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MEETING REPORT

"Feeling the force" in reproduction: Mechanotransduction in reproductive processes

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ABSTRACT

Reproductive biologists are well-versed in many types of biochemical signaling, and indeed, there are almost innumerable examples in reproduction, including steroid and peptide hormone signaling, receptor-ligand and secondary messenger-mediated signaling, signaling regulated by membrane channels, and many others. Among reproductive scientists, a perhaps lesser-known but comparably important mode of signaling is mechanotransduction: the concept that cells can sense and respond to externally applied or internally generated mechanical forces. Given the cell shape changes and tissue morphogenesis events that are components of many phenomena in reproductive function, it should be no surprise that mechanotransduction has major impacts in reproductive health and pathophysiology. The conference on "Mechanotransduction in the Reproductive Tract" was a valuable launch pad to bring this hot issue in development, cell biology, biophysics, and tissue regeneration to the realm of reproductive biology. The goal of the meeting was to stimulate interest and increased mechanotransduction research in the reproductive field by presenting a broad spectrum of responses impacted by this process. The meeting highlighted the importance of convening expert investigators, students, fellows, and young investigators from a number of research areas resulting in cross-fertilization of ideas and suggested new avenues for study. The conference included talks on tissue engineering, stem cells, and several areas of reproductive biology, from uterus and cervix to the gametes. Specific reproductive health-relevant areas, including uterine fibroids, gestation and parturition, and breast tissue morphogenesis, received particular attention.

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Introduction

The importance of cellular mechanics, and the associated process of signaling based on mechanical forces, is a well-appreciated element of tissue morphogenesis during embryonic development, and in cell physiological events, such as cell shape change during mitosis or cell motility (1–4). The mechanical forces that a cell experiences as signaling are just as significant as exposure to hormones, growth factors, and other biochemical signals. These forces can come from external pressures exerted by a cell's environment, such as pressure from the surrounding extracellular matrix (ECM) or neighboring cells, or from the cell's cytoskeleton undergoing intracellular contraction or pulling against a rigid extracellular structure. Indeed, one of the best examples of a mechanosensitive structure is focal adhesion, the contact point of a cell with its ECM (5,6). Proteins that were identified decades ago as components of focal adhesions or associated with the cytoskeleton in other ways (e.g., talin) (7) are now appreciated to be exquisitely mechanosensitive (8). Mechanical signaling and biochemical signaling can intersect as well, such as when mechanical stimulation of mechanosensitive channels (such as stretch-activated channels) allows entry of ions into the cell. Mechanosensitive channels respond to cytoskeletal contractions, shear flow, other pressures on the cell, and osmotically induced changes in cell volume. Given all this, is it any wonder that mechanotransduction-based events would be major effectors of reproductive processes? A first-of-its-kind conference entitled "Mechanotransduction in the Reproductive Tract" was hosted by The Campion Fund of the Phyllis and Mark Leppert Foundation for Fertility Research in October 2014 at Duke University. The two-day conference was attended by reproductive biologists, as well as clinicians, students, and fellows from across the US and Canada who actively participated in this informative and stimulating meeting. Cross-fertilization was stimulated by scientists from other disciplines who shared their knowledge

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regarding relevant systems. Scientific talks on tissue engineering, stem cells, and studies of degenerative disease of the joints presented concepts and specific data and details pertinent to reproductive biology.

Lessons from tissue engineering and stem cell biology

One of the first areas in biology where mechanical forces were first appreciated is developmental biology, specifically the area of tissue morphogenesis and remodeling as reviewed in several papers (1,2). By extension, an instance of "extreme" tissue morphogenesis is tissue engineering. Tissue engineering and the field of regenerative medicine have utilized the concepts of mechanotransduction for several decades and is a rapidly growing interdisciplinary scientific field devoted to the development of biological means to restore, maintain, and improve the function of tissues and organs. Regenerative medicine investigators have discovered many of the concepts of mechanical cell signaling and the biochemical pathways induced by mechanical forces. Using stem cells embedded in biologic scaffold they have developed tissues and organs and in doing so they have elucidated many of the principles underlying mechanotransduction (9).

