Pan *et al. Journal of Ovarian Research* (2024) 17:237
https://doi.org/10.1186/s13048-024-01551-9

Journal of Ovarian Research

REVIEW

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Unraveling the complexity of follicular fluid: insights into its composition, function, and clinical implications

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Abstract

Follicular fluid (FF) plays a vital role in the bidirectional communication between oocytes and granulosa cells (GCs), regulating and promoting oocyte growth and development. This fluid constitutes a complex microenvironment, rich in various molecules including hormones, growth factors, cytokines, lipids, proteins, and extracellular vesicles. Understanding the composition and metabolic profile of follicular fluid is important for investigating ovarian pathologies such as polycystic ovary syndrome (PCOS) and endometriosis. Additionally, analyzing follicular fluid can offer valuable insights into oocyte quality, aiding in optimal oocyte selection for in vitro fertilization (IVF). This review provides an overview of follicular fluid composition, classification of its components and discusses the influential components of oocyte development. It also highlights the role of follicular fluid in the pathogenesis and diagnosis of ovarian diseases, along with potential follicular fluid biomarkers for assessing oocyte quality. By understanding the intricate relationship between follicular fluid and oocyte development, we can advance fertility research and improve clinical outcomes for infertility patients.

Keywords Human ovary, Oocyte, Polycystic ovary syndrome, Endometriosis, In vitro fertilization, Follicular fluid

Introduction

The orchestration of communication between germ cells and somatic cells unfolds within the intricate milieu of Follicular fluid (FF), an encompassing microenvironment teeming with an array of complex molecules, which permits several reactions that are essential to oocyte growth to occur. FF, which emerges from the blood plasma and secretions from theca and granulosa cells (GCs), is generated by the osmotic gradient caused by hyaluronan and the versican (a chondroitin sulfate proteoglycan) [1].

The FF compartment presents itself as a biomolecule-rich reservoir, in contrast to the dynamic nature of cells. FF is a non-invasive matrix for fertility insights, reflecting changes in patients' microenvironments. Understanding FF and its metabolic profile is crucial for investigating pathologies and prognosis of diseases, such as polycystic ovary syndrome (PCOS) and endometriosis. Enhanced evaluation of oocyte quality during in vitro fertilization (IVF) can optimize the selection, facilitate the strategic transfer, and reduce embryo wastage [2].

We explore the correlation between FF and oocyte development and maturation under physiological and pathological conditions. This paper begins by providing a concise overview of the mechanism underlying FF formation. Subsequently, the composition of the FF is classified

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into various types, and the influential components of FF on oocyte development are discussed in detail. Additionally, the paper highlights the crucial role of FF in the pathological change and diagnosis of ovarian diseases, with specific emphasis on endometriosis and PCOS, two highly prevalent conditions. This offers valuable insights into the diagnosis and prognosis of these diseases. Moreover, the review emphasizes that FF analysis can offer valuable information regarding oocyte quality, aid in optimal oocyte selection, and ultimately lead to improved IVF success rates. To its end, the review also presents potential FF biomarkers for assessing oocyte quality.

Source and mechanism of follicular fluid formation

FF formation is a complex process involving the secretion activities of the oocyte and GCs, plus the transfer of blood plasma components through the blood follicular barrier. The blood follicular barrier's selectivity, allowing only proteins under 500 kDa and demonstrating higher permeability for those under 100 kDa, creates an osmotic gradient essential for FF accumulation. This selective permeability is especially pronounced at the thecal capillaries for larger molecules and is designed to prevent macromolecules produced by oocytes or GCs from leaving the follicle, thus enhancing the osmotic gradient [1].

Key components contributing to FF's osmotic potential, identified through enzymatic degradation and dialysis, include DNA, hyaluronan, chondroitin sulfate/dermatan sulfate, and their aggregates, with significant osmotic roles at molecular weight thresholds of 100 and 300 kDa. GCs synthesize glycosaminoglycans such as hyaluronan, distinguished by its large size and lack of core protein, making it a crucial component in the follicular antrum. Chondroitin sulfate proteoglycans like versican, also produced by GCs, can attach to long chondroitin sulfate side chains, enhancing FF's osmotic capacity [1]. Following the LH surge, the inter-alpha-trypsin inhibitor accumulates in FF, binding to hyaluronan to prevent its loss and forming aggregates that contribute to the osmotic gradient. Additionally, DNAs from dying GCs, possibly through a cornification-like process rather than apoptosis, contribute to osmotic pressure but are not a major factor in FF formation due to rapid degradation by cellular DNase. The established osmotic gradient draws water into the follicular cavity, facilitated by the expression of aquaporins (AQPs) in granulosa and theca cells, which govern water permeability in ovarian antral follicles. AQP9 and AQP7 in sinus follicles, and AQP1-4 in human ovulating follicles, enable the swift passive movement of water, highlighting the intricate balance of molecules and barriers orchestrating FF formation and contributing to follicular development and dominance [1].

The composition and function of follicular fluid

Extracellular vesicles (EVs)

EVs are membrane-bound particles that serve as transporters for regulatory molecules such as proteins, microRNAs (miRNAs), and lipids [6]. Among these, exosomes are small EVs (SEVs) released from intracellular compartments, while large EVs (LEVs), known as microvesicles, are discharged from the cellular surface. Both are found in FF [6]. The effect of EVs varies with the size of antral follicles, potentially enhancing oocyte function, aiding survival under stress, and promoting GC proliferation [7].

SEVs and LEVs differ significantly in number, morphology, and membrane surface-specific marker proteins, making LEVs distinct from SEVs [6]. Analysis reveals that while 25 miRNAs are common between exosomes and microvesicles, 54 are enriched in microvesicles, and 16 in exosomes [8]. These EVs provide an additional layer of regulation, carrying miRNAs targeting pathways crucial for follicular growth and oocyte maturation, including the WNT, TGFβ, MAPK, neurotrophin, ErbB, and ubiquitin-mediated pathways, suggesting role in oogenesis [9]. FF-derived EVs also promote cumulus-oocyte complex expansion by modulating gene expression related to this process, such as Ptx3, Ptgs2, and Tnfaip6, and protect against GC apoptosis [10]. LEVs specifically affect steroidogenesis, hindering the conversion of pregnenolone to progesterone (P4) by upregulating CYP11A and CYP17A mRNA levels of related enzymes and stimulate estradiol (E2) secretion through the PI3K/AKT pathway [11]. Recent studies link miRNAs in FF-derived EVs with advanced maternal age, where the upregulation of miRNA-155 and downregulation of miRNA-16 and −124 may activate P53-mediated apoptotic pathways in GCs and oocytes [13]. Additionally, it is shown that 1-unit increase in body mass index (BMI) is associated with altered FF expression of EV-linked miRNAs [14]. This altered expression of EV-miRNAs can impact the fertilization potential of oocytes [14]. While miRNAs gain stability through encapsulation in EVs, they can also interact with protein complexes. New research has identified mitochondria-derived EVs, "mitovesicles," containing mitochondrial proteins and mRNA from mitochondrial Electron Transport Chain (ETC) genes, with a positive correlation between ETC complex I subunit 1 mRNA levels and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, suggesting a link to fertilization competence [15].

Hormone

Gonadotropin

In the ovarian FF gonadotropins like FSH, human chorionic gonadotropin (hCG), and LH are key to oocyte maturation, impacting GC secretion and increasing the

likelihood of successful fertilization. LH and FSH are pivotal and complementary regulators of follicle growth and ovulation during the physiological cycle [16], with FSH promoting steroidogenesis in GCs through cAMP and ERK1/2 pathways, a process that can be influenced by external factors such as DEHP [17]. P4 stimulates follicle cells to release small antioxidant molecules into FF, which can increase the number of oocytes obtained during ICSI surgery [18].

Remarkably, FSH exhibits considerable resistance to conditional alterations neither ovarian reserve nor age appears to influence FSH levels in FF [19]. FSH stimulates the proliferation and differentiation of GCs in antral follicles via activation of the cAMP/PKA pathway, MAPK/ERK pathway, and PI3K/Akt pathway, while also inhibiting GC apoptosis through the PI3K-Akt pathway [20]. However, excessive FSH may disrupt oocyte meiosis and lead to aneuploid gametes, while its anti-apoptotic actions could hinder the selection against aneuploid oocytes [21].Theca cells and mural GCs are the sole cell types in antral follicles that express LH receptors (LHR), which are induced by LH and FSH [20].

