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# Biomechanical forces and signals operating in the ovary during folliculogenesis and their dysregulation: implications for fertility

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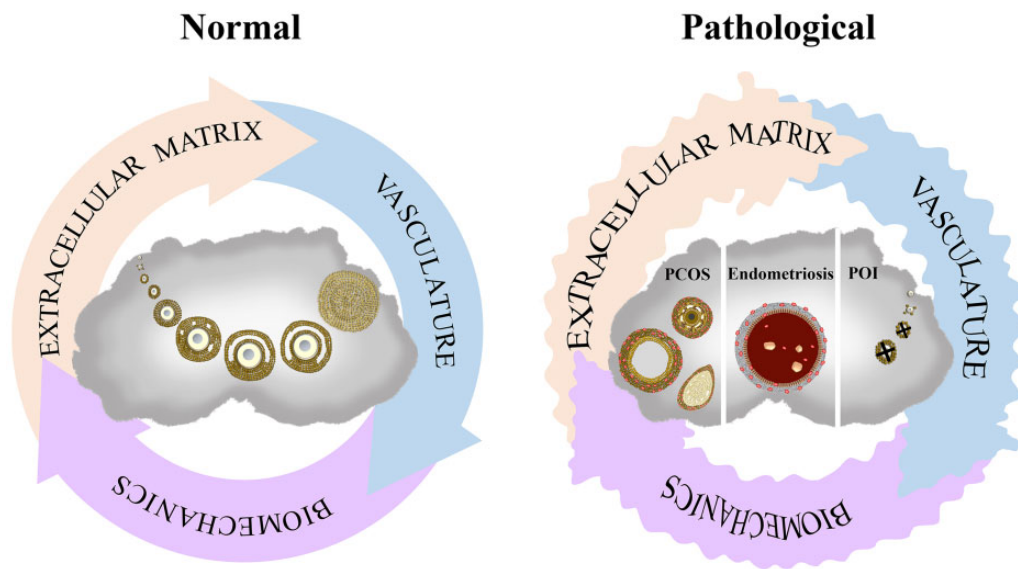
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**GRAPHICAL ABSTRACT**



**Alterations of the ovarian ECM, vasculature and biomechanical properties have implications for fertility.**

**BACKGROUND:** Folliculogenesis occurs in the highly dynamic environment of the ovary. Follicle cyclic recruitment, neo-angiogenesis, spatial displacement, follicle atresia and ovulation stand out as major events resulting from the interplay between mechanical forces and molecular signals. Morphological and functional changes to the growing follicle and to the surrounding tissue are required to produce oocytes capable of supporting preimplantation development to the blastocyst stage.

**OBJECTIVE AND RATIONALE:** This review will summarize the ovarian morphological and functional context that contributes to follicle recruitment, growth and ovulation, as well as to the acquisition of oocyte developmental competence. We will describe the changes occurring during folliculogenesis to the ovarian extracellular matrix (ECM) and to the vasculature, their influence on the mechanical properties of the ovarian tissue, and, in turn, their influence on the regulation of signal transduction. Also, we will outline how their dysregulation might be associated with pathologies such as polycystic ovary syndrome (PCOS), endometriosis or premature ovarian insufficiency (POI). Finally, for each of these three pathologies, we will highlight therapeutic strategies attempting to correct the altered biomechanical context in order to restore fertility.

**SEARCH METHODS:** For each area discussed, a systematic bibliographical search was performed, without temporal limits, using PubMed Central, Web of Science and Scopus search engines employing the keywords extracellular matrix, mechanobiology, biomechanics, vasculature, angiogenesis or signalling pathway in combination with: ovary, oogenesis, oocyte, folliculogenesis, ovarian follicle, theca, granulosa, cumulus, follicular fluid, corpus luteum, meiosis, oocyte developmental competence, preimplantation, polycystic ovary syndrome, premature ovarian insufficiency or endometriosis.

**OUTCOMES:** Through search engines queries, we yielded a total of 37 368 papers that were further selected based on our focus on mammals and, specifically, on rodents, bovine, equine, ovine, primates and human, and also were trimmed around each specific topic of the review. After the elimination of duplicates, this selection process resulted in 628 papers, of which 287 were cited in the manuscript. Among these, 89.2% were published in the past 22 years, while the remaining 8.0%, 2.4% or 0.3% were published during the 1990s, 1980s or before, respectively. During folliculogenesis, changes occur to the ovarian ECM composition and organization that, together with vasculature modelling around the growing follicle, are aimed to sustain its recruitment and growth, and the maturation of the enclosed oocyte. These events define the scenario in which mechanical forces are key to the regulation of cascades of molecular signals. Alterations to this context determine impaired folliculogenesis and decreased oocyte developmental potential, as observed in pathological conditions which are causes of infertility, such as PCOS, endometriosis or POI.

**WIDER IMPLICATIONS:** The knowledge of these mechanisms and the rules that govern them lay a sound basis to explain how follicles recruitment and growth are modulated, and stimulate insights to develop, in clinical practice, strategies to improve follicular recruitment and oocyte competence, particularly for pathologies like PCOS, endometriosis and POI.

# Introduction

Infertility affects 15% of couples worldwide and its incidence is increasing (Sun et al., 2019). The main causes might be maternal (40%), paternal (40%) or attributed to both partners (20%) (Fidler and Bernstein, 1999; Tabong and Adongo, 2013). Maternal causes of infertility include women ageing, meiotic aneuploidies, endocrine disorders, polycystic ovary syndrome (PCOS), endometriosis and premature ovarian insufficiency (POI). Common to these conditions is a reduced availability or quality of oocytes with developmental potential (Farquhar et al., 2019).

The acquisition of the oocyte's developmental capacity is a troubled odyssey (Zuccotti et al., 2011; Conti and Franciosi, 2018). In brief, from an initial pool of thousands of primordial follicles localized in the cortex, a group undergoes important morphological and functional changes with proliferating follicle cells assuming a cuboidal shape and forming a single-layered epithelium enclosing the gamete (Gougeon and Chainy, 1987). Since gonadotrophin receptors are not yet present in primordial follicles, initial recruitment appears to be pituitary independent and controlled by biomechanical, autocrine and paracrine pathways (Hsueh et al., 2015). From the primary follicle stage, an extracellular matrix (ECM), the zona pellucida (ZP), begins to be deposited (Wassarman and Litscher, 2018) and proximal stromal cells differentiate into a theca layer. Following FSH-dependent recruitment, secondary follicles are repositioned towards the nearby medulla and, once their growth in this region is terminated, they re-emerge into the cortex from where ovulation occurs (Fiorentino et al., 2020). Early antral follicle growth is accompanied by the sprouting of a calix of new capillaries that surrounds each growing follicle and convoys it to full maturation (Fraser, 2006). During subsequent follicle growth, antral fluid production is intensified by increased vascularization and capillary fenestration (Fraser, 2006), while the oocyte reaches its final size preparing for the germinal vesicle (GV) to meiosis II (MII) transition and LH-induced ovulation. The great majority of follicles will never complete maturation, but, instead, will be eliminated together with their surrounding vasculature.

Follicle cyclic recruitment, neo-angiogenesis, spatial displacement, ovulation and atresia characterize the micro-anatomical context in which oocytes, as a result of an interplay between molecular signals and physical forces, gain competence.

This review will summarize the ovarian morphological and functional context that contributes to follicle recruitment, growth and ovulation, as well as to the acquisition of oocyte developmental competence. We will describe the changes occurring to the ovarian ECM and to the vasculature during folliculogenesis, considering their effects on the mechanical properties of the ovarian tissue, which, in turn, impact on the regulation of signal transduction. Furthermore, we will outline how their dysregulation might be associated with pathologies such as PCOS, endometriosis or POI. Finally, for each of these three pathologies, therapeutic strategies aimed at correcting the altered biomechanical milieu, in order to restore fertility, will be described.

# Methods

For each area discussed, a systematic bibliographical search was performed, without temporal limits, using PubMed Central, Web of Science and Scopus search engines. The following keywords were included: extracellular matrix, mechanobiology, biomechanics, vasculature, angiogenesis, miRNA, signalling pathway, extracellular vesicle or maternal-effect gene in combination with: ovary, oogenesis, oocyte;, folliculogenesis, ovarian follicle, theca; granulosa, cumulus, follicular fluid, corpus luteum, meiosis, oocyte developmental competence, preimplantation, polycystic ovary syndrome, premature ovarian insufficiency/failure or endometriosis.

Through search engines queries, we yielded a total of 37 368 papers that were further filtered according to our focus on Mammals and, specifically, on rodents, bovines, equines, ovines, primates and humans, and eventually trimmed around each specific topic of the review. After manual elimination of duplicates, this selection process resulted in 628 papers, of which 287 were cited in the manuscript specifying, when needed, the corresponding species. Among these, 89.2% were published in the past 22 years, while the remaining 8.0%, 2.4% or 0.3% were published during the 1990s, 1980s or before, respectively.

# Ovarian extracellular matrix: main components, their spatial localization and remodelling during folliculogenesis

The ECM, with its specific meshwork of macromolecules such as proteoglycans, non-proteoglycan polysaccharides and fibrous proteins, provides mechanical support to the ovary, and regulates osmotic exchange of fluids, soluble nutrients, hormones and other extracellular signals. These factors cross the follicular basal lamina (BL), guiding follicle growth and appropriate oocyte maturation (Berkholtz et al., 2006). Changes to the ECM functional environment may cause derangements of local biochemical pathways, resulting in disturbed folliculogenesis and decreased fertility.

Both the ovarian cortex and medulla are characterized by follicular and stromal compartments in which ECM is observed.

## Follicular ECM

During folliculogenesis, the ECM composition varies along with follicles growth and repositioning across cortex and medulla.

There are three main ECM components: the BL surrounding the granulosa layer of all follicle types, the ZP which begins to be deposited between the oolemma and the first layer of follicle cells in primary/secondary follicles (Litscher and Wassarman, 2020), and the follicular fluid secreted from the early antral stage onwards (Fig. 1).

Most of our knowledge on the BL derives from studies on the bovine ovary, although a similar organization is observed in other mammals. Common BL components are laminins and collagen type IV and XVIII fibres (bovine: Rodgers and Irving-Rodgers, 2010c), whose concentration and interactions change during follicle growth, displaying a progressive decrease of collagens and increase of laminins (primate: Hornick *et al.*, 2012). More specifically, the BL of

primordial follicles is a network of laminin $\alpha1$, $\beta2$ and $\gamma1$ and a lattice-like matrix of collagen type IV $\alpha1$, $\alpha2$, $\alpha3$, $\alpha4$, $\alpha5$ and $\alpha6$ (Fig. 1A) (bovine: van Wezel *et al.*, 1998; Rodgers *et al.*, 1998; human: Heeren *et al.*, 2015). After follicle recruitment, at the preantral stage, BL composition changes with the production of nidogen, a sulphated monomeric glycoprotein, and of perlecan, a heparan sulphate proteoglycan, synthesized by both vascular endothelial and smooth muscle cells (Fig. 1B) (bovine: Rodgers *et al.*, 2003). The functional role of the highly conserved perlecan and nidogen in the ovary has not yet been elucidated. However, in other tissues, perlecan plays a regulatory function in the paracrine activity of fibroblast growth factor (FGF) pathway, by binding to both FGF and its receptor (FGFR), thus stabilizing the FGF-FGFR complex (Whitelock
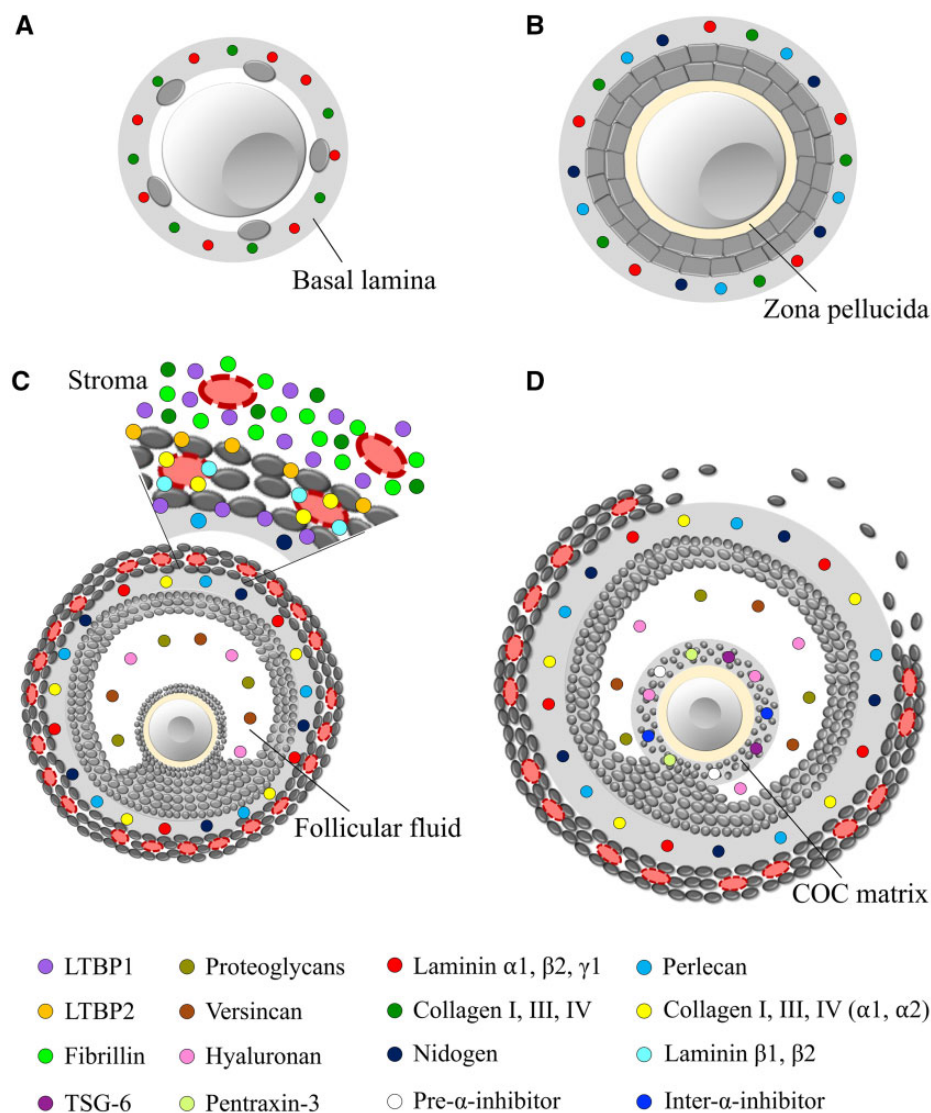


**Figure 1. Ovarian extracellular matrix (ECM) components.** Changes in follicular ECM molecules and their spatial localization during folliculogenesis, from the (**A**) primordial to the (**B**) secondary, (**C**) antral and (**D**) preovulatory follicle. The enlargement in (**C**) gives a detail of the theca and of the surrounding stroma. COC, cumulus–oocyte complex.

et al., 2008); the latter, as observed in human keratinocytes, plays a crucial role, through its central G2 domain, in the maintenance of a strong link between laminins, collagen IV and perlecan, thus preserving the BL structural integrity (Sage et al., 2012).

BL composition remains similar at the antral stage in bovines, with the exception of a loss of collagen type IV α3-α6, whose downstream effect is a marked reduction of BL stiffness. This is a biomechanical requirement for follicle size expansion and for preparation for ovulation (Fig. 1C) (bovine: van Wezel et al., 1998; Rodgers et al., 1998; McArthur et al., 2000).

Alterations to this ultrastructural organization with the addition of further BL layers result in a phenotype named 'loopy' BL, observed in both bovine and human follicles, which has been associated with oocytes with a lower developmental competence (bovine: Irving-Rodgers and Rodgers, 2000; Rodgers and Irving-Rodgers, 2010b). Experimental evidence showed that bovine oocytes with a normal BL display twice the capacity to reach the blastocyst stage, compared to those with a 'loopy' BL (bovine and human: Irving-Rodgers et al., 2009b).

A further interesting variation in the BL organization, described in mice (Zalewski et al., 2012), rats (Bagavandoss, 2014), bovines (Irving-Rodgers et al., 2004, 2006; Matti et al., 2010) and humans (Alexopoulos et al., 2000), and correlated to follicle quality, is represented by a specialized BL-type matrix which forms, at the time of dominant follicle selection, focal intra-epithelial aggregates (focimatrix) interposed amid granulosa cells. Typical focimatrix components are collagen type IV α1 and α2, laminin α1, β2 and γ1, perlecan and nidogen 1 and 2. Importantly, the volume density of focimatrix components is higher in follicles with high levels of molecular markers for dominance (bovine: Irving-Rodgers et al., 2009a).

A further follicular ECM component is the ZP, a layer of three/four main glycoproteins (e.g. ZP1-3 in the mouse or ZP1-4 in human) (mouse: Wassarman et al., 2004; mouse and human: Gupta et al., 2012), with a well-known role at the time of fertilization, when the oocyte encounters sperm and their binding with ZP3 prompts the acrosome reaction and subsequent crossing (mouse: Wassarman and Litscher, 2018). Just a few seconds after sperm-oocyte plasma membrane fusion, the oocyte's release of its enzymatic content of ~4000 cortical granules (mouse: Ducibella et al., 1988) into the perivitelline space induces ZP hardening (ZPH), an evolutionary conserved mechanism that blocks polyspermy (Liu, 2011). Among the enzymes encased inside the cortical granules, ovostacin, an astacin-family metalloproteinase, cleaves ZP2, thus preventing further sperm binding (mouse: Burkart et al., 2012). On the contrary, the ovastacin-inhibitor, fetuin-B, released by the liver into the plasma, blocks the occurrence of ZPH prior to fertilization, thus maintaining oocytes' potential for fertilization (mouse: Dietzel et al., 2013). The timely occurrence of this process and the interplay between ovostacin and its inhibitor fetuin-B are considered of crucial relevance, on one side to avoid polyspermy, and, on the other, to allow correct fertilization (mouse: Korschgen et al., 2017).