This knowledge from these systems provides insights into the mechanisms of mechanotransduction in the reproductive tract, as many of the mechanisms of cell–matrix interaction are similar across tissues. Two featured talks of the conference came from the field of orthopedics, an area that has contributed significantly to our knowledge of mechanobiology. These presentations addressed how tissue engineering is confronting major issues in osteoarthritis, a painful and debilitating disease of the joints that involves degeneration in both bone and cartilage.

In the keynote address, Rocky S. Tuan (University of Pittsburgh) presented the application of adult stem cells and biometric matrices for tissue engineering and modeling to understand osteoarthritis. Cartilage has low reparative capacity and thus represents a significant clinical challenge. Adult stem cells, with their potential for differentiation into multiple lineages and recently discovered trophic activities, can be used in combination with biomimetic scaffolds and thus provide a powerful platform for regenerative, therapeutic, and disease-modeling applications. Tuan's group has shown that biomimetic scaffolds that simulate the structure of native ECM, especially the nanoscalar fibrous nature of collagen, are effective for tissue engineering *in vivo* and *in vitro* using mesenchymal stem cells (MSCs; harvested from adult tissues such as bone marrow and adipose tissue). Custom-designed, photocross-linked hydrogel scaffolds allow cell encapsulation during fabrication and demonstrate high fidelity reproduction of internal structure and excellent cell retention. He has applied a 3D printing approach and a custom-designed bioreactor for the construction of a microtissue analogue of the biphasic osteochondral junction, which can serve as a model of the pathogenesis of osteoarthritis in which the effects of biological, hormonal, pharmacological, and mechanical perturbations can be studied. Osteoarthritis involves degeneration in both bone and cartilage. Tuan and colleagues fabricated a multichamber bioreactor in which specific microenvironments can be created by the utilization of different culture media streams. In this system, chondral and osseous tissue can develop and mature in separate chambers in culture conditions optimized for the distinct cell types (e.g., chrondrocytes thriving in hypoxic conditions, and osteoblasts needing normoxic conditions). Human bone marrow stem cells (hBMSCs)-derived constructs were fabricated *in situ*, cultured in the bioreactor chambers, and induced to undergo spatially defined chondrogeneic and osteogenic differentiation for four weeks. Tuan and colleagues conducted experiments to determine the response to Interleukin-1, a cytokine implicated in the pathogenesis of osteoarthritis (10,11). Tuan's work provides a challenge to reproductive biologists suggesting approaches to problems encountered in the reproductive sciences and raises the possibility that many of his techniques could be utilized to further study ovulation, implantation, spermatogenesis, and other aspects of reproduction.

Plenary speaker Farshid Guilak presented, "Understanding mechanotransduction to engineer new biologic therapies for arthritis: lessons for reproductive biologists." While the etiology of osteoarthritis is poorly understood, it is well accepted that biomechanical factors play an important role in the onset and progression of the disease. Chondrocytes in cartilage sense compression and osmotic stress, and regulate their metabolism in response to these mechanical forces. However, pathological mechanical stress will lead to maladaptive cellular responses and eventual cartilage destruction. Guilak and colleagues have asked how cells sense mechanical changes, seeking to determine the molecular mechanisms of mechanotransduction in these cells. Their work has examined distinct mechanosensitive ion channels, transient receptor potential vanilloid (TRP) channels, and PIEZO channels, and identified distinct roles in chondrocyte mechanotransduction during compression. TRPV4 is a Ca^{2+} cation channel that serves as a sensor of

mechanical and/or osmotic signals in the cartilage, bone, and synovium. Mice with genetic deletion of *Trpv4* develop osteoarthritis, with failure of cells to respond to osmolar changes, and humans with mutations in the *TRPV4* gene develop an inherited arthropathy that worsens over time (12,13). In engineered cartilage replacements, chemical activation of TRPV4 can reproduce many of the anabolic effects of mechanical loading and tissue growth and regeneration. Thus, TRPV4 plays a key role in transducing pain and inflammatory signals in joint tissue via mechanical forces. Guilak and colleagues also examined PIEZO1 and PIEZO2 function in injurious strain. Ca^{2+} transients produced by atomic force microscopy were blocked by GsMTx4, a PIEZO blocking peptide, or by RNAi-mediated knockdown of PIEZO expression. In an explant cartilage-injury model, GsMTx4 reduced chondrocyte cell death after mechanical injury, suggesting that direct targeting of these channels might be a possible therapy for reducing cartilage injury and posttraumatic osteoarthritis (14). Finally, their work using a woven composite scaffold reveals that an anisotrophic 3D woven structure consolidated into a chondrocyte-hydrogel will function as a tissue support for regeneration for immediate load bearing without having to culture the tissue before implantation. These studies show how tissue and biomaterial microenvironments provide architecture cues to direct important cell behaviors such as shape, alignment, migration, and tissue formation (15).