Through paracrine activity from theca cells, FF LH can induce LHR and aromatase expression, activate the Insulin-like Growth Factor (IGF) system (i.e., IGF-1, IGF-2, and IGF-1R), and suppress mural GC apoptosis [20]. LH also stimulates the EGFR pathway in cumulus cells through the adenylate cyclase/cAMP/PKA cascade, facilitating oocyte meiosis resumption and inhibiting aneuploidy [22]. Despite LH levels peaking at menopause in serum, they remain stable in FF across all ages, indicating no direct link to fertility status [19].

Steroid hormone

In FF, steroids like P4 and E2 produced by GCs play crucial roles in oocyte development folliculogenesis, and ovulation. Reduced ovarian reserve and advanced maternal age cause elevated P4 levels and reduced E2 concentrations in FF, which is associated with compromised oocyte competence [23]. E2 promotes cell proliferation and inhibits apoptosis in GCs, while also inducing the expression of FSHR, LHR, aromatase, and IGF1 [20]. During the regression of subordinate follicles, E2, along with inhibin in FF produced by the dominant follicle, suppresses FSH secretion from the pituitary gland, thereby influencing the fate of the subordinate follicles [24]. E2 stimulates the release of small antioxidant molecules by follicular cells to elevate FF HPSC, thus protecting GCs from oxidative stress-induced apoptosis and prevent follicular atresia [18].

Ovaries lacking a corpus luteum exhibited a significantly higher ratio of E2/P4 in the FF compared to ovaries containing a corpus luteum. Follicles growing adjacent to the corpus luteum may have higher FF P4 levels than those that develop in the opposite ovary of a single animal, which may lead to abnormal fertilization and adversely impact later embryo development [24]. Moreover, HPSC is negatively associated with FF P levels, and a positive correlation between the P4 is positively associated with FF thiol levels [18]. Additionally, P4 is involved in various LH-initiated periovulatory events, such as GC luteinization and oocyte maturation, through autocrine and paracrine mechanisms. A sufficient follicular rupture is also associated with an increase in P4 [25]. Theca cells are the sole source of androgen in the ovaries [26]. Androgens are crucial for follicular development, serving as substrates for the synthesis of E2 in GCs and playing various trophic roles during the early stages of follicular growth before selection [27]. Previous studies indicate that testosterone can increase GC proliferation, and the expression of the androgen receptor in GCs correlates positively with early follicle recruitment and growth but negatively with follicle maturation and ovulation in later stages [28]. The androgen-based internal follicular milieu is thought to contribute to follicular atresia, particularly concerning early follicular atresia, which negatively affects oocyte viability, fertilization, and pregnancy probability, possibly due to reduced estrogen/androgen ratios in FF [29].

Melatonin

Melatonin production occurs in GCs, cumulus oophorus, and oocytes, where it serves as an antioxidant and functions as an autocrine or paracrine agent, benefiting both these cells and their neighboring counterparts. Its levels in FF significantly exceed those in serum, through ovarian production and the active transport from blood, correlating positively with follicular diameters and indicating a role in follicular development [30], exhibiting a daily rhythm that reflects its systemic circadian modulation [31]. As organisms age, the protective effect of melatonin against reactive oxygen species (ROS) in FF diminishes, which may contribute to reduced oocyte quality and infertility. This is because Melatonin mitigates oxidative stress in the ovary through both direct free radical scavenging and indirect upregulation of antioxidant enzymes, potentially via the activation of the Sirt1/Sod2 pathway which decreases the incidence of aneuploidy of oocytes [32]. Furthermore, melatonin enhances growth arrest and DNA-damage-inducible 45 (GADD45) signaling, which is crucial for DNA repair and checkpoint functions, as well as eukaryotic initiation factor 2 (eIF2) signaling essential for translation initiation and protein synthesis in ribosomes. Additionally, melatonin suppresses autophagy-related proteins through eIF2, GADD45, and alternative reading frame (ARF) pathways [33].

Melatonin facilitates follicular maturation by promoting angiogenesis through the upregulation of vascular

endothelial growth factor A (VEGFA) expression in secondary follicles. Conversely, melatonin promotes the expression of heme oxygenase-1, which in turn upregulates VEGF synthesis by smooth muscle cells in the secondary follicle, rather than enhancing HIF-1 expression [34].

Melatonin may increase P4 synthesis in human lutein cells by upregulating the expression of RUNX2 and StAR while suppressing the expression of CYP11A1 and CYP19A1.The stimulation on StAR expression is through both MT1 and MT2 receptors, as well as the PI3K/AKT signaling pathway [35].In addition Melatonin also enhances aromatase expression and estrogen synthesis by human GCs [37]. However, a recent study showed that melatonin, at both low and high dosages (10−5 M and 10−9 M, respectively), significantly increases P4 production while not affecting E2 production [35], suggesting that varying melatonin quantities may not consistently result in the same consequences for E2 synthesis.

Growth factor

AMH

AMH plays a crucial role in the growth and differentiation of ovarian follicles [38]. Its FF level is positively correlated with oocyte and embryo quality [39]. AMH acts as a gatekeeper of ovarian steroidogenesis, and can inhibit the aromatase (i.e., CYP19) expression, leading to a decrease in the conversion of androgens to estrogens in follicles [40]. FF AMH concentrations gradually decrease as follicle diameter rises, with a significant decline observed at 8–10 mm, which allows a direct ovarian/pituitary dialog for dominant follicle selection, but in small follicles, AMH concentrations are especially high and are remarkably higher than that of serum AMH [40]. However, AMH expression still exists in cumulus cells of the preovulatory follicle with lower expression in GCs, indicating that these cells still can secrete AMH into the FF at these later stages of folliculogenesis [41]. FF AMH levels, mirroring serum levels, show a negative correlation with age but remain consistent within individual follicles of a patient [42]. Furthermore, another hypothesis suggests that AMH serves as a mere marker of GC function. The specific role of AMH in mature follicles remains unclear [43]. It is noteworthy that FF AMH may exert inhibitory effects on endometrial development, implying a potential inverse relationship between FF AMH concentration and endometrial thickness [44].

Activin and inhibin

Activins and inhibins are pivotal ovarian hormones from GCs that can be detected in FF, which can regulate FSH production [45]. Activin B is the predominant variant, exhibiting a concentration at least an order of magnitude higher than that of activin-AB and activin-A. Additionally, the concentration of inhibin B in FF significantly surpasses that of inhibin A [46]. To maintain a stable concentration, inhibin A is actively synthesized throughout follicular development. In contrast, inhibin B levels decrease as follicles grow, despite its absolute concentration remaining constant [47]. Interestingly, even though activin A is actively generated, its concentration in the FF is inversely related to the follicular diameter, indicating that its active secretion is insufficient to entirely offset the dilution impact [47].

All the isoforms of activin enhance FSH receptor and aromatase expression and E2 production while downregulating LH receptor and P4 levels through the ALK4-mediated SMAD2/SMAD4 pathway [48]. While promoting steroidogenesis in immature follicles, FF Activin A inhibits this process in mature follicles in androgen-rich FF. FF Activin A is important in inhibiting follicle luteinization by blocking the JNK pathway, and it also assists ovulation by increasing prostaglandin E2 synthesis through COX2 expression promotion [49]. In addition, activin can promote preantral follicle growth, GC proliferation, as well as antral formation. It downregulates miR-181a expression and upregulated the p-Smad3 and transforming growth factor beta (TGF-beta) receptors protein levels, which can promote proliferation and repress GC apoptosis [51]. Furthermore, a recent study discovered that activin A may reduce GC apoptosis via the ER-mediated autophagy pathway in bovine atretic follicles [52].