Disturbances to this sequence of events leads to an early ZPH and, as a consequence, to infertility. Early ZPH has been observed during in vitro maturation of mouse (Larman et al., 2006), rat (Zhang et al., 1991) and human (Zhang et al., 1991; Schiewe et al., 1995) oocytes, but it has also been reported following cryopreservation of mouse (Carroll et al., 1990) and human oocytes (Coticchio et al., 2007; De Santis et al., 2020) and in mouse postovulatory aged oocytes (Xu et al., 1997). Lastly, worth mentioning is the case of the rare, but severe, genuine empty follicle syndrome, whereby mutations of ZP1 and ZP3 genes hamper ZP assembly, leading to human oocyte degeneration (Dai et al., 2019).

Parallel to the changes occurring to the BL organization and to the events involved in ZP deposition, during antral follicle growth, granulosa cells synthesize large ECM molecules such as proteoglycans, versican and hyaluronan (HA), whose relocation into the follicular antrum (Fig. 1C) induces an increase of osmotic pressure and, as a consequence, the recruitment of plasma fluid from the capillaries irrorating the theca layer (bovine: Clarke et al., 2006; Rodgers and Irving-Rodgers, 2010a). The follicular fluid contains other ECM components, such as laminin and fibronectin. High concentrations of fibronectin in antral preovulatory human follicles correlate positively with oocyte quality (Honda et al., 2004).

At the time of ovulation (Fig. 1D), the LH surge initiates a cascade of events beginning with granulosa cell production of epidermal growth factor-like peptides, and oocyte synthesis of soluble oocyte-secreted factors [e.g. Bone morphogenetic protein 15 (BMP-15) and Growth differentiation factor 9 (GDF9), belonging to the transforming growth factor β (TGFβ) superfamily], all of which bind to their respective cumulus cells receptors and, via Mothers against decapentaplegic homolog 2/3 (SMAD2/3) or SMAD1/5/8, activate cumulus cells expression of hyaluronan synthase 2 and, in turn, the production of HA. HA accumulation, stabilized by proteins like pentraxin-3 (PTX3), Tumor necrosis factor-inducible gene 6 protein (TSG-6) and the heavy chains of inter-α-inhibitor and pre-α-inhibitor, produces a soft viscoelastic ECM which creates the physiological biomechanical condition for mouse cumulus–oocyte complex (COC) expansion (Chen et al., 2016; Lo et al., 2019). While these events are occurring, both BL and cortical stromal ECM are degraded at the follicle apex to facilitate COC ovulation (see below) (ovine: Murdoch, 1998; Mammals: Curry and Smith, 2006).

This process of ECM degradation determines an abrupt decrease in collagen type XVIII α1 in bovine developmentally competent COCs, as opposed to incompetent COCs which maintain higher collagen levels (bovine: Melo et al., 2017).

## Stromal ECM

The stroma includes the tunica albuginea, the cortical and medullar stroma, and the theca externa and interna (mammals: Kinnear et al., 2020).

The main stromal components of ECM are collagen I, III and IV fibres, fibrillin 1 (FBN1) and latent TGF-β-Binding Proteins 1 and 2 (LTBP1, LTBP2) (Fig. 1C, enlargement) (human: Lind et al., 2006; Mammals: Kinnear et al., 2020). FBNs are glycoproteins with both structural and regulative roles. In association with elastin fibres or extracellular microfilaments, they form microfibrils (Ramirez and Pereira, 1999; Kielty et al., 2002); furthermore, they play a key role in TGFβ pathway regulation given their association with LTBPs. In epithelial cells of the ovarian stroma, TGFβ 1, 2 or 3 pro-peptides bind to LTBP1 forming large latent complexes (LLCs), which are then secreted into the ECM (bovine: Rodgers and Irving-Rodgers, 2010c). Once inside the ECM, the interaction of LLCs with the N-terminal domain of FBN1 inhibits the release of TGFβs (Hirani et al., 2007); instead, the

association of LTBP2 to the complex prompts TGFβ bioavailability (Mammals: Robertson *et al.*, 2015).

The stromal compartment hosts a rich vasculature that may penetrate through to the bovine follicle theca layers. Its endothelium is surrounded by a BL made of laminin β1 and β2, collagen type IV α1 and α2 and collagen type XVIII α1 (Fig. 1C, enlargement) (bovine: Rodgers *et al.*, 2003).

# A dynamic network of blood vessels accompanies and supports follicle growth and oocyte maturation

Within the ovary, a further paramount regulative component is represented by a rich blood vasculature that, through a process of neo-angiogenesis, surrounds newly recruited follicles during the whole growth phase and, following ovulation, undergoes regression in the corpora lutea. Notably, physiological neo-angiogenesis is a remarkable and unique feature of the ovary, which has not been described in any other adult organ (Mammals: Fraser, 2006).

Before entering the ovary at the hilum site, the ovarian artery divides into uterine and ovarian branches. Once inside the ovary, the artery further divides into smaller arterioles which cross the medulla region and envelop the growing follicle within a meshwork of new capillaries (Fig. 2A) (rodents: Macchiarelli *et al.*, 1992; Mammals: Redmer and Reynolds, 1996). Thanks to this abundant and dynamic network of blood vessels, follicles are delivered with oxygen, growth factors, gonadotrophins and steroid precursors, which are necessary to sustain their growth and to regulate their selection and acquisition of developmental competence (bovine: Kosaka *et al.*, 2007; Mammals: Rizov *et al.*, 2017). The vascular system is also made of a multitude of venules responsible for draining out of the follicles those by-products derived from their active metabolism. Although a flourishing vasculature is associated with the development of a healthy follicle, and a regressed vessels network is an early sign of atresia (hamster: Greenwald, 1989), the cause–effect relationship remains poorly understood. Similarly, the dominant follicle is characterized by a denser and more permeable vascular network (porcine: Barboni *et al.*, 2000). In clinical practice, ultrasonographic assessment of high blood flow rate around single follicles is associated with both a higher developmental potential of the enclosed oocyte and higher chance of a viable pregnancy (human: Bhal *et al.*, 1999; Borini *et al.*, 2001; Costello *et al.*, 2005).

At the beginning of folliculogenesis, a group of arterioles extends into the cortical region forming the stromal vasculature that will supply, by passive diffusion (Fig. 2B; wavy red arrows), all follicles from the primordial to the secondary with nutrients and oxygen (primate: Fraser and Duncan, 2005). The formation of new vasculature in the proximity of mouse primordial follicles determines an increase in blood supply which contributes to the activation of dormant primordial follicles (mouse: Komatsu and Masubuchi, 2020).

After recruitment, when follicles reach the pre-antral stage, FSH stimulates the surrounding theca cells to produce and release several pro-angiogenic factors outside the follicle into the stromal compartment (Mammals: Fraser, 2006). In the pig, these factors promote,

within the thecal layers, the formation of two concentric anastomotic calyx-like networks of vessels surrounding the follicle (porcine: Reynolds *et al.*, 1992). Theca cells generate a diffusion gradient of vascular endothelial growth factor A (VEGFA) which prompts endothelial cells migration towards the growing mouse follicles and capillaries sprouting (Zhang *et al.*, 2019) (for a detailed description of the signalling pathways involved, see Fig. 2C).

Alongside these major events, two other crucial pathways favour the angiogenetic process. Key to a proper tip ECs migration is the concomitant matrix metalloproteinase (MMP)-mediated degradation of the surrounding ECM induced by local hypoxia via hypoxia-inducible factor 1α (Krock *et al.*, 2011; mouse: Zhang *et al.*, 2019). Also, both the ratio between the production of angiopoietin 1 (ANPT1) and 2 (ANPT2) by theca cells and the expression of VEGF regulate vessel stabilization or destabilization. High concentrations of VEGF, associated with an increased ANPT2:ANPT1 ratio, leads to the formation of new vessels; conversely, vessels destabilization is associated with low VEGF levels (bovine: Hayashi *et al.*, 2003; primate: Hazzard *et al.*, 1999; human: Wulff *et al.*, 2000).

At the time of ovulation, the inner vessel network of the theca undergoes intensive sprouting towards the granulosa, whereas the BL, located in between, undergoes MMP-mediated fragmentation (ovine: Murdoch, 1998; Mammals: Curry and Smith, 2006). This remodelling facilitates the invasion of fenestrated capillaries from the theca to the granulosa and their subsequent extensive proliferation, a process that warrants the required supply of oxygen and metabolites to both the somatic and germ cell components of the porcine preovulatory follicle (porcine: Martelli *et al.*, 2006). Interestingly, at least in the pig, this intensive capillary proliferation represents a mechanical force that might explain the observed change in follicular morphology, from the characteristic roundish to a more irregular shape. In addition to its trophic role, different regional contractions of the vasculature around the preovulatory follicle determine blood flow changes and contribute to apical rupture and subsequent oocyte ovulation (see below).

Immediately after ovulation, the corpus luteum is the site of a crescendo of blood vessel proliferation mediated by increased activity of the VEGFA-VEGFR2 signalling pathway (Mammals: Lu *et al.*, 2019), needed for structural and endocrine functional differentiation of the corpus luteum (bovine: Shirasuna *et al.*, 2012; human: Sugino *et al.*, 2008; Mammals: Lu *et al.*, 2019). A further key factor in the corpus luteum development is ANPT2, whose high levels allow for vasculature remodelling during the early stages of corpus luteum regression (human: Sugino *et al.*, 2005; bovine: Mishra *et al.*, 2016).

# Mechanical properties of the ovarian tissue regulate signal transduction during folliculogenesis

Each histological context defines specific mechanical properties key to the regulation of cell maintenance, differentiation, growth, motility and apoptosis, or its deviance to a pathological state (Ingber *et al.*, 2014; Ingber, 2018; Janmey *et al.*, 2020). Growing follicles are subject to mechanical forces from their surrounding environment and exhibit variations in their own mechanical features during folliculogenesis. This was
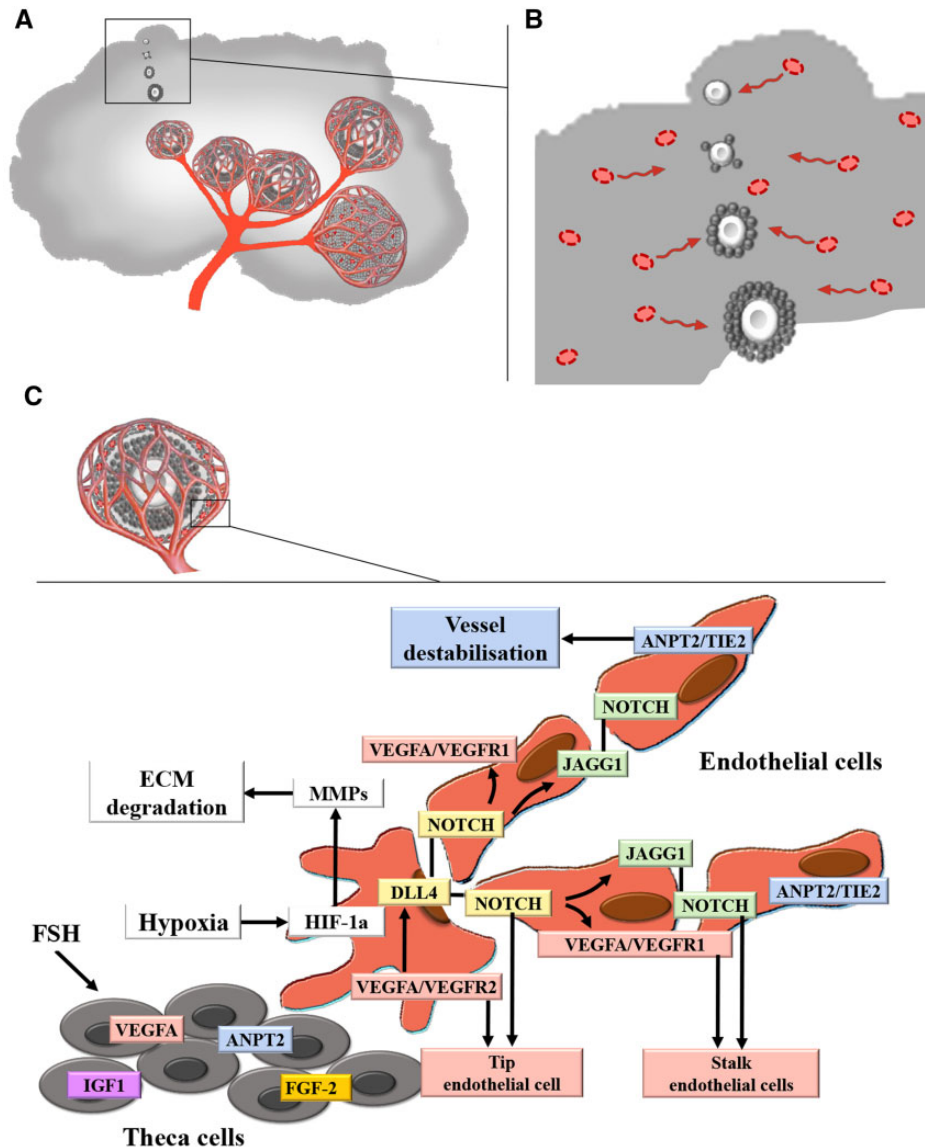
**Figure 2. Remodelling of the blood vasculature during folliculogenesis. (A)** A rich network of capillaries contributes to supply follicles with oxygen, nutrients and signalling molecules throughout folliculogenesis. **(B)** During the initial stages of follicle growth, from the primordial to secondary follicle, this contribution occurs through a passive diffusion mechanism (wavy arrows). **(C)** Then, after recruitment, a meshwork of capillaries, is generated thanks to a process of neo-angiogenesis, encapsulates each follicle. A diffusion gradient of vascular endothelial growth factor A (VEGFA) is sensed by the plasma membrane VEGFR2 receptor on tip endothelial cells (ECs), and triggers their amoeboid migration towards the growing follicles, and, in turn, promotes proliferation and reallocation of stalk ECs, contributing to capillary sprouting (Robinson et al., 2009; Xie et al., 2017). These early events lead to a signalling cascade that involves tip ECs expression of the transmembrane ligand Delta-like 4 (DLL4) and subsequent binding with the Neurogenic locus notch homolog protein 1 (NOTCH) on proximal stalk ECs (lateral inhibition). Then, stalk ECs are prevented from differentiating into tip ECs through the activation of two main pathways. The first pathway promotes the binding between VEGFR1 receptor and VEGFA, with the inactivation of the latter; the second, which involves the binding between the ligand Jagged 1 (JAGG1) and NOTCH on adjacent cells, replaces the DLL4-NOTCH interaction (lateral induction). ECM, extracellular matrix.

particularly clear when observing, in alginate hydrogel 3D follicle *in vitro* cultures, how follicle growth, theca development, antrum formation, hormone production and the overall oocyte quality are influenced by an interplay of physical forces and signalling pathways (mouse: Smith et al., 2014; Tagler et al., 2014; human: Laronda et al., 2014; Yin et al., 2016).