Mechanobiology of uterine fibroids

One of the plenary talks and one meeting session addressed uterine fibroids, which are benign, stiff, nodular tumors of the myometrium that may cause significant morbidity including severe pain, bleeding, infertility, and pregnancy complications. Fibroids are characterized by an accumulation of altered collagen, as detected by electron microscopy and the increased presence of hydroxyproline, noted by amino acid analysis (16,17). Along with differing amounts of glycosaminoglycans (GAGs) compared to the myometrium and increased interstitial fluid, this altered collagen accumulation exerts mechanical forces on cells leading to the initiation of chemical pathways within the cells through mechanical sensors on their membranes (18).

Plenary speaker James Segars (NICHD, now at Johns Hopkins) dubbed his talk, "Do fibroids feel the force, Luke?" He stated that uterine myometrial cells possess exquisite sensitivity to mechanical stress and produce a unique ECM. He reiterated what others have stated: cell compression changes gene expression (19). Uterine fibroids are clonal growths that arise in the uterus, a highly plastic organ. Segars declared that although the etiology and development of fibroids are obviously complex, science cannot deny that mechanotransduction has an important role in fibroid growth and development. In fact mechanotransduction may account for fibroid-to-fibroid variability in growth patterns, whereby in the same uterus, some tumors grow, others regress, and others are stable. Uterine fibroids produce a stiff ECM that is abnormal in amount, content, structure, and organization compared to normal myometrium (16,20,21). They also have increased fluid content relative to the myometrium and the contribution of hydration to fibroid stiffness is supported by viscoelastic measurements of the tumors that take into account the contribution of water to their stiffness (22). Studies of fibroid cells *in vivo* suggest an elevated state of activation of mechanical signaling featuring increased levels of focal adhesion kinase (FAK), active RhoA, and the Rho-guanine exchange factor, AKAP-13 (22). Paradoxically, uterine fibroid cells exhibit an attenuated functional response to external mechanical cues (22). Probing of the mechanical signaling with the inhibitor of integrin ß1, inhibition of Rho-kinase (ROCK), or siRNA supports the conclusion that uterine fibroid cells demonstrate impaired mechanical signaling. These studies demonstrate that alteration of mechanical signaling in uterine fibroid cells was associated with altered expression of key ECM genes and a reduction in mechanical stress. All available data indicate that uterine fibroid cells reside in a milieu of increased mechanical stress, but exhibit impaired mechanical signaling. Segars ended his talk by presenting a recent study of the effect of an anti-fibrotic, Fasudil, on uterine fibroid cells (23). Treatment of fibroid cells in 3D culture with Fasudil relaxed the contraction of the collagen gels caused by myometrial and fibroid cell growth. It also caused a decrease in Rho, versican and procollagen 1α, and fibronectin as well as an increase in Bax, suggesting that Fasudil could be useful in the treatment of uterine fibroids.

Liping Feng (Duke University) presented data from a number of studies conducted demonstrating that there is considerable heterogeneity between and within uterine fibroids. She showed that structural and biomechanical properties, gene profiles, and the amount of collagen in fibroids differ from one to another, and even that a single fibroid can have distinct areas across it. Strikingly, there were variations in the gross appearance and in addition to the classical whorled pattern of fibroid tissue, a trabecular pattern was observed in some fibroids and some fibroids exhibited protruding nodules. Nodules varied in size and were stiffer

compared to the surrounding areas of the tumors, as detected by both palpation and rheometry. Further proof of heterogeneity was the fact that when two different biopsy specimens within one fibroid were compared with gene profiling, 15 genes were differentially expressed. Analysis of type I, type III, and type V collagen composition revealed different collagen I/III ratios within and between fibroids, suggesting that the degree of collagen turnover in these areas is different. While in most fibroids, the overall collagen deposits appeared to be type I and type III, in one specimen, type V collagen predominated. These data should alert researchers of the fact that attention needs to be paid to characteristics of surgical specimens chosen for study and characteristics of samples taken from within those specimens as not all samples are the same and thus cannot be compared unless clearly described.