Inhibin B, relative to inhibin A, appears more crucial for dominant follicle selection, acting through negative feedback involving both paracrine and endocrine pathways to suppress FSH [49]. Elevated FF inhibin levels during the fertile window have been associated with enhanced oocyte meiotic maturation and the initiation of androgen release by theca cells [54].Previous research has shown that inhibin B dominates in preantral follicles and is gradually replaced by inhibin A as the follicle expands. As a result, inhibin A acquires dominance in the preovulatory phase [55]. Inhibin A is implicated in promoting GC proliferation during E2 secretion by upregulating proliferating cell nuclear antigen and CyclinB1, while simultaneously inhibiting GC apoptosis through a mitochondrial-mediated pathway [56].

GDF9 and BMP-15

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are secreted by the oocytes into the ovarian follicles, which supporting follicle selection and growth [57]. Their levels in FF are significantly higher than in serum, with GDF9 being more predominant than BMP15, demonstrating that GDF acts largely as a single molecule as opposed to as a component of a heterodimer [58]. Additionally, the FF levels of

the GDF9/BMP15 heterodimer and BMP15 are nearly equivalent [58]. BMP15, GDF9 and GDF9/BMP15 levels are related to the follicle diameter and associated to concentrations of other TGF-βmembers [58]. FF before follicle selection has the highest GDF9 and BMP15 and these concentrations had a negative connection with follicular size [58]. Interestingly, an age-related decline in these proteins correlates with reduced oocyte quality, especially in individuals over 40 [60].

GDF9 and BMP15 modulate the paracrine action between oocytes and GCs, contributing to pre-antral follicle recruitment, cumulus expansion, oocyte maturation, and ovulation with a negative feedback system [61].These factors stimulate folliculogenesis by enhancing FSH receptor sensitivity and promoting the IGF-1 pathway, which influences E2 synthesis and glycolysis [62]. Moreover, GDF9 and BMP15 promote GC growth while preventing apoptosis [63]. Intrafollicular GDF-9 in mice has been demonstrated to influence cumulus cell expansion by promoting gene expression in neighboring somatic cells for Has2, Tnfip6, Ptx3, and Ptgs2 genes [64]. Yan and colleagues' experiments in 2001 using GDF9/BMP15 double knockout mice revealed collaborative actions of GDF9 and BMP15, indicating their redundant or synergistic biological effects [67]. It is yet unknown whether the GDF9 and BMP15 homodimers act independently or GDF9/BMP15 heterodimers produce this cooperation. A recent in vitro study has demonstrated that the recombinant human GDF9/BMP15 heterodimer effectively stimulates the Smad2/3 signaling pathway in granulosa cells and enhances embryo development when compared to human GDF9 and BMP15 homodimers [58].

Insulin-like growth factor system

The ovarian IGF "system" includes two ligands (IGF-I and -II), two receptors (types 1 and 2), six IGF binding proteins (IGFBP-1, -2, -3, -4, -5, and −6), and at least one IGF binding protein protease [68]. IGF-I in human FF likely originates from blood circulation, whereas IGF-II levels in FF, not IGF-I, correlate with follicular maturation, highlighting IGF-II's primary role in human ovaries [70]. The IGF system plays a crucial role in the growth, development, and atresia of follicles, as well as in steroidogenesis in human ovaries [68]. IGF-I can augment the theca cell androgen response to LH activation of IGF-I receptors via insulin, which leads to increased androgen production in theca cells [71]. On the other hand, IGFBP-1 can shift follicles from androgen to estrogen dominance by blocking IGF-I-induced androgen synthesis, thus promoting E2 generation in GCs and preventing follicular atresia [72]. Also, IGF-I is necessary for the gonadotropin-induced stimulation of GCs and its preovulatory FF levels are lower than and correlate with serum levels, which indicate that both FF and serum IGF-I affect folliculogenesis and oocyte maturation [71]. A recent study discovered a significant and direct correlation between FF IGF1 levels and the top embryo rate [73]. IGF1 may stimulate GC proliferation. This effect is attributed to its ability to activate E-cadherin molecules [74]. IGF-II is pivotal for FSH-mediated actions and steroidogenesis in GCs, which is predominant in the human follicles [72]. After ovulation, the IGFBP cleavage enzyme PAPP-A is activated and thus releases IGF-II, which can act on IGF1R to regenerate the injured FTE or ovarian surface epithelium [75]. However, although high content of IGF2 in FF, on the one hand, meets the physiological need for ovulation, it can stimulate the IGF2/IGF-1R/AKT/mTOR or AKT/NANOG pathways to induce malignant transformation of high-grade serous carcinoma and fallopian tube epithelium cells [76].

VEGF

In the ovarian FF, VEGF produced by granulosa and theca cells, along with VEGF receptor 1 (VEGFR-1 or Flt-1) and VEGF receptor 2 (VEGFR-2 or KDR), play a pivotal role in folliculogenesis and angiogenesis, essential for follicle development and ovulation [77]. Additionally, soluble forms of these receptors, soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble VEGFR-2 (sVEGFR-2), regulate VEGF activity by binding to VEGF, reducing its bioavailability [78]. VEGF operates primarily through VEGFR-2 despite a higher affinity for VEGFR-1 (Flt-1), driving angiogenesis efficiently [79].VEGF levels in FF significantly increase as follicular diameter develop, peaking just before ovulation, underscoring its critical role in dominant follicle selection and the subsequent activation of primordial follicles [80]. According to a recent study, dominant follicles had lower levels of sFlt-1 and sVEGFR-2 than non-dominant follicles [82]. However, following dominant follicle selection, sVEGFR-2 expression rises while sFlt-1 expression falls, which shows a negative relationship [82]. This may indicate that one of the soluble receptors may be dominant and responsible for the regulation of biologically active VEGF levels in each phase of folliculogenesis [79]. Studies examining VEGF levels in human FF during IVF or the natural cycle consistently show an increase in VEGF levels with advancing age as well as total FSH concentration [83]. TGF-beta1 can enhance VEGF expression via SMAD2/3, ERK1/2, and p38 MAPK pathways, while melatonin activates VEGF synthesis through the MT2 receptor and PI3K/AKT signaling pathway, highlighting potential therapeutic targets for improving reproductive outcomes and understanding the pathogenesis of ovarian disorders [84].

Cytokines

Immune cells and cytokines within FF play pivotal roles in oocyte maturation and embryo implantation,

emphasizing the importance of a balanced immune system for follicle development [86]. Cytokines in FF, such as various interleukins (ILs), tumor necrosis factor (TNF), and platelet-derived growth factor, are crucial for oocyte maturation, follicle rupture, neo angiogenesis, and thereby indirectly aid in nutrient and oxygen supply for steroidogenesis [86]. IL-18 from GCs triggers essential cytokines for folliculogenesis and ovulation, including IL-1β, TNF-α, and IFN-γ,it can stimulate immune responses that depend on Th 1 and Th 2 lymphocytes based on different conditions [87]. IL-8 recruits neutrophils for pre-ovulation processes and supports corpus luteum neovascularization [54]. IL-6, associated with cumulus cell function and oocyte competence, serves as a maturation indicator, contrasting with IL-5, which negatively predicts oocyte quality [88]. The FF cytokine profile undergoes age-related variations, with FF IL-1Ra, FF IL-5, FF IL-8 and FF eotaxin increasing in younger women (below 30 years) compared to older counterparts (above 30 years) [87]. This suggests that age influences the cytokine profile associated with age-related fertility, potentially owing to depletion [87]. Additionally, a significant majority of FF cytokines exhibit regulation by FF hormones [90]. In older women, FF E2 displays preferential correlations with most FF cytokines. Conversely, in younger women, FF P4 and FF androgen preferentially correlate with FF cytokines [90].

Neurotransmitters

Various neurotransmitters have been detected in human FF, with Glutamine (Gln) and Norepinephrine (NE) being the most abundant and showing a positive correlation with one another [91]. Specifically, Gln serves as a substrate for FF protein synthesis and a primary energy ATP generation source in mitochondria, which promotes oocyte nuclear maturation and embryo development [92]. Moreover, Glutamate and NE present in the FF contribute to enhancing GCs' anti-apoptosis and proliferation ability, upregulating gene expression associated with steroidogenesis [91].