Inside the ovary, the most external cortex and the internal medulla, due to their cellular and extracellular composition, display decreasing inward stiffness (primate: Hornick et al., 2012) and different overall mechanical properties (Mammals: Woodruff and Shea, 2011; mouse: Chan et al., 2021) (Fig. 3A).
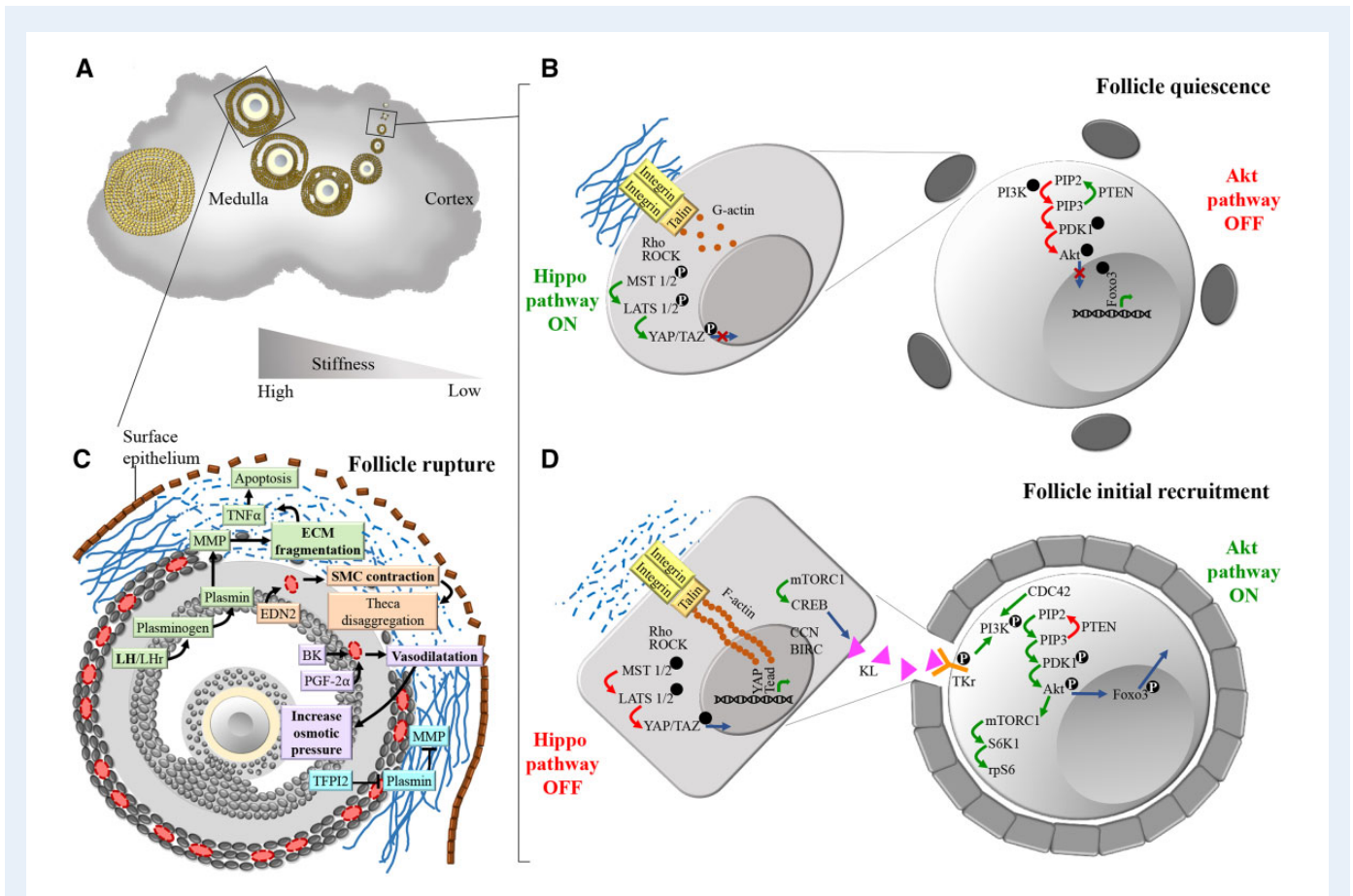
**Figure 3. Biomechanical regulation of signalling pathways during folliculogenesis.** (**A**) During folliculogenesis, follicles reposition between the cortical and medullar regions, histological environments with different degrees of stiffness. (**B**) Maintenance of primordial follicle quiescence. A higher degree of stiffness in the cortical ECM, sensed by integrin mechanoceptors on the granulosa cells plasma membrane, preserves the activity of Hippo signalling (enlargement, green arrows) and, in turn, an inactive Akt (red arrows) pathway in the oocyte. MST 1/2, serine/threonine protein kinases 1 and 2; LATS 1/2, serine/threonine-protein kinase LATS1 and 2; YAP, Yes-associated protein/Transcriptional coactivator; G-actin, globular actin; TAZ, PDZ-binding motif protein; CDC42, cell division control protein 42 homolog; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted from chromosome 10; PDK1, 3-phosphoinositide-dependent protein kinase-1; Akt, protein kinase B; FOXO3, fork-head box O3. Black circle with P, phosphorylated form; black circle, unphosphorylated form. (**C**) A reduction of ECM stiffness, regulating the inactivation of Hippo (enlargement, red arrows) and the activation of Akt (green arrows) pathways, prompts the transition from the primordial to primary follicle. TEAD, transcriptional enhancer associate domain; F-actin, filamentous actin; CCN, cellular communication network; BIRC, baculoviral inhibitors of apoptosis repeats-containing protein; mTORC1, mammalian target of rapamycin complex 1; CREB, cyclic AMP response element-binding protein; KL, Kit ligand; TKr, tyrosine kinase receptor; S6K1, ribosomal protein S6 kinase beta-1; rpS6, ribosomal protein S6. (**D**) At the time of ovulation, follicle rupture occurs through the synergic activity of four main signalling cascades. The LH surge triggers a pathway that leads to matrix metalloproteinases (MMP)-mediated extracellular matrix (ECM) fragmentation and, as a consequence, to apoptosis of surface epithelial cells at the follicle apex (green boxes). Away from the follicle rupture region, the inhibition of the MMPs activity arrests ECM fragmentation (light blue boxes). To allow COC extrusion from the theca layers, endothelin 2 (EDN2) triggers the contraction of smooth muscle cells (SMCs) surrounding the vascular endothelium and theca disaggregation (orange boxes). Also, inside the theca, capillaries are stimulated by BK and PGF-2α to vasodilatation determining a downstream increase of osmotic pressure inside the antrum that prompts ovulation (violet boxes).

During folliculogenesis, while follicles are repositioning within these regions, they encounter different molecular microenvironments which determine the activation of local biomechanical signalling pathways, guiding further follicle growth and oocyte maturation (Mammals: Woodruff and Shea, 2011; Shah *et al.*, 2018). These differences may be critical to follicle recruitment which occurs right at the interface between the cortex and medulla; furthermore, the softer medulla environment is more propitious to accompanying follicle expansion (Mammals: Woodruff and Shea, 2011). In this context, the activities of Hippo and Akt, two mechano-transduction signalling pathways, are crucial at the time of initial follicle recruitment (human: Hsueh *et al.*, 2015) and, more generally, in the control of organ size (Halder and Johnson, 2011; Zhang, 2015).

Together with hormonal signals, the local composition of the ECM surrounding early follicles plays a role in the transition from quiescence

(Fig. 3B) to recruitment (Fig. 3C). A higher degree of stiffness in the cortical region maintains the basal physiological state and follicle quiescence: mechanical forces of the cortex ECM are sensed by integrin mechanoceptors on the surface of primordial oocytes, determining the activation and inactivation of Hippo and Akt pathways, respectively (Fig. 3B). On one side, the Hippo pathway cascade induces, through negative growth factors (MST 1/2 and LATS 1/2) and via Rho-GTPase and Rho-associated protein kinase (ROCK), the phosphorylation of the Yes-associated protein/Transcriptional coactivator with PDZ-binding motif complex (YAP/TAZ) (mouse: Abbassi et al., 2016; human: Lunding et al., 2020). On the other side, the activation of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) maintains the phosphorylated and inactive form of the Akt pathway molecules favouring, thanks to Fork-head box O3 (FOXO3), the transcription of follicular quiescence regulators (mouse: Castrillon et al., 2003; John et al., 2008; ovine: Adib et al., 2019; primate: Ting and Zelinski, 2017; human: McLaughlin et al., 2014; Ernst et al., 2017; Albamonte et al., 2020).

The opposite situation occurs at the passage from the cortex to medulla (Fig. 3C). Integrins on granulosa cells surface sense a reduced collagen concentration (lower ECM stiffness) and activate a mechano-transduction cascade leading to actin polymerization and Hippo pathway inactivation (human: Lunding et al., 2020; Hsueh and Kawamura, 2020). Specifically, the dephosphorylation of Hippo intermediates induces the migration of YAP/TAZ into the nucleus, its association with transcriptional enhancer associate domain (TEAD), and the transcription of CCN growth factors and baculoviral inhibitors of apoptosis repeats-containing (BIRC) proteins, that promote follicle initial activation for growth. In parallel, granulosa cells activate the mTOR pathway and, via the cyclic AMP response element-binding protein (CREB), the secretion of KIT ligand, which binds to its tyrosine kinase receptor (TKr) on the oolemma (mouse: Zhang et al., 2014; Li et al., 2020). This signal triggers the phosphorylation of Akt signalling factors inducing Akt-dependent FOXO3 phosphorylation and its exit from the nucleus. This process ends with the initiation of oocyte maturation and BMP-15 and GDF9 synthesis (mouse and human: Kawamura et al., 2013; human: Grosbois and Demeestere, 2018; mammals: Kawashima and Kawamura, 2018). Meanwhile, by inhibiting the Tuberous sclerosis complex 1/2 (TSC1/2), Akt intermediates activate the mTORC1-S6K1-rpS6 signalling cascade, essential for protein translation and follicle activation (mouse: Tong et al., 2013; human: Yorino and Kawamura, 2020).

A very recent study of the ECM surrounding human primordial to primary follicles, before and after puberty, described higher collagen levels in the former age compared to the latter, thus explaining the non-permissive environment for follicle activation and growth of pre-pubertal ovaries (Ouni et al., 2020).

Beside the ECM differences described for cortical and medullar regions, important biomechanical events occur locally in the ECM surrounding single growing follicles, capable of inducing the activation of other signalling pathways. This is well described in preovulatory follicles at the time of ovulation, when follicle rupture occurs through the synergic activity of four main signalling cascades (Fig. 3D). The LH surge stimulates ovine granulosa cells production of plasminogen that, after its activation into plasmin, induces theca cells MMPs production and release into the surrounding ECM. Here, the enzymatic activity of MMPs degrades filaments (human: Vos et al., 2014; McCord et al.,

2012) and triggers the release of the tumour necrosis factor-alpha (TNFα) ligand that promotes apoptosis of apical superficial epithelial cells (Fig. 3D, green boxes) (ovine: Murdoch, 1998; Mammals: Curry and Smith, 2006).

Distant from the area of follicle rupture, the tissue factor pathway inhibitor 2 (TFPI2) inhibits rodents granulosa cells plasmin production contributing to the maintenance of the ECM integrity (Fig. 3D, light blue boxes) (rat and human: Puttabyatappa et al., 2017). Concurrently, granulosa cells synthesize a number of factors that contribute to ovulation. Endothelin 2 (EDN2), a mechano-transduction protein, triggers the contraction of smooth muscle cells surrounding the vascular endothelium, initiating a process of theca disaggregation which will lead to COC extrusion (Fig. 3D, orange boxes) (mouse: Ko et al., 2006). Furthermore, inside the theca, bradykinin and Placenta growth factor 2α (PGF-2α) induce capillary vasodilatation, a downstream increase of the osmotic pressure inside the antrum, and ovulation (Fig. 3D, violet boxes) (rat: Matousek et al., 2001; Mammals: Jorge et al., 2014).

With ageing, both mouse and human ovaries display an increased collagen and decreased HA deposition, which correlates with augmented stiffness and fibrosis in the stroma (mouse and human: Amargant et al., 2020; human: Ouni et al., 2020).

Alongside the mechanical forces acting at the level of the different functional compartments, the growing female gamete itself exhibits specific mechanical properties reacting to the changes occurring during maturation. The measurement of these changes during the GV-to-MII transition showed an overall reduction of human (Yanez et al., 2016) and mouse (Larson et al., 2010) oocytes stiffness; using microfluidic devices, microelectromechanical analyses showed a 2.8-fold reduction of ZP rigidity and a 6-fold decrease of oocyte cortical tension, modifications which could both favour the fertilization process. Combining time-lapse imaging with particle image velocimetry, measurements of local cytoplasmic visco-elasticity highlighted profiles of cytoplasmic movements during the GV-to-MII transition and at the time of fertilization, correlating with mouse oocytes developmental competence (Ajduk et al., 2011; Bui et al., 2017; Cavalera et al., 2018).

# Alterations of ovarian ECM, vasculature and mechanical properties associated with infertility-related pathologies and therapeutic approaches

Alterations of the ovarian morpho-functional context are associated with some of the most frequent causes of infertility-related pathologies. We will review some changes in ECM composition, biomechanical properties and vascular network, which have been described in PCOS, endometriosis and POI. For each of these pathologies, potential therapeutic strategies, aimed at correcting an altered biomechanical context and at restoring physiological folliculogenesis, will be also reviewed.

## Polycystic ovary syndrome

PCOS is the most common endocrine disorder in women of reproductive age, with a prevalence between 6% and 15%, depending on

the diagnostic criteria applied (Teede *et al.*, 2018). PCOS also represents one recognized cause of female infertility, mainly due to chronic anovulation (Teede *et al.*, 2010). Whilst the pathophysiology remains poorly understood, the main clinical features of PCOS, occurring in various combinations, are polycystic ovarian morphology at ultrasound examination, chronic anovulation resulting in irregular menstrual patterns, and clinical and/or biochemical hyperandrogenism (human: Shorakae *et al.*, 2018; Teede *et al.*, 2018). Furthermore, a significant proportion of women with PCOS display signs and symptoms related to the metabolic syndrome, such as upper body obesity, insulin resistance, hyperlipidaemia, hypertension and many suffer from type II diabetes and cardiovascular disease (human: Anagnostis *et al.*, 2018). These metabolic disturbances influence ovarian steroidogenesis and function and may aggravate the phenotypic expression of the syndrome (human: de Medeiros *et al.*, 2021).

Whilst women with PCOS show hypothalamic–pituitary abnormalities (Liao, Qiao and Pang, 2021), which often relate to metabolic aberrations and to pro-inflammatory cytokine and chemokine profiles (human: Rudnicka *et al.*, 2021), intrinsic alterations of ovarian function are considered the main culprit of the syndrome (human: Gilling-Smith *et al.*, 1997; Chang and Cook-Andersen, 2013). The distinct morphology of polycystic ovaries is characterized by an increased number of preantral and antral follicles, which tend to arrest their growth at the mid-antral stage. This in turn leads to the formation of cystic follicles, which exhibit thin granulosa cell layers and theca cell hyperplasia (human: Chang, 2007). Furthermore, polycystic ovaries display an increased number of atretic follicles and a reduced number of corpora

lutea (Fig. 4). In extreme cases of PCOS, stromal hyperplasia and hyperthecosis (nests of luteinized theca cells within ovarian stroma) can be observed (human Meczekalski *et al.*, 2021). A further feature of polycystic ovaries is disturbed angiogenesis, characterized by increased vascularization of the ovarian stroma. Indeed, increased concentrations of several angiogenic factors, including VEGF, are found in both plasma and follicular fluid of women with PCOS (Di Pietro *et al.*, 2018).

As described above (Fig. 1), the structural organization and function of the ovarian ECM is regulated by a large number of proteoglycans, non-proteoglycan polysaccharides and fibrous proteins, which are critical to correct follicle maturation, and whose localization and amount change during folliculogenesis.

Local quantitative alterations of collagen type IV, fibrillin-3, perlecan and PTX3 are correlated with the polycystic ovary phenotype (Fig. 4). More specifically, increased collagen deposition in the ovarian stroma, resulting in partial fibrosis, has been for many years considered a hallmark of the syndrome (human: Hughesdon, 1982; Papachroni *et al.*, 2010). Although the mechanisms behind the increased collagen deposition are not clearly elucidated, the evidence points to a key role of fibrillins and of the TGFβ signalling pathway in the aetiology of the disease. Among the three fibrillins found in the ECM, the ubiquitously distributed fibrillin 1 and 2, localized around antral and lytic follicles, are quantitatively similar in control and PCOS ovaries. Instead, fibrillin 3, whose distribution is restricted to the perifollicular stroma at the primordial to primary follicles transition, is less represented in human PCOS ovaries (Jordan *et al.*, 2010) (Fig. 4, left-hand side). A decrease in fibrillin 3, observed in both foetal and adult PCOS ovaries
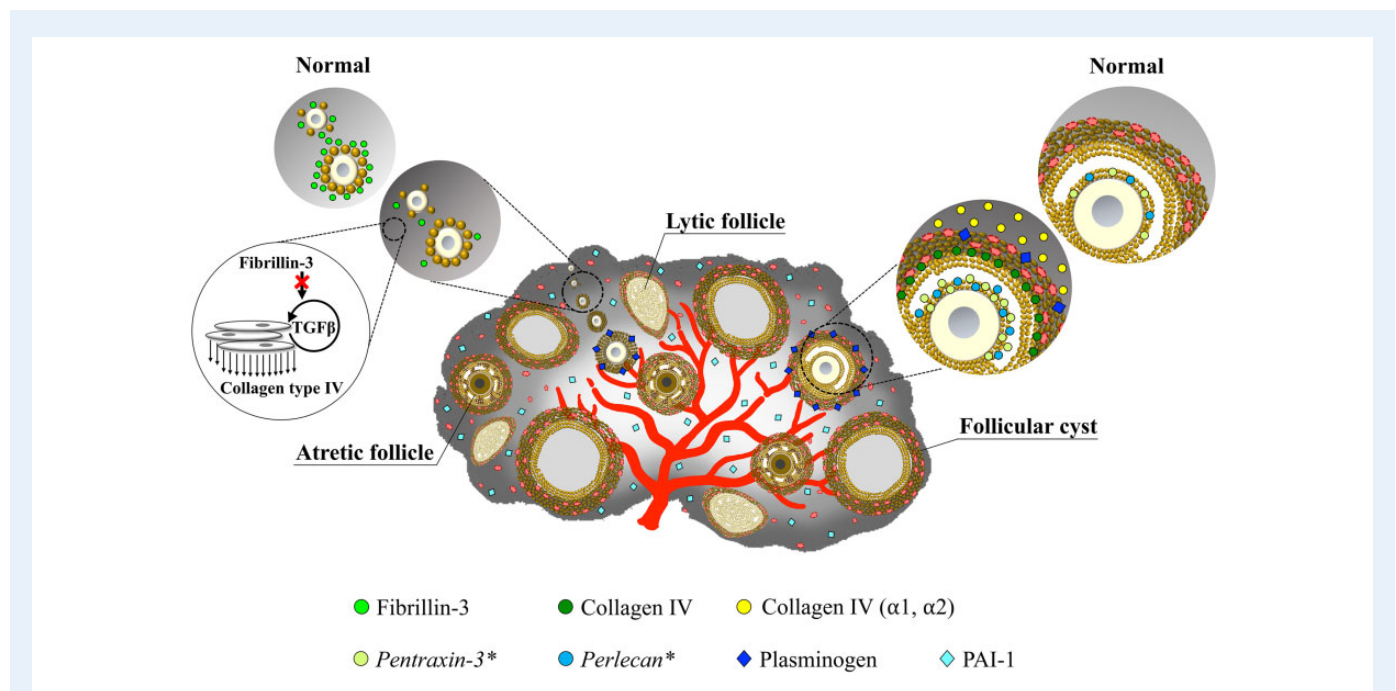


**Figure 4. Main alterations observed in the PCOS ovary.** Clearly observable are follicular cysts, enveloped by a collagen fibrous capsule, thickened theca cell layers and thin granulosa cell layers, an increased number of atretic small antral follicles, lytic follicles, the absence of corpora lutea, an increased vascular density, and altered levels of extracellular matrix (ECM) proteins or transcripts (*). PAI-1, plasminogen activator inhibitor-1; TGFβ, transforming growth factor β.