Friederike Jayes (Duke University) presented unpublished results of an *ex vivo* proof of principle study using uterine fibroid tissue (1-cm^3 cubes) injected with highly purified Collagenase *Clostridium Histolyticum* (CCH) at different doses. These were compared to vehicle-injected controls. The injected cubes were incubated for 24–96 h and then evaluated by rheometry for stiffness (complex shear modulus) or by Masson-Trichrome stain for digital analysis to estimate the degree of fibrosis. Representative samples were studied with electron microscopy to confirm collagen fibril degradation. Percent fibrosis was initially over 37%, reflecting the collagen-rich nature of uterine fibroids. Collagen fibrils were undetectable by electron microscopy, and fibrosis was as low as 2.4% in samples injected with 4/mg/ml CCH and examined after 96 h. Overall, a significant decrease in stiffness was achieved with all doses at all timepoints. Importantly, CCH does not degrade type IV collagen found in blood vessels and nerves and is currently approved by the FDA for the treatment of Dupuytren's contracture and Peyronie's disease. This study supports the possibility of conducting clinical trials of the use of injected CCH as a treatment for uterine fibroids.

Darlene Taylor (North Carolina Central University) discussed the *in vitro* characterization of a thermo-responsive injectable depot for uterine fibroids therapy. She has synthesized a drug-eluting depot (LiquoGel™) (24) and believes that this system will be useful for the delivery of anti-fibrotic drugs to uterine fibroids. The drugs can be entrapped in the gel and gradually released into the tissue. This depot substance is unique because it can be injected into living tissue as a liquid at room temperature, and it then gels at body temperature after injection. Its clinical applicability is enhanced by its construction, which allows the entrapment of multiple drugs. Taylor presented work demonstrating that pirfenidone, an anti-fibrotic, could be encapsulated into LiquoGel™ and then released. Based on these data, LiquoGel™ can be used to deliver a variety of drugs that could treat fibroids locally and could enable uterine-preserving treatments.

Mark Palmeri (Duke University) discussed methods to characterize stiffness in the organs of living persons noninvasively. His research has assessed the utility of acoustic radiation force impulse (ARFI) and shear wave elasticity imaging (SWEI), which characterize the viscoelastic properties of soft tissues and thus would be useful in several clinical arenas, including the analysis of uterine fibroids (25,26). ARFI and SWEI utilize commercial diagnostic ultrasound scanners and transducers with customized imaging and data processing sequences to generate transient micron-scale deformations of soft tissue, where the displacement amplitudes are inversely related to tissue stiffness. Shear waves are generated in response to these deformations, and the speed of the waves correlates with the tissue stiffness. ARFI and SWEI can analyze liver fibrosis and other fibrotic pathologies, and can distinguish malignant and benign disease in the prostate. These methodologies can also be used for characterizing changes in cervical compliance during pregnancy in evaluating women at risk of preterm delivery (26). It is postulated that ARFI and SWEI will aid the pretreatment evaluation of uterine fibroids.

Mechanotransduction in parturition

The uterus undergoes incredible tissue remodeling during gestation and parturition. Along with biochemical signaling, mechanical forces exerted on individual cells play a major role in this uterine remodeling. Investigations are now unraveling the complexities of parturition and the integration of mechanobiology with the biological process of inflammation, steroid and peptide hormonal signaling, and biochemical pathways.

Plenary speaker Oksana Shynlova (University of Toronto) discussed her group's work linking mechanotransduction in the myometrium with immune system function and the onset of parturition. Mechanical signaling has important effects at midgestation, when the stretching of the myometrium leads to hypoxia-induced activation of the caspase cascades; this in turn triggers the differentiation of pregnant myometrium, from a state of hyperplasia to hypertrophy (27,28). As a result of a changed endocrine environment and increased mechanical stretch, there is increased uterine production of cytokines and chemokines. These cytokines and chemokines elicit several changes, including the

expression of endothelial cell adhesion molecules, activation of peripheral immune cells, and trafficking of leukocytes to the myometrium. As these cells extravasate within the uterine muscle, the immune cells differentiate and produce more chemokines and cytokines, leading to contractile activity and the onset of labor (27–29). Mechanical signals are integrated with endocrine signals by monocyte chemotactic protein-1 (MCP-1) (also known as C-C chemokine motif ligand 2, or CCL2), mediating both term and preterm birth (30). These events are associated with the stretching of myometrial smooth muscle cells under the influence of progesterone (31). Increased expression of the rat myometrial oxytocin receptor mRNA during labor requires both mechanical and hormonal signals (32,33).