ROS and antioxidants

ROS and antioxidants are metabolic byproducts present in FF that can impact folliculogenesis [94]. ROS plays a beneficial role during key stages of reproductive development, including folliculogenesis, oocyte maturation, and embryogenesis [94]. Various molecules, including growth hormone (GH), induce signaling pathways that generate ROS, leading to the activation of essential cellular responses to environmental cues such as stress, hypoxia, antioxidant mechanisms, autophagy, and metabolic adaptations [95]. These responses are crucial for promoting oocyte maturation [95]. However, uncontrolled ROS generation can lead to detrimental effects on oocyte quality by inducing apoptosis in developing oocytes and GCs, affecting the genome and lipid membranes [96]. Consequently, compromised oocyte quality may contribute to overall declines in reproductive outcomes [96]. Nonetheless, the developing and ovulated oocyte generally maintains ROS levels in FF within a homeostatic range, despite minor fluctuations [95]. Furthermore, in older women, ROS accumulation in FF increases, while the antioxidant capacity decreases, potentially leading to altered intrafollicular hormone concentrations and decreased fertility [97]. Enzymatic antioxidant pathways play a crucial role in regulating ROS levels within acceptable ranges in FF [98]. Human ovarian FF is rich in antioxidants, including vitamin E, β-carotene, and glutathione, as well as redox-controlling enzymes such as glutathione peroxidase 3, superoxide dismutases (CuZnSOD, MnSOD, SOD3), catalase, glutathione S-transferase, and glutathione reductase [99].

Functional proteins or peptides

Irisin, identified in FF and follicle tissue, impacts GC steroidogenesis and glucose metabolism and decreases IGF-1 and FSH-dependent E2 and P4 production while enhancing cell proliferation without affecting cell viability [100]. Moreover, irisin reduces the mRNA levels of GLUT1, GLUT3, and GLUT4 in GCs, while increasing lactate release in the culture medium [100]. Osteopontin (OPN) is a glycoprotein involved in P4 and VEGF production [101]. The concentration of OPN in the FF positively correlates with its concentration in plasma and is determined by the concentration gradient [101]. In addition, OPN can partially be released into FF in response to increased LH [101]. Antinuclear antibodies (ANAs) in FF are associated with fertility loss, suggesting that aberrant autoimmunity can impact reproductive outcomes [102]. Brain-derived neurotrophic factor (BDNF) in the FF is produced by GCs, which can facilitate oocyte maturation, fertilization, and cleavage rate after oocyte activation underlining its crucial role in fertility [103]. The expression level of BDNF in FF of immature follicles was significantly lower than that of mature follicles [103] [231]. Amphiregulin, prevalent in GCs and FF and upregulated by LH/hCG, can promote oocyte meiosis resumption and VEGF expression, influencing follicular dynamics and steroidogenesis [105].

Lipid and adipokine

The FF contains two crucial components: the triglyceride and phospholipid fractions, which together constitute the majority of the fatty acid reserve in the fluid [106]. Moreover, free fatty acids (FFAs) are also present in the FF [107]. Substantial differences in FF lipid composition between follicles of different sizes include lipids from vesicular and non-vesicular parts. FF EVs from the small

follicles (SF) were particularly enriched in phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and their isoforms, while large follicles (LF) showed a higher abundance of PC, lysophosphatidylcholines (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), lysophosphatidylserines (LPS), carnitines, and ceramides [108]. Different pathways, including carbon and fatty acid (FA) energy metabolism, including the pentose phosphate pathway and FA oxidation, steroidogenesis, PI-signaling, and n-3 and n-6 polyunsaturated FA metabolism, were enriched in the ffEV lipid [108]. In addition, several lipids, including LPC, PC, and SM are more abundant in LF compared to SF [108].

Lipids serve as significant energy sources for oocyte growth and development, participating in membrane construction, cell cycle regulation, cell survival, apoptosis, and malignant transformation [109]. Age-related changes in FF lipid metabolism may impact oocyte quality [109]. However, lipid-rich FF can induce endoplasmic reticulum stress pathways in oocytes, impairing maturation and potentially affecting oocyte quality [110]. Bioactive glycerophospholipids, including prostaglandin, LPC, PC, and lysophosphatidic acid (LPA), play roles in inflammation, apoptosis, and follicular development, with LPA regulating prostaglandin levels and COX-2 activity, and LPCs contributing to apoptosis via caspase-3 activation [106]. LPA is detected in various fluids, including FF regulates prostaglandin levels and COX-2 activity [111]. Sphingosine-1-phosphate in FF can trigger FOXO1 and EREG gene expression critical for follicle development, and synergizes with gonadotropin signaling to regulate follicular growth and maturation [112]. Recent studies have shown the capacity of FF lipopolysaccharide to modulate CCAAT/enhancer-binding protein (CEBP) signaling, thereby diminishing CYP19A1 expression and consequently reducing E2 production in bovine GCs [113].

In addition, there are several adipokines present in the FF. Apelin, crucial for follicle development, is expressed in GCs and secreted into the FF, playing a vital role in the selection of dominant follicles and the regulation of GC proliferation and apoptosis [114]. The apelin/apelin-receptor system influences vascular establishment, hormone, and energy metabolism, significantly impacting follicular development [115]. Apelin levels in FF and its expression in GCs correlate positively with follicle count and BMI, suggesting its involvement in metabolic and reproductive interaction [116]. Leptin is another critical adipokine present in human FF, which is synthesized by follicular somatic cells (theca cells and GCs but not oocytes) and aids in oocyte nucleus maturation, regulated by the MAPK pathway. Abnormally low leptin levels have been associated with apoptosis of GCs, ovarian failure, and impaired gonadal function [117]. Furthermore, leptin at a concentration of 10 ng/mL has been shown to maintain the integrity of mitochondria in oocytes, which is associated with oocyte quality [118].

Key factors in follicular atresia

According to preliminary research, apoptosis is crucial for the formation of atretic follicles and the reservation of ovarian follicles [119]. The fate of follicular cells, whether they survive or die, is determined by the delicate balance between several critical pathways in the FF, including those involving TNF, the Fas/Fas ligand (Fas/FasL) system, and nitric oxide (NO). Disruption of this balance will negatively influence ovarian functions and ultimately affect women's reproductive physiology [120]. Furthermore, it was demonstrated that the main cell type in atretic follicles going through apoptosis was GCs [122].

TNF-α is released from cumulus GCs, macrophages and oocytes in the ovaries [123]. Its concentration in the FF rises with the maturation of human follicles [123]. Importantly, TNF-α regulates the process of follicular atresia by inducing the apoptosis of oocytes, stromal cells, and GCs by means of the glutathione signaling pathway, ceramide signaling pathway, and calcium release [122]. Through the breakdown of sphingomyelin, TNF-α causes the ovary to produce ceramide quickly, which in turn can cause GC apoptosis [122]. The stage of follicular maturation determines TNF-α's capacity to induce follicular atresia. The reason for this is because TNF-α preferentially binds to TNFR2 at low doses, which stimulates GC growth, whereas at high doses, TNF-α may bind to both TNFR1 and TNFR2 at the same time, which causes apoptosis [123]. The ovary's production of estradiol 17β is impacted by GC apoptosis, which inhibits the growth and development of follicular oocytes. Oocyte apoptosis and decreased oocyte quality follow as a result [94]. Furthermore, following ovulation, the cumulus cells surrounding the aging oocytes underwent time-dependent apoptosis and released soluble Fas ligand (sFasL) and soluble TNF-α (sTNF-α), which, by binding to the respective Fas receptors and TNF-R on the oocyte, accelerated oocyte aging [126]. In addition, sTNF-α and sFasL were produced in equal amounts by the aging cumulus cells, and TNF-α stimulated the production of sFasL in these cell [126].

Numerous animal species have demonstrated that NO is present in FF, and the identification of NOS expression in human luteal and GCs points to the existence of an intraovarian NO-generating system and highlights its function in regulating follicular development [128]. Nitric oxide's function in the follicle during the process of folliculogenesis is debatable because, depending on its concentration, it may exert a role in protecting or inducing follicular apoptosis [130]. An investigation revealed

that when follicles split into small (<5 mm) and large (>8 mm) sizes, the highest concentration of the NO donor markedly prevented DNA fragmentation in every cell, while the lowest concentration triggered apoptosis in GCs exclusively from large follicles [130].However, Sugino and colleagues in 1996 suggested that NO might be responsible for internucleosomal DNA cleavage in small follicles, leading to DNA fragmentation, but this effect might not be seen in larger follicles. Larger follicles likely have a tonic inhibitory system that constantly inhibits apoptotic DNA cleavage [131]. Moreover, various studies have discovered that NO can prevent rat and bovine GCs apoptosis by inhibiting the action of the Fas-FasL system, thus exert an anti-apoptotic role [132]. Futhermore, there is also a study demonstrated that NO is not involved in apoptosis under physiological condition, as its concentration in the FF is lower than that can induce DNA fragmentation [134] and (see Table 1).