(Mammals: Raja-Khan et al., 2014), is associated with an increased TGFβ bioavailability, which, as in many other fibrotic organs, may stimulate an increased fibroblast production and collagen deposition (rodents: LeClair and Lindner, 2007; Mammals: Chung et al., 2021). This condition results in a 50%, 30% and 20% thicker tunica albuginea, external cortical stroma and deep cortical or medullary stroma, respectively (human: Hughesdon, 1982). Evidence for a key role of the TGFβ pathway has been obtained by using a TGF-βRI inhibitor in a PCOS rat model. The inhibitor significantly reduced the expression of pro-fibrotic TGFβ, while increasing anti-fibrotic MMP2, thus preventing collagen deposition (rat: Wang et al., 2018).

A further alteration in the ECM composition has been described for the proteoglycan molecule perlecan, whose HSPG2 transcript in cumulus cells of preovulatory follicles is more abundant in women with PCOS than in controls. When co-detected with ADAMTS1 transcript level, the ratio ADAMTS1/HSPG2 is directly related to the oocyte's developmental competence (mouse and human: Ma et al., 2020).

PTX3 stabilizes the ECM of the cumulus oophorus, preventing proteolytic degradation (mouse: Salustri et al., 2004), and, thus, playing a crucial role for correct cumulus expansion and fertilization. The lack of PTX3 in homozygous null-PTX3 mice leads to disruption of the structural integrity of the cumulus complex and to subfertility (mouse: Varani et al., 2002). On the other hand, an excessive expression of PTX3 in both human follicle cells and follicular fluid of PCOS patients has been associated with the pro-inflammatory profile often found in these women (Pan et al., 2018, 2021).

The impairment of ECM remodelling is among the potential mechanisms behind oligo/anovulation in women with PCOS (Fig. 3D). The ECM of these patients shows down-regulation of key genes (ECM1, CTNNA1, ITGA5, LAMA3, LAMB1, FN1 and ITGA7) and the absence of MMPs (Tarkun et al., 2004; Gomes et al., 2011; Hassani et al., 2019). Studies in animal models suggest that a further potential cause of disturbed ovulation involves both the distribution and quantity of molecules of the plasminogen system, i.e. plasminogen activator inhibitor-1 (PAI-1) and plasminogen (Fig. 3D, right-hand side). The former is distributed throughout the PCOS mouse ovary, but its presence is limited to the cortex in the normal gonad; the latter is detected from the late preantral to the fully-grown preovulatory follicles in PCOS, whereas it is restricted to the preovulatory phase in control ovaries (mouse: Burchall et al., 2019). The changes in amount and localization of these proteins suggest the involvement of the proteolytic/fibrinolytic plasminogen system in PCOS ovaries not only at the time of follicle rupture but also during the initial phases of follicle growth. Whilst immunohistochemical studies have not yet been performed on human ovaries, it has been known for some time that PAI-1 concentrations are increased in the plasma of women with PCOS (Koiou et al., 2012; Koiou et al., 2013; Mammals: Cesari et al., 2010).

All the evidence listed so far supports the notion that increased density of collagen fibres within the ovary, augmented cortical stiffness and altered ECM composition determine a biomechanically non-permissive framework for follicle recruitment and growth (human: Takahashi et al., 1994; Swanson et al., 1981; Azziz et al., 2009; Mammals: Woodruff and Shea, 2011).

A further distinct feature of PCOS ovaries is an increased capillary density within the extrafollicular tissue, a condition which decreases cortical stiffness and may contribute to the lower recruitment rate of primordial and primary follicles (human: Delgado-Rosas et al., 2009)

(Fig. 4). Due to the larger stromal vascularization, the ovarian blood flow is significantly increased in patients with PCOS compared to that in control women (Zaidi et al., 1998; Battaglia et al., 2008; Dwivedi et al., 2020).

These anatomic and functional changes are matched by changes at a molecular level. Modifications in the concentration and activity of proangiogenic factors belonging to the VEGF family (Fig. 3C) are associated with PCOS (Mammals: Tal et al., 2015). Several VEGF gene polymorphisms have been described in association with either increased (human: Zhao et al., 2020) or decreased (human: Liu et al., 2021) PCOS susceptibility. In addition, there is a general consensus that both VEGF follicular production and bioavailability are increased in PCOS ovaries (human: Artini et al., 2009; Scotti et al., 2014; Tal et al., 2014). From a clinical standpoint, up-regulation of VEGF-related factors, both in serum and within the ovary, likely contributes to the dysregulation of the early stages of folliculogenesis in women with PCOS (Agrawal et al., 2002; Abd El Aal et al., 2005; Artini et al., 2006; Patil et al., 2021).

### Therapeutic strategies

The first-line approach for women with PCOS seeking pregnancy is induction of ovulation. This is usually obtained by administering either antioestrogens (clomifene citrate), aromatase inhibitors (letrozole) or exogenous gonadotrophins (Teede et al., 2018). While ovulation can be accomplished in a significant proportion of cases, some women with PCOS will either resist the therapy or display a multifollicular response, thus incurring the risk of multiple pregnancies and of ovarian hyperstimulation syndrome (OHSS). In order to overcome these problems, an alternative approach is represented by ovarian laparoscopic drilling (LOD), a surgical procedure aimed at correcting the altered biomechanical context of the PCOS ovary. LOD consists of multiple punctures on the ovarian surface, performed by either unipolar diathermy or laser vaporization (Bordewijk et al., 2020). Although he efficacy of LOD in terms of increased ovulatory cycles is established, the true impact on live birth rate is still a matter of debate and the exact mechanisms by which ovulation is restored are not fully elucidated (human: Bordewijk et al., 2020). Clearly, LOD implies destruction of the ovarian tissue, in particular within the thickened cortical and subcortical stroma (Kinnear et al., 2020).

Clinical studies have shown that androgen serum concentrations decrease dramatically after LOD, and, as a consequence, conversion of androgens to oestrogens is also decreased (human: Flyckt and Goldberg, 2011). Thus, one potential mechanism of LOD could be the partial normalization of the hypothalamic–pituitary–ovarian (HPO) axis.

A second mechanism, which could explain the efficacy of LOD, is normalization of ovarian angiogenesis and vasculature; in fact, studies in women with PCOS have shown decreased VEGF serum concentrations (Amin et al., 2003; El Behery et al., 2011) and decreased ovarian blood flow after LOD (Giampaolino et al., 2017).

A further alternative mechanism that improves ovarian function after LOD is that activated by a decreased Anti-Müllerian hormone (AMH) serum level, reported after this procedure, which contributes to increased follicle recruitment (human: Garg and Tal, 2016).

Of major interest in the context of the present review is the notion that LOD could induce ovulation by mechanical disruption of the Hippo signalling, in turn resulting in the promotion of follicle growth

(human: Farquhar *et al.*, 2012; Abu Hashim, 2015). This mechanism is supported by *in vitro* studies of entire murine ovaries and human ovarian cortical strips. Actin polymerization-promoting drugs, such as jasplakinolide and sphingosine-1-phosphate, increase the conversion of actin from a globular to a filamentous form, promoting YAP translocation into the nucleus, and stimulate CCN expression and follicle growth (mouse: Cheng *et al.*, 2015). On the basis of these observations, alternative future pharmacological treatments of anovulation in PCOS can be foreseen.

Due to the high prevalence of metabolic aberrations and insulin resistance in women with PCOS, hypoglycaemic drugs have been used in the last 30 years to induce ovulation directly or, at least, to facilitate clomiphene/gonadotropin-induced ovulation. The most used is metformin (MET), a biguanide originally introduced for the treatment of type II diabetes (Notaro and Neto, 2022), and currently used in various pathologies, in particular as anticancer drugs for its antiangiogenic activity (human: Fujita and Inagaki, 2017; Yang *et al.*, 2021; Park *et al.*, 2021). When administered to women with PCOS, MET reduces both ovarian volume and blood flow, improving hormonal profiles (Makled *et al.*, 2014). Indirect evidence of an effect of MET on ovarian vasculature and blood flow is the reduction of OHSS after ovarian stimulation for IVF (human: Palomba *et al.*, 2013). In a rat model of PCOS, MET restored the ovarian endothelium and normalized VEGF and ANPT1 protein levels, decreasing follicular cyst formation, and improving follicle maturation and ovulation (Di Pietro *et al.*, 2015).

Accumulating evidence for the role of ovarian angiogenesis and blood flow in the pathogenesis of PCOS anovulation points to VEGF production and biological activity as a potential target for future pharmacological therapies. Studies in PCOS model animals have shown that local injection of the platelet-derived growth factor B decreased intraovarian concentrations of VEGF, and partially re-established the number of small follicles, limiting cystic follicle formation (rat: Di Pietro *et al.*, 2016).

## Ovarian endometriosis

Endometriosis is a hormone-dependent gynaecologic disease affecting up to 5–10% of women of reproductive age (Bulun, 2009; Macer and Taylor, 2012). It is characterized by the presence of endometrial tissue outside the uterine cavity, most frequently in the pelvis (i.e. peritoneum, rectovaginal septum, fallopian tubes and ovaries), and occasionally outside the pelvis (i.e. colon, liver, lungs) (human: Rock and Markham, 1992; Giudice, 2010; Vercellini *et al.*, 2014). Hypotheses on the origin, the pathogenesis and the classification of the disease are still controversial. The most credited theory is based on the concept of retrograde menstruation. The core of this theory is based on the observation of physical displacement of endometrial fragments (retrograde transportation of menstrual debris via fallopian tubes), which would escape immune clearance, invading the surrounding tissues and establishing local vascularization (human: Sampson, 1927).

In the ovary, endometrioma is a common lesion that affects 30–50% women diagnosed with endometriosis (Macer and Taylor, 2012). Endometriomas have a major negative impact on the reserve of dormant primordial follicles. This is aggravated when surgical interventions are performed in order to remove the lesions. The reduction of ovarian reserve becomes particularly evident in patients with endometriomas undergoing assisted reproductive technologies. In fact, whilst the proportion of good quality embryos is maintained, the overall number of oocytes retrieved and those at the MII stage is significantly lower than that in healthy women (Yland *et al.*, 2020; Alshehre *et al.*, 2021; Bishop *et al.*, 2021; Vaiarelli *et al.*, 2021).

Similarly to other endometriotic lesions, endometriomas may originate from fragments of endometrial tissue reaching the ovarian cortex (Fig. 5, left-hand side). Fragments localize immediately beneath the surface epithelium and develop into cysts surrounded by stroma and a single layer of columnar endometrial epithelial cells. These implants maintain responsiveness to oestrogenic stimulation, and display cyclic proliferation, shedding and bleeding, similarly to the eutopic endometrium (human: Brosens *et al.*, 1994).

The implants grow and gradually accumulate cytokines, TGFβs, MMPs, factors of the plasminogen system and VEGF, together with menstrual debris (human: Exacoustos *et al.*, 2018). At some point, the epithelial wall of the endometrioma outbreaks, releasing its content in the surrounding stroma. The release of cytokines stimulates chronic inflammation, which in concert with VEGF, intensifies the process of neo-angiogenesis around the endometrial cyst, supporting its survival and further growth (human: Cooper and Rathbone, 1990; McLaren *et al.*, 1996; Mahnke *et al.*, 2000; Gazvani and Templeton, 2002; Goteri *et al.*, 2004; Ceyhan *et al.*, 2008; Goteri *et al.*, 2010). At the same time, TGFβ1 induces fibrosis via the TGFβ1/Smad signalling pathway (human: Shi *et al.*, 2017; Zeng *et al.*, 2018), whereas the enzymatic activity of MMPs contributes to tearing endometrial tissue, favouring new implants and spreading of the disease (human: Kokorine *et al.*, 1997; Chung *et al.*, 2001; Luddi *et al.*, 2020). Aberrations in the plasminogen system are a characteristic common to all types of endometriotic lesions (human: Gilabert-Estellés *et al.*, 2003; Alotaibi *et al.*, 2019). Since PAI-1 regulates the activity of plasmin and plasmin-dependent MMPs in the ECM remodelling (human: Silva *et al.*, 2013), the invasiveness and pro-fibrotic properties of endometriomas may be further explained by the higher concentration of PAI-1 found in both cyst fluid and inner epithelium (human: Boss *et al.*, 2002; Alotaibi *et al.*, 2019).

In summary, the physical invasiveness of the cyst in combination with the release of its stored factors and an inflammation outburst are the main aspects that concur to reduce the reserve of dormant primordial follicles (human: Kitajima *et al.*, 2011; Kitajima *et al.*, 2014; Carrillo *et al.*, 2016). Importantly, as shown in non-endometriotic benign ovarian cysts (human: Kuroda *et al.*, 2012), the growing endometrioma is not able to alter the biomechanical properties of the cortex per se and determine a reduction in primordial follicles. In endometriosis, the latter is also induced by the local activity of (i) TGF-βs, MMPs and PAI-1 factors, which determine a radical perturbation of the major ECM components (a cause of subsequent fibrosis), (ii) VEGF, which contributes to a flourishing of capillaries and (iii) cytokines, which stimulate an inflammatory response. Although the molecular mechanisms behind the follicle reduction are still unknown, some have observed an accelerated follicular recruitment (human: Kitajima *et al.*, 2014), that, perhaps, could be explained by a local inactivation of the Hippo pathway (Fig. 3C), and, concurrently, by increased follicles atresia (human: Kitajima *et al.*, 2014).

### Therapeutic strategies

In patients with endometrioma, milder forms of the disease can be treated with pharmacological therapies targeted at reducing both pain
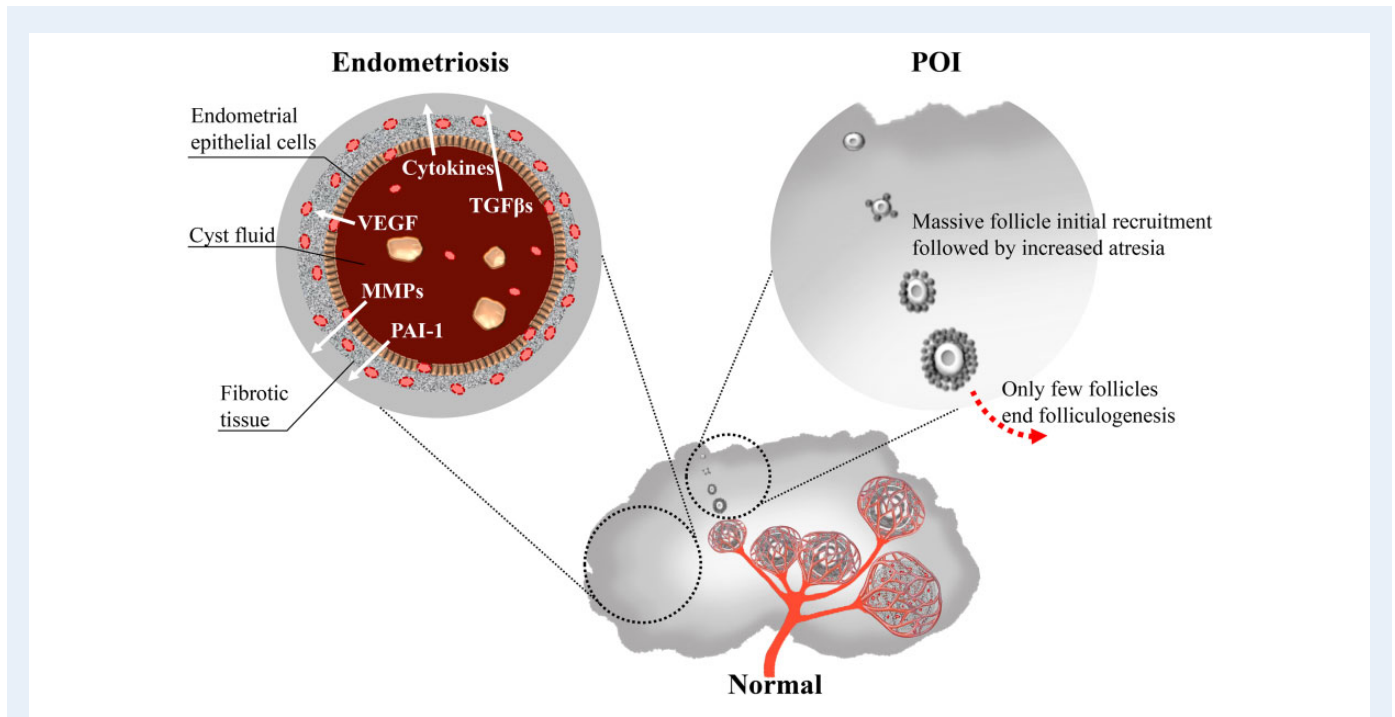
**Figure 5. Main alterations observed in the endometriotic or POI ovary.** The endometrioma is surrounded by stroma and a single layer of columnar endometrial epithelial cells. These cysts keep their receptivity to oestrogens, cyclic proliferation, shedding and bleeding. The release of cytokines, VEGF, TGFβs, MMPs and PAI-1 and an inflammation outburst cause local reduction of the primordial follicles reserve. Premature ovarian insufficiency is mainly characterized by a massive and uncontrolled initial follicle recruitment, followed by increased atresia, and terminating with an overall depauperated follicular pool. POI, primary ovarian insufficiency; VEGF, vascular endothelial growth factor; TGFβ, transforming growth factor β; MMPs, matrix metalloproteinases; PAI-1, plasminogen activator inhibitor-1.

and the size of the cysts. Contraceptive pills, progesterone and progestins, GnRH agonists (e.g. leuprolide) or androgen-derived compounds (e.g. danazol) are the approaches most frequently used (human: Guzick et al., 2011; Hull et al., 1987; Uludag et al., 2021). Common to all these therapies is the inhibition of the HPO axis, whereas some of them (e.g. progestins or danazol) exert also a modulation of oestradiol receptor function and of oestrogen synthesis, resulting in a reduction of both the oestrogenic stimulation and oestrogen sensitivity of endometriotic tissues.