Mechanical influences in uterine cervical remodeling and ripening

While the mammalian uterine body and fundus are enlarging during gestation, the cervix provides the strength to retain the growing fetus. As parturition advances, the cervix then must soften and remodel to allow for dilation in delivery. This dynamic tissue remodeling is dependent on mechanical changes in the tissue integrated by complex hormonal and inflammatory responses.

Nathan Mowa (Appalachian State University) presented proteomic analysis that revealed a novel cluster of cytoskeleton-related proteins during gestational cervical remodeling in mice. Seventy-three different proteins were detected as upregulated during cervical remodeling, including proteins associated with collagen organization (e.g., biglycan, lumican), proteins involved in immune scavenging, and proteins involved in cytoskeletal function. Increased expression of immune-related proteins at day 11 and increased cytoskeletal proteins at day 17 suggested a complex process of integrated immune response, and extracellular mechanical forces on the cervical remodeling process of gestation (34).

Shanmugasundaram Nallasamy (University of Texas Southwestern) discussed new insights into the role of decorin in collagen fibrillogensis in the cervix. During gestation, decorin and collagen have a close alignment before and during cervical ripening in the mouse. Decorin interacts with collagen to contribute to the formation of inter-fibrillar bridges between adjacent collagen fibrils. Decorin was considered previously to play a role in the dissociation with collagen molecules during cervical ripening. However, Nallasamy observed that decorin-null females have normal parturition with cervical collagen fibril ultrastructure, similar to wild-type cervices. Interestingly, collagen ultrastructure in nonpregnant decorin-null mice was abnormal with irregularly shaped fibrils and variable sized interspersed with normal fibrils. Tissue biomechanics showed reduced cervical strength in nonpregnant decorin-null mice compared to wild-type control females. This raises the possibility that lack of decorin during early pregnancy predisposes the cervix to cervical insufficiency. The studies presented suggest that decorin regulates cervical collagen architecture and thus biomechanical properties by different mechanisms at different stages of gestation.

Mala Mahendroo (University of Texas, Southwestern) presented studies on the ECM of the cervical epithelia in preterm birth. In a normal pregnancy, the total GAG levels increase at term. The increase in GAG levels is due to an increase in hyaluronan (HA), which forms large structures that stimulate viscosity, hydration, and matrix disorganization. Cyclic mechanical stretch augments HA production in cultured human cervical fibroblasts (35), suggesting that mechanotransduction is involved in the molecular mechanisms of cervical remodeling during gestation. Mahendroo has quantified the HA increase in the cervix in mice from 19% of the total GAG at day 6 to 71% of total GAG at day 18 (36), thus allowing for maximal compliance and dilation of the cervix during parturition. The increased synthesis of HA from early to late pregnancy has been long proposed to play an essential role in the disorganization of collagen-rich ECM of the uterine cervix, but the story is complex (37). However, studies of conditional knockout mice deficient in cervical HA with targeted mutations in the genes that encode Hyaluronic acid synthatases (*Has1, Has2, Has 3*) show that it is not essential for the increased distensibilty of the cervix during late pregnancy (36). However, studies using these mouse models as well as the human ectocervical ECT-1 cell line reveal that HA contributes to epithelial barrier protection in the cervical epithelium to limit pathogen infiltration in the lower reproductive tract during pregnancy (38). Interestingly, when *E. coli* were inoculated into the vaginas of wild-type and HA-deficient mice on day 16 of gestation, 100% of the HA-deficient mice delivered within 48 hours, whereas only 50% of the wild-type mice delivered during this time window. Collectively these results reveal that while HA is not essential for cervical compliance, HA appears to be protective against infection-mediated preterm birth.

