after 10 to 12 weeks of gestation.[205] Furthermore, the strongest durotactic migratory response was observed on the softest regions of a stiffness gradient (2–7 kPa), with decreased responsiveness on the stiffer regions of the gradients, in all cancer cell lines tested (U87-MG, T98G, MDA-MB-231, and HT1080), as well as in noninvasive human fibroblasts (BJ-5ta normal fibroblasts).[189] EVTs may not migrate beyond the inner third of the myometrium due to a stiff endometrium along with a marked decrease in the level of activated FAK. In vitro experiments to investigate whether durotaxis is involved in the female reproductive system, such as in migration of growing ovarian follicles, embryo implantation, and/or EVT migration, will be required to test these speculations.

5.5 | Experimental tools

Huse clearly stated, "We can only study what we can measure, and as the field of mechanobiology moves forwards, it will be advisable to keep this obvious dictum in mind (Figure 1).[175] Progress in mechanobiology is dependent on development of technologies that enable precise physical measurements in living cells".[135] To fully understand the mechanobiology of the female reproductive system in humans, in which the dynamic, complex architectural multiscale organization of tissues is elaborate, noninvasive techniques that provide precise high-resolution mechanical properties at multiple scales, from molecules, to single cells, tissues, and organs, are essential and indispensable.[136]

Today, microfluidic technologies are still far from routine biological experiments, even in basic research, partly because of the technical limitations of most biological laboratories.[149-153] However, in the future, microfluidic technologies will be an indispensable tool for investigating the cellular microenvironment in development and disease for the following reasons. The cellular microenvironment is defined by physical and chemical signals that can influence cellular behavior, either directly or indirectly.[3] Recent advances in microfluidic technologies may enable precise control of the mechanical and chemical microenvironments of cultured cells.[149,150] Microfluidics can be used to apply both solid forces (substrate mechanics, strain, and compression) and fluid forces (luminal and interstitial) to cells.[149,150] Furthermore, advances in fabrication methods will enable integration of solid and fluid forces within a single device to recapitulate the tissue microenvironment more accurately.[149,150] Both mechanical and biochemical signals play important roles in development and disease.[3] Furthermore, there are feedback loops between mechanical and biochemical signals, termed mechanochemical feedback loops, and these bidirectional interactions play key roles in morphogenesis and pattern formation in both development and disease.[22]

Furthermore, given the importance of cell-ECM interactions, a major technological challenge is to develop in vitro ECM tools that mimic both normal and pathological ECM in the female, reproductive system in vivo.

[187,206,207] Previous studies showed that synthetic hydrogels are a useful tool to investigate the effects of ECM stiffness on cell function.[155-157] However, naïve ECMs are viscoelastic and thus exhibit stress relaxation.[208-211] Both stiffness and substrate stress relaxation are fundamental physical properties that can affect cell behavior and function.[208-211] Furthermore, substrate topography and stiffness interact to regulate the mechanical properties of cells.[212,213] A significant interaction between substrate stiffness and topography was found in the elongation and orientation of cardiac fibroblasts and in the viscoelastic behavior and smooth muscle gene expression of mesenchymal stem cells.[212] Recently, a microfabrication method was developed to independently control the stiffness (3–145 kPa) and topographical structures (5–10 μm in size) of polyacrylamide hydrogels.[213] The design of well-defined micro- and nanostructural characteristics of ECM topography, including the architecture, geometry, size, and organization of the ECM network, is required to simulate the native ECM.[214] Decellularization techniques support the creation of biocompatible ECM hydrogels, providing tissue-specific environments for both in vitro cell culture and in vivo tissue regeneration.[215,216] However, a major limitation of mechanobiology experiments is that the mechanical strength of decellularized ECM hydrogels is lower than that of native tissue.[215,216] Decellularized ECM hydrogels lose essential mechanical and structural stability during decellularization and digestion processes.[215,216] Tuning the mechanical properties of decellularized ECM hydrogels to mimic native tissue in vivo without compromising biocompatibility is required.[215,216] Genetic engineering and hybrid scaffolds offer potential solutions.[217] A recent study showed that using MDA-MB-231 cells (human invasive breast cancer cell line) with varying LOX expression levels by genetic engineering, decellularized ECM scaffolds with different stiffness levels can be generated.[217] Another study developed tunable printable hydrogels composed of decellularized human heart ECM using either methacrylated gelatin (GelMA) or GelMA-methacrylated hyaluronic acid (MeHA) hydrogels, which were dual crosslinked using UV and microbial transglutaminase, to create cardiac tissue-like constructs.[218] By altering the concentrations of GelMA and MeHA individually and mixing them at different ratios, the Young's modulus values can be adjusted over a wide range.[218]