More recently, a number of VEGF antagonists have been identified for their ability to inhibit angiogenesis and, thus, reduce the vascular network that supplies the cysts, preventing their growth. In a murine model of endometriosis obtained by implanting human endometrium into nude mice, two VEGFA antagonists, a truncated inhibitory receptor and an antibody, inhibited the growth of endometriotic implants (Hull et al., 2003).

In addition to synthetic drugs, natural compounds have been also considered. Epigallocatechin-3-gallate (EGCG), is a pleiotropic polyphenol, found in large concentrations in green tea, which is currently studied for its potential application in several fields of medicine (Chu et al., 2017). EGCG suppresses VEGF expression in cultured endometrial cells. In vivo, 14 days of EGCG administration to hamsters selectively inhibited angiogenesis around the cysts without affecting blood vessel development in ovarian follicles. Eventually, the treatment reduced the volume of endometriotic lesions (Laschke et al., 2008).

When endometriomas are very large and/or rapidly expanding, surgical drainage and excision might be more indicated (human: Hayasaka et al., 2011). However, there is a long-lasting debate about the consequences of such procedures on the ovarian reserve. Although several studies suggest that cystectomy provides good results in terms of recurrence, pain symptoms and subsequent spontaneous pregnancy, the traditional approach of cyst stripping leads to a reduction of the ovarian reserve caused by an unintentional excision of the surrounding ovarian cortex (human: Muzii et al., 2005, 2016; Rius et al., 2020). Furthermore, the use of energy-based technologies to guarantee haemostasis during surgery, negatively affects ovarian blood supply, thus worsening the risk of follicle loss (human: Reich and Abrao, 2006; Peters et al., 2017). As an alternative, the use of $CO_2$ lasers and plasma energy devices is associated with a minimal interference with ovarian biomechanics and a reduced loss of primordial follicles (human: Wyns and Donnez, 2003; Chen et al., 2021).

## Premature ovarian insufficiency

POI, also known as premature ovarian failure, is a degenerative disease of the ovary that affects up to 1% of women under 40 years of age and causes infertility (Coulam, 1982; Cohen et al., 2015; Gleicher et al., 2015; Tucker et al., 2016). POI occurs when the pool of primordial follicles is depleted or inhibited in activation (human: Kawashima and Kawamura, 2017). The aetiological and pathophysiological

mechanisms underlying spontaneous POI have not yet been determined, whereas chemo- and radiotherapy represent known causes of iatrogenic POI (human: Bao *et al.*, 2018; Blumenfeld, 2019). Clinically, POI is associated with amenorrhoea, increased gonadotropin production and low serum concentrations of ovarian steroids (mouse: Wang *et al.*, 2013; human: Sullivan *et al.*, 2016; Kozub *et al.*, 2017; Ishizuka *et al.*, 2021).

One of the putative mechanisms involved in the pathogenesis of POI is compromised vascularity. Recent evidence shows that some allelic variants of the pro-angiogenic *VEGFA* gene, are associated with low serum levels of VEGFA and with the risk of POI (human: Li *et al.*, 2021).

Of relevance for the present review, POI correlates also with biomechanical variations of the cortex that affect the early stages of folliculogenesis and lead to a diminished follicle pool (human: de Koning *et al.*, 2008). Disturbances in the Hippo and Akt mechano-transduction pathways, which regulate follicle quiescence/recruitment (Fig. 3B and C), have been indicated as the molecular context in which POI develops. One example comes from studies in a mouse model of POI, in which animals lacking a major negative regulator of phosphatidylinositol 3-kinase (PI3K), display Akt hyperactivation in primordial follicles. As a consequence, follicles undergo massive and uncontrolled initial recruitment, which is followed by increased atresia, eventually leading to an overall diminished follicular pool (Fig. 5, right-hand side) (mouse: Reddy *et al.*, 2008).

*Therapeutic strategies*

Traditionally, the treatment for women with POI is aimed at restoring physiological concentrations of sex hormones, by exogenous hormone replacement. While this treatment alleviates the symptoms and prevents several long-term systemic complications related to oestrogen depletion, it does not reconstitute the ovarian reserve (human: Webber *et al.*, 2017; Kozub *et al.*, 2017). Expanding knowledge about the physiology of biomechanics and of paracrine pathways within the ovary has paved the way for recent attempts to rescue the small number of primordial follicles still remaining in the ovaries of POI patients.

One of the methods most explored is *in vitro* activation (IVA) of primordial dormant follicles (human: Li *et al.*, 2010). In recent clinical studies, the surgical isolation of ovarian fragments, followed by *in vitro* culture with Akt stimulators, led to the disruption of the Hippo signalling pathway by conversion of the G-actin into F-actin, which resulted in activation of primordial follicles. When the ovarian fragments were auto-transplanted in POI patients, follicular growth was restored, mature oocytes were recovered, and healthy babies were delivered following IVF (human: Kawamura *et al.*, 2013, Suzuki *et al.*, 2015; Zhai *et al.*, 2016).

As a further development, the possibility of a drug-free IVA has been successfully explored. Laparoscopic removal of part of the ovarian cortex, simply cut in small cubes and immediately repositioned as autograft within either the tubal serosa or between the ovarian cortex and medulla, proved to be sufficient to disrupt the Hippo signalling, activating follicle recruitment and growth in POI patients (Tanaka *et al.*, 2020) and in women with diminished ovarian reserve (DOR) (Lunding *et al.*, 2019; Kawamura *et al.*, 2020; Tanaka *et al.*, 2020). This allowed either spontaneous pregnancy or successful IVF in a proportion of treated women. In some cases of POI, the procedure might result in resumption of spontaneous ovulation even for several months post-

surgery. The relevance of biomechanics in ovarian follicle dynamics is further supported by the results of a clinical study performed in women with a syndrome of ovarian resistance to exogenous gonadotropins (Kawamura *et al.*, 2013). A simple laparoscopic ovarian incision resulted in improved follicular responsiveness and growth in 7 out of 10 women (human: Tanaka *et al.*, 2020). Although these surgical techniques have not yet reached the stage for general clinical applicability, and attitudes towards such an approach are controversial (Steiner, 2019), these studies nevertheless represent a proof-of-concept for future therapies focused on ovarian biomechanics.

To date, follicle activation has been obtained in clinical settings by surgery and mechanical injury to the ovary. However, studies in rodents demonstrated the ability of actin polymerization-enhancing drugs to promote YAP nuclear translocation and follicle growth (Cheng *et al.*, 2015). Basis on these results, future studies might evaluate the efficacy of intraovarian injection of drugs for Hippo signalling disruption in human ovarian pathologies.

An alternative line of research is focused on the ovarian vascular network and on the putative role of growth factors within the ovary. Following intraovarian injection of either platelet-rich plasma or mesenchymal stem cells, recent studies reported successful stimulation of folliculogenesis and induction of ovulation via remodelling of the ovarian microvasculature (rat: Vural *et al.*, 2019; Ahmadian *et al.*, 2020; Cho *et al.*, 2021; mouse: Feng *et al.*, 2019; human: Park *et al.*, 2019). This approach has also been used in patients with DOR or poor ovarian response to FSH, but so far with conflicting results (Sills *et al.*, 2018; Atkinson *et al.*, 2021; Hajipour *et al.*, 2021).

# Conclusions

Our understanding of the biomechanical interactions occurring within the ovary is far from exhaustive. However, the evidence collected so far support the notion of a crucial role of the ovarian mechanical environment in both the development of the gonad and its function during the reproductive years (Ouni *et al.*, 2020). Indeed, dynamic is the most appropriate adjective to describe the adult mammalian ovary, as it portrays an organ in continuous histological re-organization. The changes occurring during folliculogenesis to the ovarian ECM and the vasculature remodelling around the growing follicle are aimed to sustain its recruitment and growth, together with the maturation of the enclosed companion oocyte. These events define the scenario in which mechanical forces are key to the regulation of cascades of known, or still poorly known, molecular signals. Alterations to this context determine impair folliculogenesis and oocyte developmental potential, as observed in pathological conditions like PCOS, endometriosis or POI that lead to infertility.

Improving our knowledge in this specific field of ovarian physiology has implications for future clinical applications. As an example, a better understanding of the biomechanical properties of follicles could help to refine ART. Applying certain mechanical inputs to oocytes cultured *in vitro* might improve the quality of the embryos obtained, thus increasing pregnancy rates (He, 2017; Yanez and Camarillo, 2017). Furthermore, expanding our knowledge on the mechanisms behind follicular activation and on the modulation of the Hippo signalling pathway, might help developing new clinical protocols for fertility-sparing treatments in patients with POI or in women who face gonadotoxic

therapies. To date, fertility preservation in pre-pubertal women is performed by ovarian tissue freezing. This procedure has been available over the last two decades, and the technique has been progressively improved. However, when ovarian fragments are thawed and transplanted, it is estimated that 50–90% of primordial follicles are lost (Celik et al., 2020). Protocols using either pharmacological or mechanical follicular activation might overcome this crucial problem. Similarly, these techniques could help in the clinical management of women with large and/or rapidly growing endometriomas, who face the same risk of ovarian depletion. Even wider applications in reproductive medicine can be predicted, considering the rapidly increasing number of women of advanced reproductive age and DOR, who resort to ART (Choi et al., 2022). Finally, since the same conditions apply to PCOS, alternative, less invasive and more efficient strategies for ovulation induction in women unresponsive to conventional therapies, including ovarian drilling, might be foreseen.

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# Conflict of interest

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# References

Abbassi L, Malki S, Cockburn K, Macaulay A, Robert C, Rossant J, Clarke HJ. Multiple mechanisms cooperate to constitutively exclude the transcriptional co-activator YAP from the nucleus during murine oogenesis. Biol Reprod 2016;**94**:102.

Abd El Aal DE, Mohamed SA, Amine AF, Meki AR. Vascular endothelial growth factor and insulin-like growth factor-1 in polycystic ovary syndrome and their relation to ovarian blood flow. Eur J Obstet Gynecol Reprod Biol 2005;**118**:219–224.

Abu Hashim H. Predictors of success of laparoscopic ovarian drilling in women with polycystic ovary syndrome: an evidence-based approach. Arch Gynecol Obstet 2015;**291**:11–18.

Adib S, Valojerdi MR, Alikhani M. Dose optimisation of PTEN inhibitor, bpV (HOpic), and SCF for the in-vitro activation of sheep primordial follicles. Growth Factors 2019;**37**:178–189.

Agrawal R, Jacobs H, Payne N, Conway G. Concentration of vascular endothelial growth factor released by cultured human luteinized granulosa cells is higher in women with polycystic ovaries than in women with normal ovaries. Fertil Steril 2002;**78**:1164–1169.

Ahmadian S, Sheshpari S, Pazhang M, Bedate AM, Beheshti R, Abbasi MM, Nouri M, Rahbarghazi R, Mahdipour M. Intra-ovarian injection of platelet-rich plasma into ovarian tissue promoted rejuvenation in the rat model of premature ovarian insufficiency and restored ovulation rate via angiogenesis modulation. Reprod Biol Endocrinol 2020;**18**:78.

Ajduk A, Ilozue T, Windsor S, Yu Y, Seres KB, Bomphrey RJ, Tom BD, Swann K, Thomas A, Graham C et al. Rhythmic actomyosin-driven contractions induced by sperm entry predict mammalian embryo viability. Nat Commun 2011;**2**:417.

Albamonte MI, Calabró LY, Albamonte MS, Zuccardi L, Stella I, Halperin J, Vitullo AD. PTEN and FOXO3 expression in the prenatal and postnatal human ovary. J Assist Reprod Genet 2020;**37**:1613–1622.

Alexopoulos E, Shahid J, Ongley HZ, Richardson MC. Luteinized human granulosa cells are associated with endogenous basement membrane-like components in culture. Mol Hum Reprod 2000;**6**:324–330.

Alotaibi FT, Peng B, Klausen C, Lee AF, Abdelkareem AO, Orr NL, Noga H, Bedaiwy MA, Yong PJ. Plasminogen activator inhibitor-1 (PAI-1) expression in endometriosis. PLoS One 2019;**14**:e0219064.

Alshehre SM, Narice BF, Fenwick MA, Metwally M. The impact of endometrioma on in vitro fertilisation/intra-cytoplasmic injection IVF/ICSI reproductive outcomes: a systematic review and meta-analysis. Arch Gynecol Obstet 2021;**303**:3–16.

Amargant F, Manuel SL, Tu Q, Parkes WS, Rivas F, Zhou LT, Rowley JE, Villanueva CE, Hornick JE, Shekhawat GS et al. Ovarian stiffness increases with age in the mammalian ovary and depends on collagen and hyaluronan matrices. Aging Cell 2020;**19**:e13259.

Amin AF, Abd el-Aal DE, Darwish AM, Meki AR. Evaluation of the impact of laparoscopic ovarian drilling on Doppler indices of ovarian stromal blood flow, serum vascular endothelial growth factor, and insulin-like growth factor-1 in women with polycystic ovary syndrome. Fertil Steril 2003;**79**:938–941.

Anagnostis P, Tarlatzis BC, Kauffman RP. Polycystic ovarian syndrome (PCOS): long-term metabolic consequences. Metabolism 2018;**86**:33–43.

Artini PG, Monti M, Matteucci C, Valentino V, Cristello F, Genazzani AR. Vascular endothelial growth factor and basic fibroblast growth factor in polycystic ovary syndrome during controlled ovarian hyperstimulation. Gynecol Endocrinol 2006;**22**:465–470.

Artini PG, Ruggiero M, Parisen Toldin MR, Monteleone P, Monti M, Cela V, Genazzani AR. Vascular endothelial growth factor and its soluble receptor in patients with polycystic ovary syndrome undergoing IVF. Hum Fertil (Camb) 2009;**12**:40–44.

Atkinson L, Martin F, Sturmey RG. Intraovarian injection of platelet-rich plasma in assisted reproduction: too much too soon? Hum Reprod 2021;**36**:1737–1750.

Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE et al.; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 2009;**91**:456–488.

Bagavandoss P. Temporal expression of tenascin-C and type I collagen in response to gonadotropins in the immature rat ovary. *Acta Histochem* 2014;**116**:1125–1133.

Bao R, Xu P, Wang Y, Wang J, Xiao L, Li G, Zhang C. Bone marrow derived mesenchymal stem cells transplantation rescues premature ovarian insufficiency induced by chemotherapy. *Gynecol Endocrinol* 2018;**34**:320–326.

Barboni B, Turriani M, Galeati G, Spinaci M, Bacci ML, Forni M, Mattioli M. Vascular endothelial growth factor production in growing pig antral follicles. *Biol Reprod* 2000;**63**:858–864.

Battaglia C, Mancini F, Cianciosi A, Busacchi P, Facchinetti F, Marchesini GR, Marzocchi R, de Aloysio D. Vascular risk in young women with polycystic ovary and polycystic ovary syndrome. *Obstet Gynecol* 2008;**111**:385–395.

Berkholtz CB, Shea LD, Woodruff TK. Extracellular matrix functions in follicle maturation. *Semin Reprod Med* 2006;**24**:262–269.

Bhal PS, Pugh ND, Chui DK, Gregory L, Walker SM, Shaw RW. The use of transvaginal power Doppler ultrasonography to evaluate the relationship between perifollicular vascularity and outcome in in-vitro fertilization treatment cycles. *Hum Reprod* 1999;**14**:939–945.

Bishop LA, Gunn J, Jahandideh S, Devine K, Decherney AH, Hill MJ. Endometriosis does not impact live-birth rates in frozen embryo transfers of euploid blastocysts. *Fertil Steril* 2021;**115**:416–422.

Blumenfeld Z. Fertility preservation in women with malignancy: future endeavors. *Clin Med Insights Reprod Health* 2019;**13**: 1179558119872490.

Bordewijk EM, Ng KYB, Rakic L, Mol BWJ, Brown J, Crawford TJ, van Wely M. Laparoscopic ovarian drilling for ovulation induction in women with anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev* 2020;**2**:CD001122.

Borini A, Maccolini A, Tallarini A, Bonu MA, Sciajno R, Flamigni C. Perifollicular vascularity and its relationship with oocyte maturity and IVF outcome. *Ann N Y Acad Sci* 2001;**943**:64–67.

Boss EA, Massuger LF, Thomas CM, Geurts-Moespot A, van Schaik JH, Boonstra H, Sweep CG. Clinical value of components of the plasminogen activation system in ovarian cyst fluid. *Anticancer Res* 2002;**22**:275–282.

Brosens IA, Puttemans PJ, Deprest J. The endoscopic localization of endometrial implants in the ovarian chocolate cyst. *Fertil Steril* 1994;**61**:1034–1038.

Bui TTH, Belli M, Fassina L, Vigone G, Merico V, Garagna S, Zuccotti M. Cytoplasmic movement profiles of mouse surrounding nucleolus and not-surrounding nucleolus antral oocytes during meiotic resumption. *Mol Reprod Dev* 2017;**84**:356–362.

Bulun SE. Endometriosis. *N Engl J Med* 2009;**360**:268–279.

Burchall GF, Pouniotis DS, Teede HJ, Ranasinha S, Walters KA, Piva TJ. Expression of the plasminogen system in the physiological mouse ovary and in the pathological polycystic ovary syndrome (PCOS) state. *Reprod Biol Endocrinol* 2019;**17**:33.

Burkart AD, Xiong B, Baibakov B, Jimenez-Movilla M, Dean J. Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. *J Cell Biol* 2012;**197**:37–44.

Carrillo L, Seidman DS, Cittadini E, Meirow D. The role of fertility preservation in patients with endometriosis. *J Assist Reprod Genet* 2016;**33**:317–323.

Carroll J, Depypere H, Matthews CD. Freeze-thaw-induced changes of the zona pellucida explains decreased rates of fertilization in frozen-thawed mouse oocytes. *J Reprod Fertil* 1990;**90**:547–553.

Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* 2003;**301**:215–218.