Kyoko Yoshida (Columbia University) presented a biomechanical material model for cervical tissue dependent on collagen content and collagen crosslink density. Noting that abnormal cervical ripening presents a significant clinical problem in the management of pregnancy, Dr. Yoshida envisions that a biomechanical

model for cervical tissue that correlates quantitative ECM measures to tissue mechanical behavior will be helpful to clinicians and researchers. Cervical tissue is modeled as a porous fiber composite where the stress–strain relationship is dependent on quantitative ECM measures. In normal gestation mature collagen crosslink density decreases while collagen content per dry weight remains constant. During this time, hydration and thus the total tissue volume increase. In the model of Yoshida and colleagues, the solid component of the cervix is cast as a cross-linked collagen fiber network embedded within a NeoHookean matrix. Quantification of mature collagen crosslinks (pyridinoline, deoxypyridinoline, hydroxylysinonorleucine, and dehydroxylysinonorleucine) and other measures of collagen matrix functionality throughout gestation reveals a correlation with tissue stiffness and strength (39), and the resulting model demonstrates the relationship between collagen fiber stiffness and ECM composition.

Studies of breast morphogenesis

The impact of cell–matrix interactions on breast tissues is widely known. Drawing on this fund of knowledge, Lucia Speroni and Tessie Paulose (Tufts University) addressed studies of breast tissue using a hormone-sensitive 3D culture model. During each ovarian cycle and in preparation for lactation, the mammary gland undergoes distinct morphological changes. Estrogen induces ductal elongation in the follicular phase, progesterone combined with estrogen in the luteal phase induces the formation of elongated branching structures, and then during pregnancy, prolactin with estrogen and progesterone supports the development of budding structures (40,41). As revealed in an *in vitro* 3D model that mimics the effects of these three mammotropic hormones, collagen fiber organization plays a role in epithelial elongation and branching. Using a software tool that facilitates morphometric analysis of epithelial structures in this 3D model, this research team demonstrated that reproductive hormones influence the ability of epithelial cells to organize collagen fibers and to form structures with specific geometry. Differences in fiber organization surrounding mammary acini and branching ducts reflect the different mechanical microenvironments whereby the fiber alignments change due to the tensile forces applied to the cells. Manipulation of the mechanical properties of the ECM in this model influences epithelial morphogenesis. This was complemented by the quantitative analysis of collagen fiber organization around the epithelial ducts and acini. In the mammary gland, epithelial cells and fibroblasts exert physical forces on type I collagen fibers. These forces impose constraints on mammary morphogenesis affecting cell proliferation, motility, and adhesion. Experiments used T47D mammary epithelial cells in the 3D culture system in type I collagen, with or without fibroblasts. Analysis of type I collagen fibers between pairs of points at specific distances on the tissue slice revealed that type I collagen fibers lie parallel to ducts and, in contrast, have a radial symmetry around acini. Cultures containing both epithelial cells and fibroblasts tended to have more variability in the correlation of function of fibers. Questions still remain as to whether hormones act on epithelial cells and fibroblasts to differentially organize type I collagen fibers around branching and budding epithelial ducts and acini. Additional information of the role of hormones on mammary development emphasizes the importance of fetal exposure to estrogenic substances (42). Taken together, these studies highlight how mechanical forces in conjunction with hormonal signaling play important roles in morphological processing in breast tissue.

Mechanotransduction in placental development

Heewon Seo (with the lab of Greg Johnson at Texas A & M, System Health Science Center) discussed the placental development, from work using the pig as a model system and focusing on placental villi. Pigs have diffuse epitheliochordial placentae, allowing for the maintenance of intimate contact of the uterine luminal epithelium and the trophectoderm/chorion throughout gestation. During porcine gestation (114 days in length), this uterine–placental interface develops extensive folding between the uterine luminal epithelium and placental villi. Between days 25 and 35, the length of these folds increases significantly, then remains constant until day 50, and then increases again at day 60. This increased folding between the uterine epithelium and placental villi correlated with an expanding microvascular network. During this 24–35 day period of placental development, the expression of talin and vinculin, well-known components of focal adhesions, increased along the entire length of the uterine placental interface. Talin and vinculin remained elevated through day 60, and a cytoskeletal adapter protein, ezrin, showed a similar pattern. Since focal adhesions, through complexes including talin and vinculin, transmit mechanical forces between the ECM and the actin cytoskeleton, actin dynamics in porcine placental villi also were evaluated. Phalloidin staining indicated that F-actin was enriched at the tips of the uterine villi on days 35–60. This F-actin was co-localized with ARP2,

which participates in actin nucleation, and filamin, which stabilizes actin. Additionally, in the stroma underlying the developing uterine villi, α-smooth muscle actin staining increased from day 24–35 and then remained high to day 60. This suggests that differentiation of myofibroblast-like cells in the underlying stroma provides mechanical support to accommodate the increasing length of the interlocking uterine placenta villi. Thus, the expansion of the microvascular network occurring during this timeframe in placental development applies mechanical forces to the underlying surfaces of the epithelia to support folding between the villi.