Role of FF in Pathogenesis and diagnosis of ovarian disease

PCOS

PCOS represents a prevalent cause of female infertility, occurring in approximatel up to 15% of females wordwide [135]. The condition is characterized by excessive androgen levels, polycystic ovaries, and disordered ovulation, making anovulatory infertility its primary feature, potentially associated with alterations in follicular fluid [2].

Endocrine abnormalities

The etiology of PCOS remains uncertain and involves heterogeneous endocrine disorders [136].

In infertile PCOS patients, the steroid profile in the FF exhibits notable alterations, including increased estrogen and pregnenolone levels, while P4 levels are decreased [137]. Abundant heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) in FF of PCOS patients induce GCs apoptosis and mitochondrial dysfunction by encouraging excessive estrogen production through the cAMP-PKA-JNK/ERK-Ca2+-FOXO1 pathway [138].FF miRNAs can affect steroidogenesis and are associated with PCOS [139].Excessive Androgen promotes the recruitment and growth of follicles, which result in the production of multiple smaller-sized, immature follicles, ultimately culminating in polycystic ovarian changes [140]. Additionally, excessive androgen exposure has been associated with low-level inflammatory responses in PCOS patients, creating a detrimental cycle contributing to impaired follicular development [141]. Furthermore, the downregulation of miR-379-5p in FF is linked to decreased GC proliferation in PCOS, possibly attributed to elevated androgen levels [142].

Lipids disturbances

Lipid metabolism is significantly dysregulated in women with PCOS, characterized by a notable elevation in the concentrations of fatty acids and insulin in the FF [143]. The disruption in FFA biosynthesis is closely linked to insulin resistance [143]. Elevated palmitic acid strongly stimulates insulin secretion, leading to hyperinsulinemia and insulin resistance, and also contributes to the excessive secretion of VEGF [144]. Abnormal insulin levels result in decreased glucose uptake in the ovary and follicle [145]. Previous studies have shown that elevated levels of chemerin in FF can impact pre-adipocyte differentiation into adipocytes by downregulating the expression of genes involved in lipid and glucose metabolism, ultimately reducing total GLUT4 expression in hGLs and impairing insulin-induced GLUT4 translocation, thereby induce insulin resistance and impaired glucose uptake [146]. Additionally, elevated Arachidonic Acid (AA) in the FF of PCOS patients can target GCs, impairing their secretory and mitochondrial functions, weakening antioxidant capabilities, and promoting cell apoptosis. Furthermore, AA can influence steroidogenesis in GCs by upregulating CYP19A1 and downregulating STAR, CYP11A1, and HSD3B1, leading to decreased P4 and increased estrogen secretion [147]. Elevated levels of GDF8 in FF disrupt insulin-mediated glucose metabolism in hGL cells and upregulate SERPINE1 expression in thecal cells, potentially contributing to the pathogenesis of PCOS [148].

Inflammatory state

The inflammatory stress present within the microenvironment of follicles can trigger adaptive changes, resulting in an innate inflammatory response of GCs, which is proposed as a potential mechanism underlying PCOS progression and is closely intertwined with metabolic disorders [149]. In PCOS, while exogenous lipopolysaccharides enter from the peripheral circulation, IL-1β and interleukin-18 IL-18 mainly through ovarian microcirculation to accumulate in the FF [71]. As a result of the interplay between IL-1R and TLR4 on GCs, the follicular fluid inflammatory cytokines alter the follicular microenvironment, activating NLRP3 inflammasome and the nuclear factors NF-κB pathway, ultimately affecting GCs' function and impairing the development and growth of oocytes [149]. Moreover, the FF levels of FFAs exhibited a close association with the inflammatory status of the follicles [151]. Particularly, oleic acid (OA) displayed noteworthy correlations with the levels of IL-8, IL-6, and IL-18 within the follicles [151]. Upon stimulation with OA, there was a notable increase in the transcriptional levels of IL-8 and IL-6, attributed to the activation of the ERK1/2 signaling pathway, and concurrently, the AMPK signaling pathway was inhibited [151]. This

Table 1 Components in follicular fluid and their physiological roles

Composition	Chemical nature	Secretory cycle	Involved pathway	Role	Affecting factor
Extracellular vesicle	membrane-bound particles	-	Wnt, TGFβ, MAPK, ErbB, and ubiquitin-mediated pathways.Ptgs2,Ptx3 and Tnfaip6 expression.	EVs are transporters for proteins and other regulatory molecules such as microRNAs (miRNAs), proteins, and lipids.	Age
GnRH	including FSH, hCG, and LH	-	FSH stimulates cAMP/PKA, MAPK/ERK, and PI3K/Akt pathway.LH activates the IGF system and cAMP pathways	GnRH stimulates sterogenesis, proliferation of GC, and oocyte meiosis resumption., inhibit apoptosis of GCs.	
Steroid hormone	estrogen, progesterone and androgen	Before ovulation, the FF E2 reaches a significant peak.	E2 induces FSHR, LHR, aromatase, and IGF1 expression, inflammatory pathway	E2 enhances GC proliferation and HPSC, inhibits subordinate follicle and GC apoptosis. P induces granulosa cell luteinization and oocyte maturation. androgen promotes early follicle recruitment and growth but inhibits later follicle maturation and ovulation.	Age and ovarian reserve.
Melatonin	an antioxidant	Melatonin levels show a 24-hour rhythm in the follicles.	Melatonin activates the Sirt1/Sod2 pathway and PI3K/AKT signaling pathway, enhance GADD45,eIF2 and ARF signaling and VEGF expression.	Melatonin serves as a receptor-independent free radical scavenger directly and enhances antioxidant enzyme activity indirectly to reduce ovarian oxidative stress and mediate sterogenesis.	Age
AMH	glycoprotein dimer in the transforming growth factor superfamily	FF AMH decreases as follicles enlarge, with a significant decline observed at 8–10 mm.	AMH inhibits CYP19, P450scc and LHR expression	AMH inhibits sterogenesis and dominant follicular selection. Its role in ovarian reserve, follicular development, and oocyte maturity rate is controversial.	Age
Inhibin and activin	dimeric glycoproteins belonging to the transforming growth factor beta superfamily	Inhibin A and activin A are actively produced throughout follicular growth Inhibin B is not to be actively produced.	All the Activin isoforms target ALK4-mediated SMAD2/SMAD4-dependent pathway. Activin A suppresses JNK, ERβ-mediated autophagy signaling. Inhibin B mediates paracrine and endocrine pathways. inhibin A mediates the Mt-mediated pathway and promotes CNA and CyclinB1.	Inhibin and activin regulate FSH and steroid hormone production, inhibit GCS apoptosis. Inhibin activates dominant follicle selection and oocyte meiosis.	
BMP15 and GDF9	TGF-β superfamily as oocyte secretion factors(OSF)	highest FF BMP15 and GFF9 before follicle selection.	GDF9/BMP15 heterodimer activates Smad2/3 signaling. BMP15 induce KITL, GDF-9 promote Has2、Tnfip6 、Ptx3 and Ptgs2 expression.	GDF9 and BMP15 play important roles in follicular development including pre-antral follicle recruitment, cumulus expansion, oocyte maturation, and ovulation through modulation of Intrafollicular paracrine signaling between GCs and oocytes.	Age-related decline.
VEGF	angiogenic factors	FF VEGF increases as follicles enlarge, and peaks before ovulation	VEGF participates in the VEGF-VEGFR-2 pathway, being induced via SMAD2/3, ERK1/2, p38 MAPK, and PI3K/AKT signaling pathway.	VEGF induces ovarian angiogenesis, facilitates the provision of oxygen, gonadotropins, and nutrients to the follicles, ultimately leading to the activation of primordial follicles.	Age and FSH levels
ROS	O2-, H2O2, OH-, O3, 1O2	FF ROS is maintained in the itesitseostatic range by the maturing and ovulated oocyte.	H2O2- cAMP-CDK1,GH-ROSpathway	The physiological level is beneficial during folliculogenesis, oocyte maturation, and embryogenesis. Overgeneration of ROS results in a decline in oocyte quality.	Age
Lipid	phospholipid triglyceride free fatty acid	Throughout follicular development	accumulation of lipid induces ER stress pathways. LPCs mediate phospholipase A2 activation via caspase 3 .S1P induces pCREB-FOXO1/EREG.LPS can alter CEBP signaling.	Lipids serve as energy sources for oocyte growth and development and participate in GC survival and apoptosis. Lipid buildup impaired oocyte maturation.	BMI and age