Cavalera F, Zanoni M, Merico V, Bui TTH, Belli M, Fassina L, Garagna S, Zuccotti M. A neural network-based identification of developmentally competent or incompetent mouse fully-grown oocytes. *J Vis Exp* 2018;**133**:56668.

Celik S, Ozkavukcu S, Celik-Ozenci C. Altered expression of activator proteins that control follicle reserve after ovarian tissue cryo-preservation/transplantation and primordial follicle loss prevention by rapamycin. *J Assist Reprod Genet* 2020;**37**:2119–2136.

Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. *Cardiovasc Ther* 2010;**28**:e72–e91.

Ceyhan ST, Onguru O, Baser I, Gunhan O. Expression of cyclooxygenase-2 and vascular endothelial growth factor in ovarian endometriotic cysts and their relationship with angiogenesis. *Fertil Steril* 2008;**90**:988–993.

Chan CJ, Bevilacqua C, Prevedel R. Mechanical mapping of mammalian follicle development using Brillouin microscopy. *Commun Biol* 2021;**4**:1133–1133.

Chang RJ. The reproductive phenotype in polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab* 2007;**3**:688–695.

Chang RJ, Cook-Andersen H. Disordered follicle development. *Mol Cell Endocrinol* 2013;**373**:51–60.

Chen X, Bonfiglio R, Banerji S, Jackson DG, Salustri A, Richter RP. Micromechanical analysis of the hyaluronan-rich matrix surrounding the oocyte reveals a uniquely soft and elastic composition. *Biophys J* 2016;**110**:2779–2789.

Chen J, Huang D, Zhang J, Shi L, Li J, Zhang S. The effect of laparoscopic excisional and ablative surgery on ovarian reserve in patients with endometriomas: a retrospective study. *Medicine (Baltimore)* 2021;**100**:e24362.

Cheng Y, Feng Y, Jansson L, Sato Y, Deguchi M, Kawamura K, Hsueh AJ. Actin polymerization-enhancing drugs promote ovarian follicle growth mediated by the Hippo signaling effector YAP. *FASEB J* 2015;**29**:2423–2430.

Cho J, Kim TH, Seok J, Jun JH, Park H, Kweon M, Lim JY, Kim GJ. Vascular remodeling by placenta-derived mesenchymal stem cells restores ovarian function in ovariectomized rat model via the VEGF pathway. *Lab Invest* 2021;**101**:304–317.

Choi SKY, Venetis C, Ledger W, Havard A, Harris K, Norman RJ, Jorm LR, Chambers GM. Population-wide contribution of medically assisted reproductive technologies to overall births in Australia: temporal trends and parental characteristics. *Hum Reprod* 2022;**37**: 1047–1058.

Chu C, Deng J, Man Y, Qu Y. Green tea extracts epigallocatechin-3-gallate for different treatments. *Biomed Res Int* 2017;**2017**: 5615647.

Chung JY, Chan MK, Li JS, Chan AS, Tang PC, Leung KT, To KF, Lan HY, Tang PM. TGF-β signaling: from tissue fibrosis to tumor microenvironment. *IJMS* 2021;**22**:7575.

Chung H-W, Wen Y, Chun S-H, Nezhat C, Woo B-H, Lake Polan M. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-3

mRNA expression in ectopic and eutopic endometrium in women with endometriosis: a rationale for endometriotic invasiveness. *Fertil Steril* 2001;**75**:152–159.

Clarke HG, Hope SA, Byers S, Rodgers RJ. Formation of ovarian follicular fluid may be due to the osmotic potential of large glycosaminoglycans and proteoglycans. *Reproduction* 2006;**132**:119–131.

Cohen J, Chabbert-Buffet N, Darai E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder—a plea for universal definitions. *J Assist Reprod Genet* 2015;**32**:1709–1712.

Conti M, Franciosi F. Acquisition of oocyte competence to develop as an embryo: integrated nuclear and cytoplasmic events. *Hum Reprod Update* 2018;**24**:245–266.

Cooper EH, Rathbone BJ. Clinical significance of the immunometric measurements of hyaluronic acid. *Ann Clin Biochem* 1990;**27**: 444–451.

Costello MF, Shrestha SM, Sjoblom P, McNally G, Bennett MJ, Steigrad SJ, Hughes GJ. Power Doppler ultrasound assessment of ovarian perifollicular blood flow in women with polycystic ovaries and normal ovaries during in vitro fertilization treatment. *Fertil Steril* 2005;**83**:945–954.

Coticchio G, Bonu MA, Sciajno R, Sereni E, Bianchi V, Borini A. Truths and myths of oocyte sensitivity to controlled rate freezing. *Reprod Biomed Online* 2007;**15**:24–30.

Coulam CB. Premature gonadal failure. *Fertil Steril* 1982;**38**:645–655.

Curry TE Jr, Smith MF. Impact of extracellular matrix remodeling on ovulation and the folliculo-luteal transition. *Semin Reprod Med* 2006;**24**:228–241.

Dai C, Chen Y, Hu L, Du J, Gong F, Dai J, Zhang S, Wang M, Chen J, Guo J. ZP1 mutations are associated with empty follicle syndrome: evidence for the existence of an intact oocyte and a zona pellucida in follicles up to the early antral stage. A case report. *Hum Reprod* 2019;**34**:2201–2207.

de Koning CH, McDonnell J, Themmen AP, de Jong FH, Homburg R, Lambalk CB. The endocrine and follicular growth dynamics throughout the menstrual cycle in women with consistently or variably elevated early follicular phase FSH compared with controls. *Hum Reprod* 2008;**23**:1416–1423.

de Medeiros SF, Rodgers RJ, Norman RJ. Adipocyte and steroidogenic cell cross-talk in polycystic ovary syndrome. *Hum Reprod Update* 2021;**27**:771–796.

De Santis L, Nottola SA, Coticchio G, Borini A, Iussig B, Miglietta S, Macchiarelli G. Type of protein supplement in cryopreservation solutions impacts on the degree of ultrastructural damage in frozen-thawed human oocytes. *Cryobiology* 2020;**95**:143–150.

Delgado-Rosas F, Gaytan M, Morales C, Gomez R, Gaytan F. Superficial ovarian cortex vascularization is inversely related to the follicle reserve in normal cycling ovaries and is increased in polycystic ovary syndrome. *Hum Reprod* 2009;**24**:1142–1151.

Di Pietro M, Parborell F, Irusta G, Pascuali N, Bas D, Bianchi MS, Tesone M, Abramovich D. Metformin regulates ovarian angiogenesis and follicular development in a female polycystic ovary syndrome rat model. *Endocrinology* 2015;**156**:1453–1463.

Di Pietro M, Pascuali N, Parborell F, Abramovich D. Ovarian angiogenesis in polycystic ovary syndrome. *Reproduction* 2018;**155**: R199–R209.

Di Pietro M, Scotti L, Irusta G, Tesone M, Parborell F, Abramovich D. Local administration of platelet-derived growth factor B (PDGFB) improves follicular development and ovarian angiogenesis in a rat model of polycystic ovary syndrome. *Mol Cell Endocrinol* 2016;**433**:47–55.

Dietzel E, Wessling J, Floehr J, Schafer C, Ensslen S, Denecke B, Rosing B, Neulen J, Veitinger T, Spehr M *et al.* Fetuin-B, a liver-derived plasma protein is essential for fertilization. *Dev Cell* 2013; **25**:106–112.

Ducibella T, Anderson E, Albertini DF, Aalberg J, Rangarajan S. Quantitative studies of changes in cortical granule number and distribution in the mouse oocyte during meiotic maturation. *Dev Biol* 1988;**130**:184–197.

Dwivedi AND, Ganesh V, Shukla RC, Jain M, Kumar I. Colour Doppler evaluation of uterine and ovarian blood flow in patients of polycystic ovarian disease and post-treatment changes. *Clin Radiol* 2020;**75**:772–779.

El Behery MM, Diab AE, Mowafy H, Ebrahiem MA, Shehata AE. Effect of laparoscopic ovarian drilling on vascular endothelial growth factor and ovarian stromal blood flow using 3-dimensional power Doppler. *Int J Gynaecol Obstet* 2011;**112**:119–121.

Ernst EH, Grondahl ML, Grund S, Hardy K, Heuck A, Sunde L, Franks S, Andersen CY, Villesen P, Lykke-Hartmann K. Dormancy and activation of human oocytes from primordial and primary follicles: molecular clues to oocyte regulation. *Hum Reprod* 2017;**32**: 1684–1700.

Exacoustos C, De Felice G, Pizzo A, Morosetti G, Lazzeri L, Centini G, Piccione E, Zupi E. Isolated ovarian endometrioma: a history between myth and reality. *J Minim Invasive Gynecol* 2018;**25**: 884–891.

Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev* 2012;**6**:CD001122.

Farquhar CM, Bhattacharya S, Repping S, Mastenbroek S, Kamath MS, Marjoribanks J, Boivin J. Female subfertility. *Nat Rev Dis Primers* 2019;**5**:7.

Feng P, Li P, Tan J. Human menstrual blood-derived stromal cells promote recovery of premature ovarian insufficiency via regulating the ECM-dependent FAK/AKT signaling. *Stem Cell Rev Rep* 2019; **15**:241–255.

Ferreri J, Fabregues F, Calafell JM, Solernou R, Borras A, Saco A, Manau D, Carmona F. Drug-free in-vitro activation of follicles and fresh tissue autotransplantation as a therapeutic option in patients with primary ovarian insufficiency. *Reprod Biomed Online* 2020;**40**: 254–260.

Fidler AT, Bernstein J. Infertility: from a personal to a public health problem. *Public Health Rep* 1999;**114**:494–511.

Fiorentino G, Parrilli A, Garagna S, Zuccotti M. Three-dimensional micro-computed tomography of the adult mouse ovary. *Front Cell Dev Biol* 2020;**8**:566152.

Flyckt RL, Goldberg JM. Laparoscopic ovarian drilling for clomiphene-resistant polycystic ovary syndrome. *Semin Reprod Med* 2011;**29**: 138–146.

Fraser HM. Regulation of the ovarian follicular vasculature. *Reprod Biol Endocrinol* 2006;**4**:18.

Fraser HM, Duncan WC. Vascular morphogenesis in the primate ovary. *Angiogenesis* 2005;**8**:101–116.

Fujita Y, Inagaki N. Metformin: new preparations and nonglycemic benefits. *Curr Diab Rep* 2017;**17**:5.

Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. *Reprod Biomed Online* 2016;**33**:15–28.

Gazvani R, Templeton A. Peritoneal environment, cytokines and angiogenesis in the pathophysiology of endometriosis. *Reproduction* 2002;**123**:217–226.

Giampaolino P, Morra I, De Rosa N, Cagnacci A, Pellicano M, Di Carlo C, Nappi C, Bifulco G. Impact of transvaginal hydrolaparoscopy ovarian drilling on ovarian stromal blood flow and ovarian volume in clomiphene citrate-resistant PCOS patients: a case-control study. *Gynecol Endocrinol* 2017;**33**:690–693.

Gilabert-Estellés J, Estellés A, Gilabert J, Castelló R, España F, Falcó C, Romeu A, Chirivella M, Zorio E, Aznar J. Expression of several components of the plasminogen activator and matrix metalloproteinase systems in endometriosis. *Hum Reprod* 2003;**18**:1516–1522.

Gilling-Smith C, Story H, Rogers V, Franks S. Evidence for a primary abnormality of thecal cell steroidogenesis in the polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1997;**47**:93–99.

Giudice LC. Clinical practice. Endometriosis. *N Engl J Med* 2010;**362**:2389–2398.

Gleicher N, Kushnir VA, Barad DH. Prospectively assessing risk for premature ovarian senescence in young females: a new paradigm. *Reprod Biol Endocrinol* 2015;**13**:34.

Gomes VA, Vieira CS, Jacob-Ferreira AL, Belo VA, Soares GM, Fernandes JB, Ferriani RA, Tanus-Santos JE. Imbalanced circulating matrix metalloproteinases in polycystic ovary syndrome. *Mol Cell Biochem* 2011;**353**:251–257.

Goteri G, Lucarini G, Filosa A, Pierantoni A, Montik N, Biagini G, Fabris G, Ciavattini A. Immunohistochemical analysis of vascular endothelial growth factor cellular expression in ovarian endometriomata. *Fertil Steril* 2004;**81**:1528–1533.

Goteri G, Lucarini G, Zizzi A, Rubini C, Di Primio R, Tranquilli AL, Ciavattini A. Proangiogenetic molecules, hypoxia-inducible factor-1alpha and nitric oxide synthase isoforms in ovarian endometriotic cysts. *Virchows Arch* 2010;**456**:703–710.

Gougeon A, Chainy GB. Morphometric studies of small follicles in ovaries of women at different ages. *J Reprod Fertil* 1987;**81**:433–442.

Greenwald GS. Temporal and topographic changes in DNA synthesis after induced follicular atresia. *Biol Reprod* 1989;**41**:175–181.

Grosbois J, Demeestere I. Dynamics of PI3K and Hippo signaling pathways during in vitro human follicle activation. *Hum Reprod* 2018;**33**:1705–1714.

Gupta SK, Bhandari B, Shrestha A, Biswal BK, Palaniappan C, Malhotra SS, Gupta N. Mammalian zona pellucida glycoproteins: structure and function during fertilization. *Cell Tissue Res* 2012;**349**:665–678.

Guzick DS, Huang LS, Broadman BA, Nealon M, Hornstein MD. Randomized trial of leuprolide versus continuous oral contraceptives in the treatment of endometriosis-associated pelvic pain. *Fertil Steril* 2011;**95**:1568–1573.

Hajipour H, Farzadi L, Latifi Z, Keyhanvar N, Navali N, Fattahi A, Nouri M, Dittrich R. An update on platelet-rich plasma (PRP) therapy in endometrium and ovary related infertilities: clinical and molecular aspects. *Syst Biol Reprod Med* 2021;**67**:177–188.

Halder G, Johnson RL. Hippo signaling: growth control and beyond. *Development* 2011;**138**:9–22.

Hassani F, Oryan S, Eftekhari-Yazdi P, Bazrgar M, Moini A, Nasiri N, Sharifi-Zarchi A. Downregulation of extracellular matrix and cell adhesion molecules in cumulus cells of infertile polycystic ovary syndrome women with and without insulin resistance. *Cell J* 2019;**21**:35–42.

Hayasaka S, Ugajin T, Fujii O, Nabeshima H, Utsunomiya H, Yokomizo R, Yuki H, Terada Y, Murakami T, Yaegashi N. Risk factors for recurrence and re-recurrence of ovarian endometriomas after laparoscopic excision. *J Obstet Gynaecol Res* 2011;**37**:581–585.

Hayashi KG, Acosta TJ, Tetsuka M, Berisha B, Matsui M, Schams D, Ohtani M, Miyamoto A. Involvement of angiopoietin-tie system in bovine follicular development and atresia: messenger RNA expression in theca interna and effect on steroid secretion. *Biol Reprod* 2003;**69**:2078–2084.

Hazzard TM, Molskness TA, Chaffin CL, Stouffer RL. Vascular endothelial growth factor (VEGF) and angiopoietin regulation by gonadotrophin and steroids in macaque granulosa cells during the periovulatory interval. *Mol Hum Reprod* 1999;**5**:1115–1121.

He X. Microfluidic encapsulation of ovarian follicles for 3D culture. *Ann Biomed Eng* 2017;**45**:1676–1684.

Heeren AM, van Iperen L, Klootwijk DB, de Melo Bernardo A, Roost MS, Gomes Fernandes MM, Louwe LA, Hilders CG, Helmerhorst FM, van der Westerlaken LA *et al.* Development of the follicular basement membrane during human gametogenesis and early folliculogenesis. *BMC Dev Biol* 2015;**15**:4.

Hirani R, Hanssen E, Gibson MA. LTBP-2 specifically interacts with the amino-terminal region of fibrillin-1 and competes with LTBP-1 for binding to this microfibrillar protein. *Matrix Biol* 2007;**26**:213–223.

Honda T, Fujiwara H, Yoshioka S, Yamada S, Nakayama T, Egawa M, Nishioka Y, Takahashi A, Fujii S. Laminin and fibronectin concentrations of the follicular fluid correlate with granulosa cell luteinization and oocyte quality. *Reprod Med Biol* 2004;**3**:43–49.

Hornick JE, Duncan FE, Shea LD, Woodruff TK. Isolated primate primordial follicles require a rigid physical environment to survive and grow in vitro. *Hum Reprod* 2012;**27**:1801–1810.

Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. *Endocr Rev* 2015;**36**:1–24.

Hsueh AJW, Kawamura K. Hippo signaling disruption and ovarian follicle activation in infertile patients. *Fertil Steril* 2020;**114**:458–464.

Hughesdon PE. Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called "hyperthecosis". *Obstet Gynecol Surv* 1982;**37**:59–77.

Hull ML, Charnock-Jones DS, Chan CL, Bruner-Tran KL, Osteen KG, Tom BD, Fan TP, Smith SK. Antiangiogenic agents are effective inhibitors of endometriosis. *J Clin Endocrinol Metab* 2003;**88**:2889–2899.

Hull ME, Moghissi KS, Magyar DF, Hayes MF. Comparison of different treatment modalities of endometriosis in infertile women. *Fertil Steril* 1987;**47**:40–44.

Ingber DE. From mechanobiology to developmentally inspired engineering. *Phil Trans R Soc B* 2018;**373**:20170323.

Ingber DE, Wang N, Stamenovic D. Tensegrity, cellular biophysics, and the mechanics of living systems. *Rep Prog Phys* 2014;**77**:046603.

Irving-Rodgers HF, Catanzariti KD, Aspden WJ, D'Occhio MJ, Rodgers RJ. Remodeling of extracellular matrix at ovulation of the bovine ovarian follicle. *Mol Reprod Dev* 2006;**73**:1292–1302.