Mechanical signaling in other reproductive processes

While the studies noted above focus on the mechanotransduction aspects of uterine function and pathophysiology, there are other examples of the impact of mechanical forces in reproductive processes; these include the male reproductive tract and individual gametes. Jun Zhou (University of Georgia) discussed how a member of the tetraspan superfamily of proteins is implicated in the development of the male distal reproductive tract (i.e., the vas deferens and ejaculatory duct) in a mouse model. He presented unpublished findings revealing a spontaneous point mutation in exon 2 of the tetraspan lipoma HMGIC fusion partner-like 2 (*Lhfpl2*) leads to complete infertility in ~70% of males carrying this mutation. These infertile males had normal epididymal sperm counts and normal mating behavior, but could not transmit sperms to wild-type females in natural matings. Interestingly, even fertile mutant males had structural abnormalities in the distal vas deferens. Although the functions of many tetraspan proteins are not well-understood, these proteins are implicated in membrane order, and potentially in mechanotransduction, as a closely related protein lipoma HMGIC fusion partner-like 5 (LHFPL5) has been identified in the mechanotransduction machinery in cochlear hair cells (43,44). Thus, LHFPL2 appears to be involved in male distal reproductive tract development, raising the possibility that mechanotransduction is involved in this process.

Plenary speaker Janice Evans (Johns Hopkins University) addressed mechanics in mammalian oocytes, including the finding that cortical tension in the oocyte is a contributor to successful mammalian female meiosis (45). Oocyte cortical tension changes dramatically through meiotic maturation and egg activation, and is altered upon perturbation of actin, the actin-associated motor protein non-muscle myosin-II, and the family of actin-to-membrane crosslinking proteins known as ERMs (ezrin, radixin, and moesin) (46). Abnormal cortical tension is linked with aberrant spindle function and cytokinesis during the completion of meiosis. The metaphase II egg has mechanical polarity, with the domain over the metaphase II spindle being ~2.5-fold more rigid than the cortex on the opposite side of the oocyte. Interestingly, this mechanical polarity differs in eggs that reach metaphase II by *in vitro* maturation, with the spindle-sequestering domain in *in vitro*-matured eggs being less rigid than this domain in ovulated eggs, which correlates with the difference in spindle size and morphology (46,47). Oocyte cortical tension also is correlated with the ability of the oocyte to establish the membrane block to polyspermy (48). Finally, oocyte cortical tension may play a role in ion homeostasis, potentially through the regulation of mechanosensitive channels (49), complementing an emerging paradigm that Ca^{2+} entry plays a crucial role in the egg-to-embryo transition (50,51).

Closing perspectives for the future in this field

With much of reproductive physiology and pathophysiology rooted in tissue morphology and morphogenesis, mechanotransduction appears destined to be a major issue in reproductive biology research. To complement work that has examined known molecular players (e.g., cytoskeletal elements, mechanosensitive channels such as TRP and PIEZO channels), research in this arena will be advanced by improved tools to assess and manipulate the mechanical properties of tissues and cells (1,52). Greater knowledge of the mechanical properties and mechanotransduction in reproductive tissues and cells will certainly open the door to an improved understanding of a wide range of reproductive diseases. In terms of improving reproductive health, we can look to augmenting the substantial number of therapeutics that have exploited classic physiological and biochemical signals (e.g., combined oral contraceptives, selective steroid receptor modulators, 5-α-reductase and aromatase inhibitors, and phosphodiesterase inhibitors) with the manipulation of mechanically associated signals as new avenues for the treatment of diseases of the reproductive tracts.

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Declaration of interest

Dr. Palmeri is an inventor of several ARFI and SWEI technologies and holds intellectual property related to these technologies. Dr. Taylor holds intellectual property related to LiquioGel. A patent application has been filed by BioSpecifics Technologies Corp. for CCH treatment of uterine fibroids. Dr. Leppert is the President of The Campion Fund, a 501(c)3 public charity with a mission to educate scientists and the public about reproductive biology.

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