led to the induction of ROS production and inflammasome activation, ultimately resulting in elevated mature IL-18 protein secretion [151]. Additionally, a study conducted by Fabjan et al. revealed a decrease in the levels of 8-hydroxy-2'-deoxyguanosine in the PCOS patients' FF, indicative of ROS accumulation which can induce oxidative stress in the context of PCOS [152]. Significantly, 8-hydroxy-2'-deoxyguanosine emerged as a valuable

predictor for oocyte maturation and fertilization in PCOS patients [152].

miRNA and protein dysregulations

miRNA and protein dysregulations within the FF-derived exosomes are closely associated with various aspects of the pathogenesis of PCOS, encompassing neurotransmitter regulation, hormone metabolism, insulin secretion, and fatty factor expression and secretion [153].

For instance, in PCOS patients, the FF exhibits a low level of miR-18b-5p and a high level of PTEN, suggesting that the advantageous effect of exosomal miR-18b-5p in activating the PI3K/Akt/mTOR pathway through inhibiting PTEN downregulation, thereby inhibiting GC proliferation and promoting apoptosis [154]. Furthermore, via the downregulation of GRAMD1 protein in FF-EVs of PCOS patients, miRNA expression is reduced [155]. Consequently, this triggers accessible cholesterol to accumulate in the plasma membrane and perturbates steroid hormone production and activity, thereby contributing to the pathogenesis of PCOS [155]. Notably, PCOS patients also exhibit lower expression of SPTLC2 in FF-EVs, highlighting a potential link between PCOS and insulin resistance [155]. Moreover, FF-EVs play a role in the regulation of E2 production via CYP19A1 in PCOS by reducing circLDLR levels, thereby derepressing the function of miR-1294 [156]. Additionally, a study revealed that the exosomal miR-424-5p derived from FF of PCOS patients hindered the proliferation of GCs as well as induced their senescence by acting on the CDCA4-mediated Rb/E2F1 signaling [157]. Furthermore, proteomic analysis of FF-EVs identified the presence of the S100-A9 protein, which promotes inflammation through the activation of the NF-κB pathway in PCOS [158]. These findings collectively underscore the significance of FF-EVs in influencing critical molecular pathways implicated in PCOS pathogenesis [154]. Another observed difference in PCOS patients is the significantly lower expression of HIF-1α protein in the FF, indicating its association with impaired development of follicles [159]. Moreover, the decreased ratio of pro-angiogenic EMs (2-OHE2, 4-OHE1, and 16-kE2) to anti-angiogenic EMs in antral follicles of PCOS patients might be linked to the lower FF quantity of VEGF, resulting to decreased vascularity and, ultimately, halting follicle development [160]. However, another study indicated that in patients with PCOS, FF APLN levels surpassed both serum levels and those in FF of healthy women. This can precipitate ovarian dysfunctions, characterized by elevated estrogen levels induced by IGF-1, intensified VEGF-mediated ovarian angiogenesis, and disturbance in energy metabolism within PCOS [161]. Additionally, PCOS patients exhibit elevated levels of AMH in FF, which is linked to the dysregulated FF miR-199b-5p and contributes to reduced intrafollicular VEGF levels in PCOS [160]. Furthermore, higher levels of FF GDF8 are positively correlated with BMI and inversely related to IVF outcome [148] (See Fig. 1).

Endometriosis

Endometriosis is a common disease affecting 5–10% of women of reproductive age globally [163]. It is characterized by endometrial-like tissue present outside of the uterine cavity, which is a chronic and inflammatory gynecologic disorders. This disease is known to be estrogen-dependent and is associated with infertility [164] Among the various locations where endometriosis can manifest, the ovary is the most commonly affected site [165].

Endocrine abnormalities

In PCOS, the FF level of P4 is markedly elevated, while cholesteryl sulfate, LH, 17α-hydroxypregnenolone and pregnenolone exhibit diminished levels in patients with endometriosis [166]. Through the suppression of the NF-κB signaling pathway, elevated intrafollicular P4 may downregulate COX-2 and HPGD expression in GCs [166]. Consequently, this downregulation results in reduced FF prostaglandin E2 levels, contributing to ovulatory dysfunction in patients with endometriosis [166].

Metabolic disorders and inflammatory processes

Patients with endometriosis have alterations in energy metabolism and inflammatory processes in the FF [168]. There is a distinct change in the glycosidic balance within the FF, characterized by reduced glucose and elevated pyruvate and lactate levels [169]. This shift is associated with heightened GC activity to meet the increased energy demands in a relatively low oxygen environment, leading to enhanced glycolysis [93].

Furthermore, due to inadequate glucose supply for cellular energy demands, endometriosis patients display higher concentrations of FFAs and ketone bodies present in the FF, indicating potential activation of the pathway for ketone body production [93]. Additionally, FF from endometriosis patients demonstrates enrichment of pathways related to steroidogenesis and lipid metabolism, which may serve to mobilize lipid metabolism for adapting to the inflammatory response [170]. Notably, specific amino acids crucially involved in aberrantly energy metabolism, such as valine, leucine, and lysine, are also reduced in the FF of endometriosis patients [172].

Elevated markers of oxidative stress and reduced antioxidant levels are demonstrated in the FF of endometriosis patients [173]. Lower levels of vitamins A, C, and E are found in the FF of infertile cases [174]. Additionally, FF from endometriosis patients exhibits lower levels of selenium and zinc, respectively [174]. Conversely, higher levels of cadmium and lead positively associated with intrafollicular ROS, are observed [174]. Furthermore, FF