Irving-Rodgers HF, Harland ML, Rodgers RJ. A novel basal lamina matrix of the stratified epithelium of the ovarian follicle. *Matrix Biol* 2004;**23**:207–217.

Irving-Rodgers HF, Harland ML, Sullivan TR, Rodgers RJ. Studies of granulosa cell maturation in dominant and subordinate bovine follicles: novel extracellular matrix focimatrix is co-ordinately regulated with cholesterol side-chain cleavage CYP11A1. *Reproduction* 2009a;**137**:825–834.

Irving-Rodgers HF, Morris S, Collett RA, Peura TT, Davy M, Thompson JG, Mason HD, Rodgers RJ. Phenotypes of the ovarian follicular basal lamina predict developmental competence of oocytes. *Hum Reprod* 2009b;**24**:936–944.

Irving-Rodgers HF, Rodgers RJ. Ultrastructure of the basal lamina of bovine ovarian follicles and its relationship to the membrana granulosa. *J Reprod Fertil* 2000;**118**:221–228.

Ishizuka B, Furuya M, Kimura M, Kamioka E, Kawamura K. Live birth rate in patients with premature ovarian insufficiency during long-term follow-up under hormone replacement with or without ovarian stimulation. *Front Endocrinol* 2021;**12**:795724.

Janmey PA, Fletcher DA, Reinhart-King CA. Stiffness sensing by cells. *Physiol Rev* 2020;**100**:695–724.

John GB, Gallardo TD, Shirley LJ, Castrillon DH. Foxo3 is a PI3K-dependent molecular switch controlling the initiation of oocyte growth. *Dev Biol* 2008;**321**:197–204.

Jordan CD, Bohling SD, Charbonneau NL, Sakai LY. Fibrillins in adult human ovary and polycystic ovary syndrome: is fibrillin-3 affected in PCOS?. *J Histochem Cytochem* 2010;**58**:903–915.

Jorge S, Chang S, Barzilai JJ, Leppert P, Segars JH. Mechanical signaling in reproductive tissues: mechanisms and importance. *Reprod Sci* 2014;**21**:1093–1107.

Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, Ho CH, Kawamura N, Tamura M, Hashimoto S et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA* 2013;**110**:17474–17479.

Kawamura K, Ishizuka B, Hsueh AJW. Drug-free in-vitro activation of follicles for infertility treatment in poor ovarian response patients with decreased ovarian reserve. *Reprod Biomed Online* 2020;**40**:245–253.

Kawashima I, Kawamura K. Disorganization of the germ cell pool leads to primary ovarian insufficiency. *Reproduction* 2017;**153**:R205–R213.

Kawashima I, Kawamura K. Regulation of follicle growth through hormonal factors and mechanical cues mediated by Hippo signaling pathway. *Syst Biol Reprod Med* 2018;**64**:3–11.

Kielty CM, Wess TJ, Haston L, Ashworth JL, Sherratt MJ, Shuttleworth CA. Fibrillin-rich microfibrils: elastic biopolymers of the extracellular matrix. *J Muscle Res Cell Motil* 2002;**23**:581–596.

Kinnear HM, Tomaszewski CE, Chang FL, Moravek MB, Xu M, Padmanabhan V, Shikanov A. The ovarian stroma as a new frontier. *Reproduction* 2020;**160**:R25–R39.

Kitajima M, Defrère S, Dolmans MM, Colette S, Squifflet J, Van Langendonckt A, Donnez J. Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. *Fertil Steril* 2011;**96**:685–691.

Kitajima M, Dolmans MM, Donnez O, Masuzaki H, Soares M, Donnez J. Enhanced follicular recruitment and atresia in cortex derived from ovaries with endometriomas. *Fertil Steril* 2014;**101**:1031–1037.

Ko C, Gieske MC, Al-Alem L, Hahn Y, Su W, Gong MC, Iglarz M, Koo Y. Endothelin-2 in ovarian follicle rupture. *Endocrinology* 2006;**147**:1770–1779.

Koiou E, Tziomalos K, Dinas K, Katsikis I, Kandaraki EA, Tsourdi E, Mavridis S, Panidis D. Plasma plasminogen activator inhibitor-1 levels in the different phenotypes of the polycystic ovary syndrome. *Endocr J* 2012;**59**:21–29.

Koiou E, Tziomalos K, Katsikis I, Delkos D, Tsourdi EA, Panidis D. Disparate effects of pharmacotherapy on plasma plasminogen activator inhibitor-1 levels in women with the polycystic ovary syndrome. *HJ* 2013;**12**:559–566.

Kokorine I, Nisolle M, Donnez J, Eeckhout Y, Courtoy PJ, Marbaix E. Expression of interstitial collagenase (matrix metalloproteinase-1) is related to the activity of human endometriotic lesions. *Fertil Steril* 1997;**68**:246–251.

Komatsu K, Masubuchi S. Increased supply from blood vessels promotes the activation of dormant primordial follicles in mouse ovaries. *J Reprod Dev* 2020;**66**:105–113.

Korschgen H, Kuske M, Karmilin K, Yiallouros I, Balbach M, Floehr J, Wachten D, Jahnen-Dechent W, Stocker W. Intracellular activation of ovastacin mediates pre-fertilization hardening of the zona pellucida. *Mol Hum Reprod* 2017;**23**:607–616.

Kosaka N, Sudo N, Miyamoto A, Shimizu T. Vascular endothelial growth factor (VEGF) suppresses ovarian granulosa cell apoptosis in vitro. *Biochem Biophys Res Commun* 2007;**363**:733–737.

Kozub MM, Prokopiuk VY, Skibina KP, Prokopiuk OV, Kozub NI. Comparison of various tissue and cell therapy approaches when restoring ovarian, hepatic and kidney's function after chemotherapy-induced ovarian failure. *Exp Oncol* 2017;**39**:181–185.

Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: good and evil. *Genes Cancer* 2011;**2**:1117–1133.

Kuroda M, Kuroda K, Arakawa A, Fukumura Y, Kitade M, Kikuchi I, Kumakiri J, Matsuoka S, Brosens IA, Brosens JJ et al. Histological assessment of impact of ovarian endometrioma and laparoscopic cystectomy on ovarian reserve. *J Obstet Gynaecol Res* 2012;**38**:1187–1193.

Larman MG, Sheehan CB, Gardner DK. Calcium-free vitrification reduces cryoprotectant-induced zona pellucida hardening and increases fertilization rates in mouse oocytes. *Reproduction* 2006;**131**:53–61.

Laronda MM, Duncan FE, Hornick JE, Xu M, Pahnke JE, Whelan KA, Shea LD, Woodruff TK. Alginate encapsulation supports the growth and differentiation of human primordial follicles within ovarian cortical tissue. *J Assist Reprod Genet* 2014;**31**:1013–1028.

Larson SM, Lee HJ, Hung PH, Matthews LM, Robinson DN, Evans JP. Cortical mechanics and meiosis II completion in mammalian oocytes are mediated by myosin-II and Ezrin-Radixin-Moesin (ERM) proteins. *Mol Biol Cell* 2010;**21**:3182–3192.

Laschke MW, Schwender C, Scheuer C, Vollmar B, Menger MD. Epigallocatechin-3-gallate inhibits estrogen-induced activation of endometrial cells in vitro and causes regression of endometriotic lesions in vivo. *Hum Reprod* 2008;**23**:2308–2318.

LeClair R, Lindner V. The role of collagen triple helix repeat containing 1 in injured arteries, collagen expression, and transforming growth factor beta signaling. *Trends Cardiovasc Med* 2007;**17**: 202–205.

Li J, Kawamura K, Cheng Y, Liu S, Klein C, Liu S, Duan EK, Hsueh AJ. Activation of dormant ovarian follicles to generate mature eggs. *Proc Natl Acad Sci USA* 2010;**107**:10280–10284.

Li Y, Liu F, Li S, Tan S. Association of variants in vascular endothelial growth factor A gene and VEGFA serum levels with the risk of primary ovarian insufficiency: a case-control study. *Gynecol Obstet Invest* 2021;**86**:469–476.

Li J, Zhang Y, Zheng N, Li B, Yang J, Zhang C, Xia G, Zhang M. CREB activity is required for mTORC1 signaling-induced primordial follicle activation in mice. *Histochem Cell Biol* 2020;**154**:287–299.

Liao B, Qiao J, Pang Y. Central regulation of PCOS: abnormal neuronal-reproductive-metabolic circuits in PCOS pathophysiology. *Front Endocrinol (Lausanne)* 2021;**12**:667422.

Lind AK, Weijdegård B, Dahm-Kähler P, Mölne J, Sundfeldt K, Brännström M. Collagens in the human ovary and their changes in the perifollicular stroma during ovulation. *Acta Obstet Gynecol Scand* 2006;**85**:1476–1484.

Litscher ES, Wassarman PM. Zona pellucida proteins, fibrils, and matrix. *Annu Rev Biochem* 2020;**89**:695–715.

Liu M. The biology and dynamics of mammalian cortical granules. *Reprod Biol Endocrinol* 2011;**9**:149.

Liu MM, Chen XH, Lu XM, Wang FF, Wang C, Liu Y, Li PL, Du BT, Liang S, Gong PD *et al.* Variations in the profiles of vascular-related factors among different sub-types of polycystic ovarian syndrome in Northern China. *Front Endocrinol (Lausanne)* 2021;**11**: 527592.

Lo BKM, Archibong-Omon A, Ploutarchou P, Day AJ, Milner CM, Williams SA. Oocyte-specific ablation of N- and O-glycans alters cumulus cell signalling and extracellular matrix composition. *Reprod Fertil Dev* 2019;**31**:529–537.

Lu E, Li C, Wang J, Zhang C. Inflammation and angiogenesis in the corpus luteum. *J Obstet Gynaecol Res* 2019;**45**:1967–1974.

Luddi A, Marrocco C, Governini L, Semplici B, Pavone V, Luisi S, Petraglia F, Piomboni P. Expression of matrix metalloproteinases and their inhibitors in endometrium: high levels in endometriotic lesions. *IJMS* 2020;**21**:2840.

Lunding SA, Andersen AN, Hardardottir L, Olesen HO, Kristensen SG, Andersen CY, Pors SE. Hippo signaling, actin polymerization, and follicle activation in fragmented human ovarian cortex. *Mol Reprod Dev* 2020;**87**:711–719.

Lunding SA, Pors SE, Kristensen SG, Landersoe SK, Jeppesen JV, Flachs EM, Pinborg A, Macklon KT, Pedersen AT, Andersen CY *et al.* Biopsying, fragmentation and autotransplantation of fresh ovarian cortical tissue in infertile women with diminished ovarian reserve. *Hum Reprod* 2019;**34**:1924–1936.

Ma Y, Jin J, Tong X, Yang W, Ren P, Dai Y, Pan Y, Zhang Y, Zhang S. ADAMTS1 and HSPG2 mRNA levels in cumulus cells are related to human oocyte quality and controlled ovarian hyperstimulation outcomes. *J Assist Reprod Genet* 2020;**37**:657–667.

Macchiarelli G, Vizza E, Nottola SA, Familiari G, Motta PM. Cellular and microvascular changes of the ovarian follicle during folliculogenesis: a scanning electron microscopic study. *Arch Histol Cytol* 1992;**55**:191–204.

Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. *Obstet Gynecol Clin North Am* 2012;**39**:535–549.

Mahnke JL, Dawood MY, Huang JC. Vascular endothelial growth factor and interleukin-6 in peritoneal fluid of women with endometriosis. *Fertil Steril* 2000;**73**:166–170.

Makled AK, El Sherbiny M, Elkabarity R. Assessment of ovarian stromal blood flow after metformin treatment in women with polycystic ovary syndrome. *Arch Gynecol Obstet* 2014;**289**:883–891.

Martelli A, Berardinelli P, Russo V, Mauro A, Bernabo N, Gioia L, Mattioli M, Barboni B. Spatio-temporal analysis of vascular endothelial growth factor expression and blood vessel remodelling in pig ovarian follicles during the periovulatory period. *J Mol Endocrinol* 2006;**36**:107–119.

Matousek M, Carati C, Gannon B, Brannstrom M. Novel method for intrafollicular pressure measurements in the rat ovary: increased intrafollicular pressure after hCG stimulation. *Reproduction* 2001; **121**:307–314.

Matti N, Irving-Rodgers HF, Hatzirodos N, Sullivan TR, Rodgers RJ. Differential expression of focimatrix and steroidogenic enzymes before size deviation during waves of follicular development in bovine ovarian follicles. *Mol Cell Endocrinol* 2010;**321**:207–214.

McArthur ME, Irving-Rodgers HF, Byers S, Rodgers RJ. Identification and immunolocalization of decorin, versican, perlecan, nidogen, and chondroitin sulfate proteoglycans in bovine small-antral ovarian follicles. *Biol Reprod* 2000;**63**:913–924.

McCord LA, Li F, Rosewell KL, Brännström M, Curry TE. Ovarian expression and regulation of the stromelysins during the periovulatory period in the human and the rat. *Biol Reprod* 2012;**86**:78.

McLaren J, Prentice A, Charnock-Jones DS, Smith SK. Vascular endothelial growth factor (VEGF) concentrations are elevated in peritoneal fluid of women with endometriosis. *Hum Reprod* 1996;**11**:220–223.

McLaughlin M, Kinnell HL, Anderson RA, Telfer EE. Inhibition of phosphatase and tensin homologue (PTEN) in human ovary in vitro results in increased activation of primordial follicles but compromises development of growing follicles. *Mol Hum Reprod* 2014;**20**:736–744.

Meczekalski B, Szeliga A, Maciejewska-Jeske M, Podfigurna A, Cornetti P, Bala G, Adashi EY. Hyperthecosis: an underestimated nontumorous cause of hyperandrogenism. *Gynecol Endocrinol* 2021; **37**:677–682.

Melo EO, Cordeiro DM, Pellegrino R, Wei Z, Daye ZJ, Nishimura RC, Dode MA. Identification of molecular markers for oocyte competence in bovine cumulus cells. *Anim Genet* 2017;**48**:19–29.

Mishra SR, Parmar MS, Yadav VP, Reshma R, Bharati J, Bharti MK, Paul A, Chouhan VS, Taru Sharma G, Singh G *et al.* Expression and localization of angiopoietin family in corpus luteum during different stages of oestrous cycle and modulatory role of angiopoietins on steroidogenesis, angiogenesis and survivability of cultured buffalo luteal cells. *Reprod Dom Anim* 2016;**51**:855–869.

Murdoch WJ. Regulation of collagenolysis and cell death by plasmin within the formative stigma of preovulatory ovine follicles. *J Reprod Fertil* 1998;**113**:331–336.

Muzii L, Achilli C, Bergamini V, Candiani M, Garavaglia E, Lazzeri L, Lecce F, Maiorana A, Maneschi F, Marana R *et al.* Comparison between the stripping technique and the combined excisional/ablative

technique for the treatment of bilateral ovarian endometriomas: a multicentre RCT. *Hum Reprod* 2016;**31**:339–344.

Muzii L, Bellati F, Palaia I, Plotti F, Manci N, Zullo MA, Angioli R, Panici PB. Laparoscopic stripping of endometriomas: a randomized trial on different surgical techniques. Part I: clinical results. *Hum Reprod* 2005;**20**:1981–1986.

Notaro ALG, Neto FTL. The use of metformin in women with polycystic ovary syndrome: an updated review. *J Assist Reprod Genet* 2022;**39**:573–579.

Ouni E, Bouzin C, Dolmans MM, Marbaix E, Pyr Dit Ruys S, Vertommen D, Amorim CA. Spatiotemporal changes in mechanical matrisome components of the human ovary from prepuberty to menopause. *Hum Reprod* 2020;**35**:1391–1410.

Palomba S, Falbo A, La Sala GB. Effects of metformin in women with polycystic ovary syndrome treated with gonadotrophins for in vitro fertilisation and intracytoplasmic sperm injection cycles: a systematic review and meta-analysis of randomised controlled trials. *BJOG* 2013;**120**:267–276.

Pan JX, Tan YJ, Wang FF, Hou NN, Xiang YQ, Zhang JY, Liu Y, Qu F, Meng Q, Xu J et al. Aberrant expression and DNA methylation of lipid metabolism genes in PCOS: a new insight into its pathogenesis. *Clin Epigenetics* 2018;**10**:6.

Pan J, Zhou C, Zhou Z, Yang Z, Dai T, Huang H, Jin L. Elevated ovarian pentraxin 3 in polycystic ovary syndrome. *J Assist Reprod Genet* 2021;**38**:1231–1237.

Papachroni KK, Piperi C, Levidou G, Korkolopoulou P, Pawelczyk L, Diamanti-Kandarakis E, Papavassiliou AG. Lysyl oxidase interacts with AGE signalling to modulate collagen synthesis in polycystic ovarian tissue. *J Cell Mol Med* 2010;**14**:2460–2469.

Park HS, Ashour D, Elsharoud A, Chugh RM, Ismail N, El Andaloussi A, Al-Hendy A. Towards cell free therapy of premature ovarian insufficiency: human bone marrow mesenchymal stem cells secretome enhances angiogenesis in human ovarian microvascular endothelial cells. *HSOA J Stem Cells Res Dev Ther* 2019;**5**:1–8.

Park JY, Lim MC, Baek MH, Park YH, Kim S. Impact of metformin on survival outcome in ovarian cancer: a nationwide population-based cohort study. *J Gynecol Oncol* 2021;**32**:e65.

Patil K, Hinduja I, Mukherjee S. Alteration in angiogenic potential of granulosa-lutein cells and follicular fluid contributes to luteal defects in polycystic ovary syndrome. *Hum Reprod* 2021;**36**:1052–1064.

Peters A, Rindos NB, Lee T. Hemostasis during ovarian cystectomy: systematic review of the impact of suturing versus surgical energy on ovarian function. *J Minim Invasive Gynecol* 2017;**24**:235–246.

