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Running title: Cancer-follicular fluid interplay

The interplay between cancer and follicular fluid: molecular changes and tumor-promoting properties

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Follicular fluid is a complex biological microenvironment essential for oocyte maturation and folliculogenesis. Alterations in follicular fluid composition have been associated with reproductive disorders reflecting compromised oocyte quality. Emerging evidence suggests that malignancies also significantly alter the composition of follicular fluid, potentially compromising the follicular microenvironment and impacting fertility preservation outcomes. Interestingly, the follicular fluid itself exhibits tumor-initiating and tumor-promoting properties, inducing DNA damage, pro-inflammatory signaling, mild proliferation, and suppressing apoptosis. Understanding cancer-associated follicular fluid alterations may improve fertility care, identify early biomarkers, and inform strategies for ovarian cancer prevention and therapy. This review aims to summarize current knowledge on how cancer can alter follicular fluid composition and its role in ovarian malignancies.

Key words: follicular fluid; follicular fluid composition; malignancy; high-grade serous carcinoma; ovulation

Follicular fluid (FF) is a complex biological fluid that constitutes the immediate microenvironment required for proper maturation and growth of the oocyte. Formed primarily from plasma transudate and secretions of granulosa and theca cells, FF contains a diverse array of bioactive components, including hormones, cytokines, proteins, metabolites, polysaccharides, enzymes, reactive oxygen species (ROS), anticoagulants, antioxidants, and growth factors [1, 2]. Such a composition provides the optimal microenvironment for folliculogenesis, and any alterations can directly reflect the metabolic state and overall developmental potential of the oocyte [2, 3]. FF also contains extracellular vesicles and regulatory non-coding RNAs, including microRNAs (miRNAs), which contribute to intercellular communication within the follicular microenvironment [4, 5].

Because FF directly surrounds the oocyte, its composition reflects the functional and metabolic status of the developing follicle and is closely associated with oocyte competence [1]. Changes in FF composition have been described in several reproductive disorders associated with infertility, such as polycystic ovarian syndrome (PCOS) and endometriosis, where disturbances in lipid metabolism, oxidative stress, and inflammation signaling have been linked to impaired oocyte

quality and reduced reproductive potential, thus supporting the concept that FF composition can be reflective of overall reproductive health [6-8].

Emerging evidence suggests that not only chronic reproductive health conditions, but also malignancies, have an altering effect on FF composition. This could result in an unfavorable environment for the oocyte, influencing its quality, fertility preservation outcomes, embryo development, and potentially long-term reproductive and offspring health following assisted reproductive procedures (Figure 1) [9-11].

Epithelial ovarian cancer comprises several histological subtypes, including high-grade serous carcinoma (HGSC), low-grade serous carcinoma, endometrioid carcinoma, clear cell carcinoma, and mucinous carcinoma, each characterized by distinct molecular alterations and etiological mechanisms [12]. Among these, HGSC is the most common and lethal subtype and appears to be most strongly associated with ovulation-related mechanisms and repeated exposure of the fimbrial epithelium to ovulatory FF [13]. However, FF has also been shown to promote and initiate growth of ovarian cancer, specifically HGSC, supporting the incessant ovulation theory, which states that each ovulatory cycle increases the risk of epithelial ovarian cancer [14-18].

Despite increasing evidence linking FF alterations with both reproductive dysfunction and ovarian carcinogenesis, several questions remain. It is still unclear whether different malignancies affect FF composition differently, which FF components are causative rather than associative, and whether FF profiling could serve as a predictive tool for fertility outcomes or early HGSC risk assessment. Furthermore, most mechanistic studies investigating FF-induced carcinogenic effects utilize FF collected from women undergoing IVF, where controlled ovarian stimulation may alter the physiological composition of FF compared with spontaneous ovulation [9].

This review aims to synthesize comprehensive evidence establishing the impact of FF on ovarian carcinogenesis and how malignancy alters the molecular composition of FF.