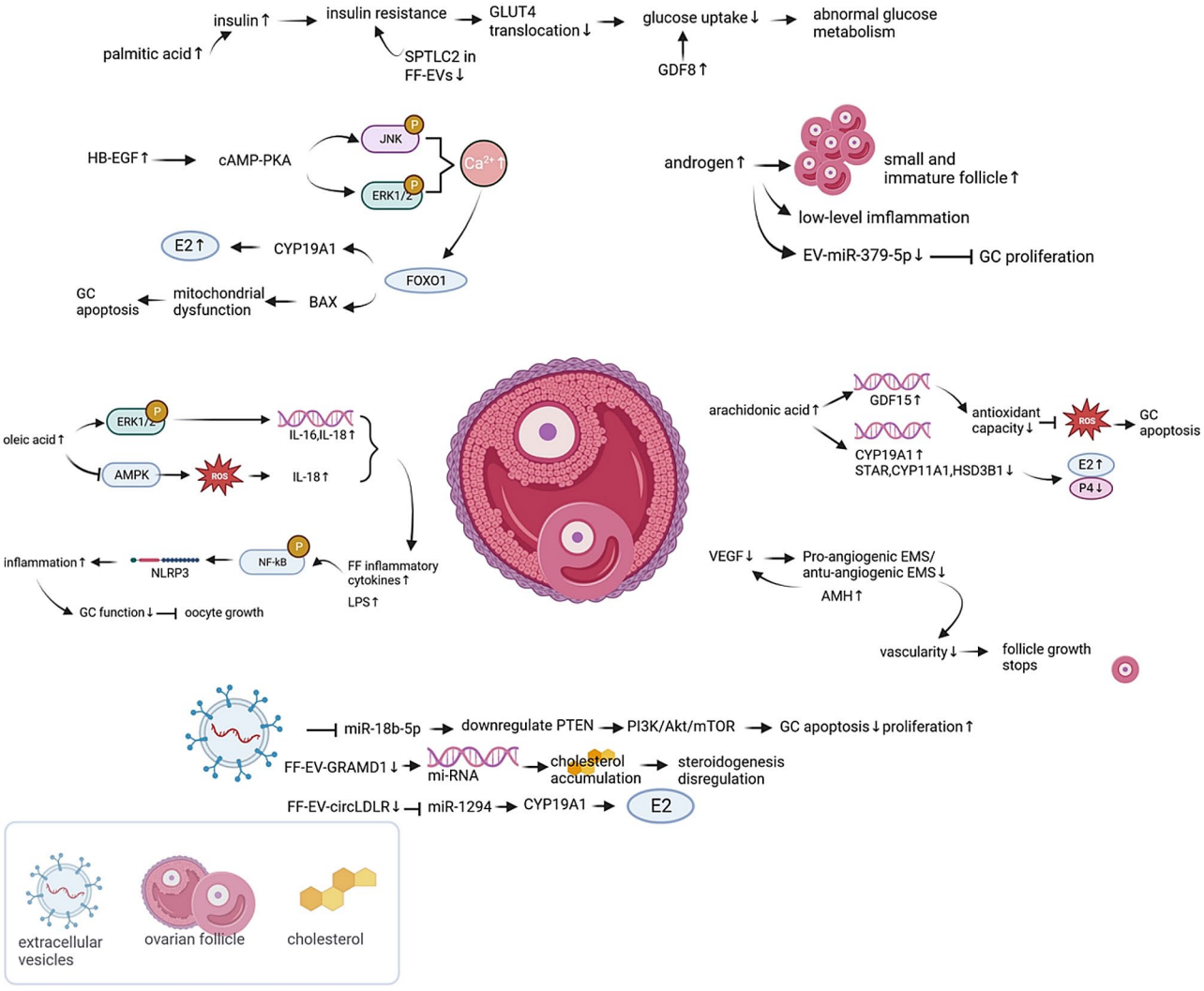

Fig. 1 Core alterations in the follicular fluid that cause PCOS (polycystic ovary syndrome). HB-GEF, androgen, arachidonic acid, oleic acid VEGF, and EV-miRNA exhibit alterations in the follicular fluid of PCOS patients. Elevated HB-EGF may induce GCs apoptosis and excessive estrogen secretion through the cAMP-PKA-JNK/ERK-Ca2+-FOXO1 pathway. Excessive androgen can promote the recruitment and growth of follicles, producing a large number of small and immature follicles, as well as induce low-level inflammatory reactions. Additionally, it downregulates FF-EV miR-379-5p, thus inhibiting GC proliferation. Arachidonic acid, through upregulating GDF15 expression in GC which weakens the antioxidant capacity, can promote GC apoptosis. Also, increased AA can affect steroidogenesis in GCs by upregulating CYP19A1 and downregulating STAR, CYP11A1, and HSD3B1, leading to decreased progesterone and increased estrogen secretion.VEGF which is decreased in FF, leads to a lower ratio of pro-angiogenic EMs (2-OHE2, 4-OHE1, and 16-kE2) to anti-angiogenic EMs, resulting in decreased vascularity and, ultimately, halting follicle development. Stimulation from oleic acid not only leads to elevated IL-16 and IL-18 expression level through the ERK1/2 signaling pathway but also inhibit the AMPK pathway, thus increasing ROS production and IL-18 secretion. These inflammatory cytokines and LPS activate NLRP3 inflammasome through the NF-κB pathway, ultimately affecting GCs' function and impairing the development and growth of oocytes. FF exhibits a low level of EV-miR-18b-5p which can activate the PI3K/Akt/mTOR pathway by inhibiting PTEN downregulation, thereby inhibiting granulosa cell (GC) proliferation and promoting apoptosis. In addition, lower FF-EV-GRAMD1 triggers accessible cholesterol accumulation and perturbates steroid hormone production and activity. Moreover, reduced circLDLR levels, derepressing the function of miR-1294, play a role in the regulation of estradiol production via CYP19A1 in PCOS. Was this figure done using BioRender or any other tool? If yes, please give credit

from endometriosis patients presents elevated levels of oxidative stress markers malondialdehyde and resolvin D1 [167]. Activation of the inflammasome within human GCs can be manifested as elevated levels of IL-1beta and IL-18 in FF from endometriosis patients [149]. Interestingly, while heightened levels of interleukin (IL)-1β usually enhance ovulation signals, this specific effect is absent in cases of endometriosis [176]. This is due to GCs actively decreasing the activity of EZH2 histone methyltransferase, which subsequently inhibits the expression of IL-1R2, thus interfering with the ovulation cascade that would typically be induced by IL-1β [176].

Alterations in miRNA

In recent investigation, miRNA expression within the FF of patients with endometriosis exhibits notable alterations [177]. These miRNAs, displaying distinct expression patterns in comparison to healthy individuals, have been suggested to potentially impact embryonic development [177]. Specifically, within the FF, seven miRNAs (miR-766, miR-133, miR-191, miR-720, miR-143, miR-29c, and miR-203) display upregulation in the context of endometriosis, while eleven miRNAs (miR-1260, miR-145, miR-125a, miR-21, miR-628, miR-542, miR-223, miR-663, miR-378, miR-23a, and miR-451) exhibit downregulation [177].

Immune Imbalance

Perturbed balances in immune content possibly underlie infertility in immune-mediated conditions like endometriosis [178]. Alterations in the distribution of immune cell populations within FF may exert ripple effects on the follicular milieu, disrupting the FF cytokine profile, which is a result of the increased inflammatory state in endometriosis. In turn, these levels could attract more immune cells into the follicle or the peritoneal cavity after ovulation [178]. Notably, FF from endometriosis patients exhibits elevated levels of NK cells, CD14+macrophages/monocytes, and B cells, with T cell levels remaining relatively stable compared to other infertility causes [178].

The outcome of IVF

Despite the progress made in assisted reproduction treatment (ART), the success rates of IVF technique remain relatively low [179]. Research into the factors that impact the IVF and intracytoplasmic sperm injection (ICSI) could potentially enhance the success rates [180]. Among these factors, Oocyte quality is a key determinant in the success of fertilization and the subsequent development of embryos [181]. The oocyte quality may depend not only on the nuclear and mitochondrial genome, but also on the microenvironment provided by the ovaries, represented by FF, mainly during follicular development and pre-ovulation follicles. These numerous effectors can alter oocyte transcription and oocyte translation, affecting the developmental potential of future embryos [182]. FF, which can be easily collected during oocyte retrieval, serves as a rich source for identifying biomarkers that might predict the competence of oocytes and the implantation potential of embryos [184]. Since proteins, which are the end products of mRNA translation, play a critical role in cell function and metabolism, the study of proteins (proteomics) and metabolites (metabolomics) in FF has been particularly informative [29]. Furthermore, the study of non-coding RNAs, including long non-coding RNAs, is emerging as a field that may offer additional insights into predicting the developmental competence of oocytes [185]. Recently, a study developed a platform to perform metabolic fingerprinting of FF (MFFF), using particle-assisted laser desorption/ionization mass spectrometry (PALDI-MS), which may help to diagnose diminished OR and identify high-quality oocytes/embryos [186].

The metabolic byproducts produced by granulosa and theca cells within FF have drawn attention to oxidative stress, which is considered a significant factor that can negatively affect the success of ART [187]. The antioxidant capacity of FF has been found to be positively associated with the number of retrieved and mature oocytes, and the number of fertilized ones [184]. Increased levels of ROS can negatively impact IVF by causing fragmentation of embryos and impeding the development of blastocysts, which ultimately reduces the success rate of pregnancies [188]. FF total sialic acid (FF-TSA) levels positively correlated with germinal vesicle oocytes and metaphase I oocytes. possibly as a part of the natural maturation process or as a response to counteract the effects of ROS during oocyte development. On the other hand, there was no correlation between the FF free sialic acid (FF-FSA) levels and number of immature oocytes [190]. Interestingly, some studies have reported a positive correlation between the levels of ROS in FF and oocyte maturation indicators [191]. NO and/or its by-products may be potential biomarkers for IVF outcome. Although the levels of NO2 and NO3 can not predict the total number of oocytes recovered from the donors or the MII oocytes count, the proportion of MII oocytes was related directly to NO2 levels and inversely to NO3 levels. In addition, there is an inverse relation between NO3 levels in FF and the potential of embryos to implant in the uterus [193]. GH administration has been observed to enhance embryo quality, and implantation rate, and mitigate OS in FF, which could potentially be associated with the Nrf2/Keap1 pathway [194].