5.6 | Organoids and organoid-on-a-chip

Recent developments in human organoid technology including endometrial epithelial cell organoids[114,115] and trophoblast organoids[112,113] have provided a deeper understanding of human biology and diseases. However, despite tremendous advances in organoid technology, there are still many limitations in traditional organoid systems.[219-221] A major limitation is that they lack key specialized cell types (vasculature, stromal, and immune cells) and fail to recapitulate the complexity of native organs.[219-221] To understand the underlying mechanisms of female reproductive system physiology and related disorders such as embryo implantation and early embryonic development, molecular and functional interactions of embryos or trophoblasts with decidua including epithelial, stromal, and immune cells should be investigated.[222-225] Complex organotypic models involving cancer organoids and endometrial organoids co-cultured with stromal and/or immune cellular components have been developed.[222,226] Interestingly, a recent study developed an air-liquid interface (ALI) method to establish patient-derived organoids (PDOs) preserving diverse endogenous immune cells and other non-epithelial cell types.[227] The cancer tissues were physically (not enzymatically) minced into fragments, and ALI PDOs cultures were established from 100 individual patient tumors representing 19 distinct tissue sites and 28 unique disease subtypes.[227] However, it remains unclear whether the ALI method can also be applied to female reproductive tissues.

The organoid-on-a-chip model, which synergistically combines the best features of organ-on-a-chip and organoid models, can help address the major technical challenges in organoid research, including microenvironmental control of organoids (biophysical and biochemical microenvironments, nutrient supply), more controllable and more conducive environments for co-culture of different cell, tissue and organ types in organoid systems, and minimizing variability.[228] Organoid-on-a-chip systems will provide a promising platform to investigate the physiology and related disorders of the human female reproductive system.[228]

5.7 | Computational mechanobiology models

Computational models have become a standard tool for testing hypotheses in biological settings.[163,229-231] They cannot prove whether a proposed mechanism is true for an observed phenomenon, but they can demonstrate whether the mechanism is sufficient to produce the observed results.[163,229-231] Because computational models allow testing of numerous hypotheses via simulated experiments and identify the most probable/correct hypotheses to prove via laboratory experiments, these models are useful before conducting laboratory experiments, especially when investigating complex relationships among biochemical and mechanical pathways at multiple levels.[163,229-231] Thus, a computational model is a promising tool to identify the most probable/correct hypotheses in the field of mechanobiology.[163,229-231] However, to prove a hypothesis, high-quality experimental data are indispensable.[163,229-231] Furthermore, to generalize the findings to the clinic, "the verification, analytical validation and clinical validation" process is essential.[232] However, in the study of the human female reproductive system, even the first step (verification) is challenging due to a lack of high-quality multiscale experimental data. Nevertheless, in the future, multiscale computational models that can recapitulate multiple complex feedback loops between mechanical and biochemical signals will be a

powerful tool to help enable true understanding of the physiology and related disorders of the female reproductive system. A close collaboration with computational biologists will be required.[163,229-231]

6 | CONCLUSION

The present review provides the current knowledge regarding the mechanobiology of the female reproductive system. Because we do not yet fully understand the mechanobiology of female reproductive system physiology, whether and how mechanical perturbations are involved in reproductive system disorders are unclear. Recent and tremendous technological advancements in mechanobiology research will help us understand the role of mechanical forces in reproductive system disorders, such as embryo implantation failure and early pregnancy loss. Today, significant efforts are still needed to fully understand the mechanobiology of the female reproductive system. However, in the near future, I hope we can determine how biochemical, genetic, and mechanical factors are integrated to regulate the female reproductive system, and how perturbations in these factors result in related disorders.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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