Puttabyatappa M, Al-Alem LF, Zakerkish F, Rosewell KL, Brannstrom M, Curry TE Jr. Induction of tissue factor pathway inhibitor 2 by hCG regulates periovulatory gene expression and plasmin activity. *Endocrinology* 2017;**158**:109–120.

Raja-Khan N, Urbanek M, Rodgers RJ, Legro RS. The role of TGF-β in polycystic ovary syndrome. *Reprod Sci* 2014;**21**:20–31.

Ramirez F, Pereira L. The fibrillins. *Int J Biochem Cell Biol* 1999;**31**:255–259.

Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, Du C, Tang W, Hamalainen T, Peng SL et al. Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. *Science* 2008;**319**:611–613.

Redmer DA, Reynolds LP. Angiogenesis in the ovary. *Rev Reprod* 1996;**1**:182–192.

Reich H, Abrao MS. Post-surgical ovarian failure after laparoscopic excision of bilateral endometriomas: is this rare problem preventable? *Am J Obstet Gynecol* 2006;**195**:339–340.

Reynolds LP, Killilea SD, Redmer DA. Angiogenesis in the female reproductive system. *FASEB J* 1992;**6**:886–892.

Rius M, Gracia M, Ros C, Martínez-Zamora MÁ, deGuirior C, Quintas L, Carmona F. Impact of endometrioma surgery on ovarian reserve: a prospective, randomized, pilot study comparing stripping with CO2 laser vaporization in patients with bilateral endometriomas. *J Int Med Res* 2020;**48**:300060520927627.

Rizov M, Andreeva P, Dimova I. Molecular regulation and role of angiogenesis in reproduction. *Taiwan J Obstet Gynecol* 2017;**56**:127–132.

Robertson IB, Horiguchi M, Zilberberg L, Dabovic B, Hadjiolova K, Rifkin DB. Latent TGF-beta-binding proteins. *Matrix Biol* 2015;**47**:44–53.

Robinson RS, Woad KJ, Hammond AJ, Laird M, Hunter MG, Mann GE. Angiogenesis and vascular function in the ovary. *Reproduction* 2009;**138**:869–881.

Rock JA, Markham SM. Pathogenesis of endometriosis. *Lancet* 1992;**340**:1264–1267.

Rodgers HF, Irvine CM, van Wezel IL, Lavranos TC, Luck MR, Sado Y, Ninomiya Y, Rodgers RJ. Distribution of the alpha1 to alpha6 chains of type IV collagen in bovine follicles. *Biol Reprod* 1998;**59**:1334–1341.

Rodgers RJ, Irving-Rodgers HF. Formation of the ovarian follicular antrum and follicular fluid. *Biol Reprod* 2010a;**82**:1021–1029.

Rodgers RJ, Irving-Rodgers HF. Morphological classification of bovine ovarian follicles. *Reproduction* 2010b;**139**:309–318.

Rodgers RJ, Irving-Rodgers HF. The roles of the ovarian extracellular matrix in fertility. *Soc Reprod Fertil Suppl* 2010c;**67**:217–230.

Rodgers RJ, Irving-Rodgers HF, Russell DL. Extracellular matrix of the developing ovarian follicle. *Reproduction* 2003;**126**:415–424.

Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, Smolarczyk R, Meczekalski B. Chronic low grade inflammation in pathogenesis of PCOS. *IJMS* 2021;**22**:3789.

Sage J, Leblanc-Noblesse E, Nizard C, Sasaki T, Schnebert S, Perrier E, Kurfurst R, Bromme D, Lalmanach G, Lecaille F. Cleavage of nidogen-1 by cathepsin S impairs its binding to basement membrane partners. *PLoS One* 2012;**7**:e43494.

Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, Doni A, Bastone A, Mantovani G, Beck Peccoz P et al. PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in in vivo fertilization. *Development* 2004;**131**:1577–1586.

Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. *Am J Pathol* 1927;**3**:93–110.43.

Schiewe MC, Araujo E Jr, Asch RH, Balmaceda JP. Enzymatic characterization of zona pellucida hardening in human eggs and embryos. *J Assist Reprod Genet* 1995;**12**:2–7.

Scotti L, Parborell F, Irusta G, De Zuñiga I, Bisioli C, Pettorossi H, Tesone M, Abramovich D. Platelet-derived growth factor BB and DD and angiopoietin1 are altered in follicular fluid from polycystic ovary syndrome patients. *Mol Reprod Dev* 2014;**81**:748–756.

Shah JS, Sabouni R, Cayton Vaught KC, Owen CM, Albertini DF, Segars JH. Biomechanics and mechanical signaling in the ovary: a systematic review. *J Assist Reprod Genet* 2018;**35**:1135–1148.

Shi LB, Zhou F, Zhu HY, Huang D, Jin XY, Li C, Dai Y, Pan YB, Zhang SY. Transforming growth factor beta1 from endometriomas promotes fibrosis in surrounding ovarian tissues via Smad2/3 signaling. *Biol Reprod* 2017;**97**:873–882.

Shorakae S, Ranasinha S, Abell S, Lambert G, Lambert E, de Courten B, Teede H. Inter-related effects of insulin resistance, hyperandrogenism, sympathetic dysfunction and chronic inflammation in PCOS. *Clin Endocrinol (Oxf)* 2018;**89**:628–633.

Shirasuna K, Nitta A, Sineenard J, Shimizu T, Bollwein H, Miyamoto A. Vascular and immune regulation of corpus luteum development, maintenance, and regression in the cow. *Domest Anim Endocrinol* 2012;**43**:198–211.

Sills ES, Rickers NS, Li X, Palermo GD. First data on in vitro fertilization and blastocyst formation after intraovarian injection of calcium gluconate-activated autologous platelet rich plasma. *Gynecol Endocrinol* 2018;**34**:756–760.

Silva N, Tennekoon K, Senanayake H, Samarakoon S. Metalloestrogen cadmium stimulates proliferation of stromal cells derived from the eutopic endometrium of women with endometriosis. *Taiwan J Obstet Gynecol* 2013;**52**:540–545.

Smith RM, Shikanov A, Kniazeva E, Ramadurai D, Woodruff TK, Shea LD. Fibrin-mediated delivery of an ovarian follicle pool in a mouse model of infertility. *Tissue Eng Part A* 2014;**20**:3021–3030.

Steiner AZ. Evidence that biopsying, fragmentation and auto-transplantation of ovarian tissue should be abandoned as a treatment of diminished ovarian reserve. *Hum Reprod* 2019;**34**: 1853–1854.

Sugino N, Matsuoka A, Taniguchi K, Tamura H. Angiogenesis in the human corpus luteum. *Reprod Med Biol* 2008;**7**:91–103.

Sugino N, Suzuki T, Sakata A, Miwa I, Asada H, Taketani T, Yamagata Y, Tamura H. Angiogenesis in the human corpus luteum: changes in expression of angiopoietins in the corpus luteum throughout the menstrual cycle and in early pregnancy. *J Clin Endocrinol Metab* 2005;**90**:6141–6148.

Sullivan SD, Sarrel PM, Nelson LM. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. *Fertil Steril* 2016;**106**:1588–1599.

Sun H, Gong TT, Jiang YT, Zhang S, Zhao YH, Wu QJ. Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990-2017: results from a global burden of disease study, 2017. *Aging (Albany NY)* 2019;**11**: 10952–10991.

Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, Morimoto Y, Kawamura K. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 2015;**30**:608–615.

Swanson M, Sauerbrei EE, Cooperberg PL. Medical implications of ultrasonically detected polycystic ovaries. *J Clin Ultrasound* 1981;**9**: 219–222.

Tabong PT, Adongo PB. Understanding the social meaning of infertility and childbearing: a qualitative study of the perception of childbearing and childlessness in Northern Ghana. *PLoS One* 2013;**8**: e54429.

Tagler D, Makanji Y, Tu T, Bernabe BP, Lee R, Zhu J, Kniazeva E, Hornick JE, Woodruff TK, Shea LD. Promoting extracellular matrix remodeling via ascorbic acid enhances the survival of primary ovarian follicles encapsulated in alginate hydrogels. *Biotechnol Bioeng* 2014;**111**:1417–1429.

Takahashi K, Ozaki T, Okada M, Uchida A, Kitao M. Relationship between ultrasonography and histopathological changes in polycystic ovarian syndrome. *Hum Reprod* 1994;**9**:2255–2258.

Tal R, Seifer DB, Arici A. The emerging role of angiogenic factor dysregulation in the pathogenesis of polycystic ovarian syndrome. *Semin Reprod Med* 2015;**33**:195–207.

Tal R, Seifer DB, Grazi RV, Malter HE. Follicular fluid placental growth factor is increased in polycystic ovarian syndrome: correlation with ovarian stimulation. *Reprod Biol Endocrinol* 2014;**12**:82.

Tanaka Y, Hsueh AJ, Kawamura K. Surgical approaches of drug-free in vitro activation and laparoscopic ovarian incision to treat patients with ovarian infertility. *Fertil Steril* 2020;**114**:1355–1357.

Tarkun I, Canturk Z, Arslan BC, Turemen E, Tarkun P. The plasminogen activator system in young and lean women with polycystic ovary syndrome. *Endocr J* 2004;**51**:467–472.

Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med* 2010; **8**:41.

Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril* 2018;**110**:364–379.

Ting AY, Zelinski MB. Characterization of FOXO1, 3 and 4 transcription factors in ovaries of fetal, prepubertal and adult rhesus macaques. *Biol Reprod* 2017;**96**:1052–1059.

Tong Y, Li F, Lu Y, Cao Y, Gao J, Liu J. Rapamycin-sensitive mTORC1 signaling is involved in physiological primordial follicle activation in mouse ovary. *Mol Reprod Dev* 2013;**80**:1018–1034.

Tucker EJ, Grover SR, Bachelot A, Touraine P, Sinclair AH. Premature ovarian insufficiency: new perspectives on genetic cause and phenotypic spectrum. *Endocr Rev* 2016;**37**:609–635.

Uludag SZ, Demirtas E, Sahin Y, Aygen EM. Dienogest reduces endometrioma volume and endometriosis-related pain symptoms. *J Obstet Gynaecol* 2021;**41**:1246–1251.

Vaiarelli A, Venturella R, Cimadomo D, Conforti A, Pedri S, Bitonti G, Iussig B, Gentile C, Alviggi E, Santopaolo S *et al.* Endometriosis shows no impact on the euploid blastocyst rate per cohort of inseminated metaphase-II oocytes: a case-control study. *Eur J Obstet Gynecol Reprod Biol* 2021;**256**:205–210.

van Wezel IL, Rodgers HF, Rodgers RJ. Differential localization of laminin chains in bovine follicles. *J Reprod Fertil* 1998;**112**:267–278.

Varani S, Elvin JA, Yan C, DeMayo J, DeMayo FJ, Horton HF, Byrne MC, Matzuk MM. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. *Mol Endocrinol* 2002;**16**:1154–1167.

Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* 2014;**10**:261–275.

Vos MC, van der Wurff AA, Last JT, de Boed EA, Smeenk JM, van Kuppevelt TH, Massuger LF. Immunohistochemical expression of

MMP-14 and MMP-2, and MMP-2 activity during human ovarian follicular development. *Reprod Biol Endocrinol* 2014;**12**:12.

Vural B, Duruksu G, Vural F, Gorguc M, Karaoz E. Effects of VEGF + mesenchymal stem cells and platelet-rich plasma on inbred rat ovarian functions in cyclophosphamide-induced premature ovarian insufficiency model. *Stem Cell Rev Rep* 2019;**15**:558–573.

Wang D, Wang W, Liang Q, He X, Xia Y, Shen S, Wang H, Gao Q, Wang Y. DHEA-induced ovarian hyperfibrosis is mediated by TGF-β signaling pathway. *J Ovarian Res* 2018;**11**:6.

Wang F, Wang L, Yao X, Lai D, Guo L. Human amniotic epithelial cells can differentiate into granulosa cells and restore folliculogenesis in a mouse model of chemotherapy-induced premature ovarian failure. *Stem Cell Res Ther* 2013;**4**:124.

Wassarman PM, Jovine L, Litscher ES. Mouse zona pellucida genes and glycoproteins. *Cytogenet Genome Res* 2004;**105**:228–234.

Wassarman PM, Litscher ES. The mouse egg's zona pellucida. *Curr Top Dev Biol* 2018;**130**:331–356.

Webber L, Anderson RA, Davies M, Janse F, Vermeulen N. HRT for women with premature ovarian insufficiency: a comprehensive review. *Hum Reprod Open* 2017;**2017**:hox007.

Whitelock JM, Melrose J, Iozzo RV. Diverse cell signaling events modulated by perlecan. *Biochemistry* 2008;**47**:11174–11183.

Woodruff TK, Shea LD. A new hypothesis regarding ovarian follicle development: ovarian rigidity as a regulator of selection and health. *J Assist Reprod Genet* 2011;**28**:3–6.

Wulff C, Wilson H, Largue P, Duncan WC, Armstrong DG, Fraser HM. Angiogenesis in the human corpus luteum: localization and changes in angiopoietins, tie-2, and vascular endothelial growth factor messenger ribonucleic acid. *J Clin Endocrinol Metab* 2000;**85**:4302–4309.

Wyns C, Donnez J. Vaporisation laser des endométriomes ovariens: impact sur la réponse aux gonadotrophines [Laser vaporization of ovarian endometriomas: the impact on the response to gonadotrophin stimulation]. *Gynecol Obstet Fertil* 2003;**31**:337–342.

Xie Q, Cheng Z, Chen X, Lobe CG, Liu J. The role of Notch signalling in ovarian angiogenesis. *J Ovarian Res* 2017;**10**:13.

Xu Z, Abbott A, Kopf GS, Schultz RM, Ducibella T. Spontaneous activation of ovulated mouse eggs: time-dependent effects on M-phase exit, cortical granule exocytosis, maternal messenger ribonucleic acid recruitment, and inositol 1,4,5-trisphosphate sensitivity. *Biol Reprod* 1997;**57**:743–750.

Yanez LZ, Camarillo DB. Microfluidic analysis of oocyte and embryo biomechanical properties to improve outcomes in assisted reproductive technologies. *Mol Hum Reprod* 2017;**23**:235–247.

Yanez LZ, Han J, Behr BB, Pera RAR, Camarillo DB. Human oocyte developmental potential is predicted by mechanical properties within hours after fertilization. *Nat Commun* 2016;**7**:10809.

Yang X, Huang M, Zhang Q, Chen J, Li J, Han Q, Zhang L, Li J, Liu S, Ma Y *et al.* Metformin antagonizes ovarian cancer cells malignancy through MSLN mediated IL-6/STAT3 signaling. *Cell Transplant* 2021;**30**:9636897211027819.

Yin H, Kristensen SG, Jiang H, Rasmussen A, Andersen CY. Survival and growth of isolated pre-antral follicles from human ovarian medulla tissue during long-term 3D culture. *Hum Reprod* 2016;**31**:1531–1539.

Yland J, Carvalho LFP, Beste M, Bailey A, Thomas C, Abrão MS, Racowsky C, Griffith L, Missmer SA. Endometrioma, the follicular fluid inflammatory network and its association with oocyte and embryo characteristics. *Reprod Biomed Online* 2020;**40**:399–408.

Yorino S, Kawamura K. Rapamycin treatment maintains developmental potential of oocytes in mice and follicle reserve in human cortical fragments grafted into immune-deficient mice. *Mol Cell Endocrinol* 2020;**504**:110694.

Zaidi J, Jacobs H, Campbell S, Tan SL. Blood flow changes in the ovarian and uterine arteries in women with polycystic ovary syndrome who respond to clomiphene citrate: correlation with serum hormone concentrations. *Ultrasound Obstet Gynecol* 1998;**12**:188–196.

Zalewski A, Cecchini EL, Deroo BJ. Expression of extracellular matrix components is disrupted in the immature and adult estrogen receptor beta-null mouse ovary. *PLoS One* 2012;**7**:e29937.

Zeng X, Yue Z, Gao Y, Jiang G, Zeng F, Shao Y, Huang J, Yin M, Li Y. NR4A1 is involved in fibrogenesis in ovarian endometriosis. *Cell Physiol Biochem* 2018;**46**:1078–1090.

Zhai J, Yao G, Dong F, Bu Z, Cheng Y, Sato Y, Hu L, Zhang Y, Wang J, Dai S *et al.* In vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. *J Clin Endocrinol Metab* 2016;**101**:4405–4412.

Zhang H, Risal S, Gorre N, Busayavalasa K, Li X, Shen Y, Bosbach B, Brannstrom M, Liu K. Somatic cells initiate primordial follicle activation and govern the development of dormant oocytes in mice. *Curr Biol* 2014;**24**:2501–2508.

Zhang J, Zhang J, Gao B, Xu Y, Liu H, Pan Z. Detection of the effects and potential interactions of FSH, VEGFA, and 2-methoxyestradiol in follicular angiogenesis, growth, and atresia in mouse ovaries. *Mol Reprod Dev* 2019;**86**:566–575.

Zhang L. Control of growth and beyond: a special issue on Hippo signaling. *Acta Biochim Biophys Sin (Shanghai)* 2015;**47**:1.

Zhang X, Rutledge J, Armstrong DT. Studies on zona hardening in rat oocytes that are matured in vitro in a serum-free medium. *Mol Reprod Dev* 1991;**28**:292–296.

Zhao J, Li D, Tang H, Tang L. Association of vascular endothelial growth factor polymorphisms with polycystic ovarian syndrome risk: a meta-analysis. *Reprod Biol Endocrinol* 2020;**18**:18.

Zuccotti M, Giorgi Rossi P, Martinez A, Garagna S, Forabosco A, Redi CA. Meiotic and developmental competence of mouse antral oocytes. *Biol Reprod* 1998;**58**:700–704.

Zuccotti M, Merico V, Cecconi S, Redi CA, Garagna S. What does it take to make a developmentally competent mammalian egg? *Hum Reprod Update* 2011;**17**:525–540.