FF AMH levels from individual follicles hold the potential to indicate ovarian reserve and accurately predict subsequent embryonic developmental competence [195]. Although a greater FF AMH concentration is linked to a higher chance of clinical pregnancy and implantation rate, neither the total nor mature oocyte yield is correlated with it [44]. Conversely, studies by Mehta et al. have revealed a negative correlation between FF AMH levels and oocyte quality, fertilization rates, pregnancy rates, and embryo implantation rates [196]. Another study suggests that oocyte maturity and development inversely relate to AMH levels within individual follicles [196]. The dynamic regulation of AMH in response to folliculogenesis and follicle quality may underlie the conflicting findings reported in previous studies [197]. Recently, investigation has revealed that diminished levels of FF-AMH are intricately linked to the presence of top-quality

oocytes, while reduced levels of FF-PAI-1 are associated with follicle maturity, as manifested in the retrieval of mature MII oocytes during IVF cycles [195].

The fatty acid encompassing phospholipids and triglycerides exerts profound implications for oocyte and embryo quality within the realm of IVF programs [198]. Stearic acid, palmitic acid, linoleic acid, and oleic acid emerge as prominent constituents of these lipid components [198]. Of these, stearic acid levels present in FF demonstrate an intriguing inverse correlation with oocyte maturation and developmental competence, particularly towards cleavage-stage embryos, independently of other predictive factors [199]. On the other hand, embryo cleavage and oocyte maturation exhibit a direct correlation with the levels of oleic acid, which is independent of the age of women [198].

The concentration of adipokine leptin in FF is intricately associated with ovarian reserve and represents a promising biomarker for predicting oocyte maturity [117]. In addition, miR-10a-5p and miR-103a-3p exert negative regulatory effects on oocyte maturation by modulating the expression of BDNF in human FF [104]. Moreover, miR-103a-3p and miR-10a-5p expression levels in FF hold the potential to determine embryo quality on both days 3 and 5 [104]. Intrafollicular NE and Gln have been demonstrated to upregulate the antioxidative gene IDH1 expression in cumulus GCs, thus establishing it as a prospective indicator for evaluating the embryonic development quality [91]. ANAs have also been strongly linked to miscarriage or IVF-ET transplantation failure. By preventing proliferation and encouraging apoptosis in trophoblasts, ANAs in FF act as prognostic indicators for unfavorable outcomes in the setting of IVF-ET [102].

The steroid composition, particularly the presence of P4, wields a profound influence on oocyte quality, as excessive levels of P4 are known to culminate in diminished cellular quality whereas appropriate P4 input is beneficial to oocyte quality [200]. One recent study has suggested that the intrafollicular concentration of E2 positively impacts implantation rates, whereas the ratio of E2 to P4 levels has been inversely associated with implantation rates [201]. However, according to another study, high levels of FF E2/P4 are associated with a greater pregnancy rate [202]. Additionally, the implantation rate benefited from intrafollicular E2/T concentration. In the realm of IVF/ICSI cycles, elevated MMP-2 levels have demonstrated a positive correlation with fertilization rates and oocyte maturation, thus positing it as a potential marker for oocyte maturation rate [203]. Dehydroepiandrosterone (DHEA) is a hormone that is converted into testosterone by the theca cells in ovarian connective tissue [204]. The enzyme SULT2A1 facilitates the reversible conversion of DHEA into its sulfate form, DHEA-S, which has been identified as a key precursor for estrogen synthesis in GC [205]. The interconversion between DHEA and DHEA-S, along with their roles in ovarian androgen and testosterone synthesis, positions them as important biomarkers to assess during IVF treatments [206]. Research has indicated that elevated levels of DHEA in FF are inversely related to the number of oocytes retrieved and the success of fertilization [207]. Additionally, oocytes originating from follicles with high levels of LH and GH in the FF exhibited superior developmental potential [208]. High concentrations of IGF-1 showed similar outcomes, but it was found to be deficient in follicles of patients with poor response to ovarian stimulation [208]. This suggests that the intra-follicular effects of GH might be exerted through IGF-1 [208]. Moreover, an increased concentration of homocysteine in FF detrimentally affects oocyte growth and maturation, potentially leading to a lower implantation rate [209].

Overall, oocyte fertilization is facilitated by a microenvironment that is low in proinflammatory molecules. In one study, the levels of proinflammatory cytokines (IL-6, IL-8, and IL-12) and related inflammatory molecules (TGF-β and HIF-1α) in FF produced from fertilized oocytes was considerably lower than that of unfertilized oocytes. Furthermore, FF produced from fertilized oocytes had a considerably higher amount of the anti-inflammatory cytokine IL-10 than FF derived from unfertilized oocytes. The levels of the other proinflammatory molecules (IL-8, IL-12, HIF-1α, and TGF-β) and the concentration of IL-6 were positively connected, while the levels of IL-10 were inversely correlated [184]. On the other hand, some research has shown that there is no relationship between the number of oocytes and the rate of fertilization and the preovulatory FF concentration of IL-10 or IL-8 [210]. The influence of FF IL-6 appears to be biphasic, contingent upon its dosage. Several studies have attested to the positive impact of FF IL-6 on the quality of oocytes and embryos, as well as the overall outcome of IVF [212]. Since it is known that gonadotrophin stimulation during IVF modifies the immune cell profile in the FF as well as the cytokine concentrations in the FF and serum, the various stimulation protocols used during controlled ovarian stimulation may also be relevant in this regard [213].In addition, although there is no difference of FF TNF-α concentrations between pregnant and non-pregnant cycles, significantly higher TNF-α concentrations were found in follicles containing poor quality oocytes [214].

Conclusion and discussion

FF is the sole biological matrix connected to the oocyte, essential for the formation of female gametes. It comprises bioactive chemicals that fluctuate in quantity and quality throughout follicle growth, as well as specific follicular microenvironmental changes that contribute

to follicle and oocyte maturation and development. The oocyte microenvironment, mainly composed of FF, reflects the physiological and pathological states of ovarian follicles. In this review, we first introduce the formation mechanism for FF formation. The formation mechanism for FF formation involves the diffusion of plasma, secretions of follicle somite cells, and oocytes. An osmotic gradient is generated in the follicular antrum to attract the capillary vasculature's fluid for FF formation. Furthermore, we conclude the complex contents of the microenvironment formed by FF and detail these components' function on oocyte development respectively. FF has various abundant components, including large hormones, GH, cytokines, growth factors, reactive oxygen species, antioxidants, antiapoptotic factors, various lipids, functional proteins, and EVs. Each component plays a role in follicle and oocyte maturation and development, either by interacting with each other or alone. GCs and their communication with oocytes are vital in determining the fate of follicles, serving molecules essential for follicular growth and maintenance.

Finally, we briefly demonstrated the two prevalent diseases associated with infertility, PCOS and endometriosis. We focus on how FF can act on pathogenesis, mainly including endocrine and metabolite disturbance, as well as provide insight into the diagnosis using FF biomarkers. Moreover, we discussed some FF biomarkers related to oocyte quality in IVF, which help to optimize oocyte selection, allowing for the optimal choice of oocytes for transfer or cryostorage.

Acknowledgements
The figure was created at BioRender.com.

Author contributions
C.Z. conceived and designed the approach； Y.P. was a major contributor in writing the manuscript. Y.P. and C.P.designed and prepared figures. C.Z., Y.P. and C.P. carried out the final edits. All authors read and approved the final manuscript.

Funding
This study was supported by the National Natural Science Foundation of China, grant numbers No. 32160176 and 81960272, and was supported by School of Basic Medical Sciences, Nanchang University.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 5 July 2024 / Accepted: 31 October 2024
Published online: 26 November 2024

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