Influence of cancer on FF composition

Cancer is increasingly recognized as a systemic disease capable of altering metabolic, inflammatory, and oxidative signaling pathways beyond the primary tumor site [19]. Because FF reflects the functional and metabolic status of the developing follicle, systemic cancer-associated alterations may also affect the follicular microenvironment [1].

Distinct metabolic changes in FF have been reported in breast cancer (BC) and lymphoma patients undergoing cryopreservation prior to receiving cancer treatment [9, 20]. Although 12 differential metabolites were identified in the FF of these patients, no statistically significant metabolomic differences were observed between the BC and lymphoma cohorts. A significant increase in citrate, creatine, glycerol, glycerophosphocholine, and glucose was observed in the patients, alongside

decreased levels of amino acids (asparagine, aspartate, proline), cholesterol, choline, lactate, and lipids [9]. Importantly, while BC patients received different hormonal stimulation, lymphoma patients received the same hormonal stimulation as the healthy controls. Despite this, lymphoma patients still exhibited different FF metabolic profiles compared with the controls, indicating that these differences could be attributed to the disease rather than the hormonal stimulation [9]. A study focusing on BC patients with estrogen-sensitive tumors confirmed the dysregulation of energy-related metabolites, including increased glucose and decreased levels of amino acids, glutamine, and lipids. Additionally, stratification of patients by disease progression has revealed further metabolic alterations in FF, with patients with lymph node metastasis exhibiting significantly lower levels of β-hydroxybutyrate and trimethylamine N-oxide than healthy controls. These findings suggest that cancer-associated metabolic changes in FF are not uniform and may vary depending on the stage of the disease [20].

Glutamine, being one of the most abundant metabolites in the human body, plays a crucial role in cancer metabolism, influencing tumor growth and progression. Some cancer types become "addicted" to this normally nonessential/conditionally essential amino acid, increasing its uptake as they cannot proliferate without it [21]. Cancer cells reprogram their glucose metabolism to generate sufficient energy for their uncontrolled proliferation, with glycolysis being typically elevated under anaerobic conditions, making it the primary energy source in malignant tumors even in the presence of oxygen (also known as the Warburg effect) [22]. Such metabolic alterations in glucose and glutamine levels could suggest dysregulation of the energy supply within the oocyte microenvironment, but further research is required. Together, these findings indicate that cancer can induce a metabolic shift in FF, potentially compromising the energy support required for proper oocyte development and maturation.

Altered glucose and lipid metabolism can contribute to oxidative stress within the follicular microenvironment, as mitochondrial metabolism and redox homeostasis are closely interconnected [23, 24]. Impaired lipid metabolism has also been associated with increased oxidative stress, mitochondrial dysfunction, and reduced developmental competence of the oocytes [25]. Therefore, cancer-associated metabolic alterations in FF may not only disrupt the energy supply for the oocyte but also promote a pro-oxidative follicular microenvironment, disrupting redox balance and inflammatory signaling. Oxidative stress and inflammation are closely interconnected, as excessive ROS production can activate pro-inflammatory signaling pathways, while inflammatory mediators may further amplify oxidative damage within the follicular microenvironment [8]. Indeed, alterations in antioxidant defense pathways have been observed in FF of BC patients [20]. Expression of Nrf2, a master regulator of the antioxidant response that upregulates the expression of antioxidant enzymes and cytoprotective proteins, such as NADPH quinone oxidoreductase 1

(NQO1) and superoxide dismutase (SOD), under conditions of cellular stress has been found to be significantly decreased. This finding is consistent with the absence of compensatory changes in NQO1 and SOD levels. Failure to activate these enzymes may have contributed to the increased levels of the pro-inflammatory chemokine CXCL10 that were observed in BC patients [20].

In addition to metabolic, oxidative, and inflammatory alterations, cancer-associated changes in FF also involve deregulation of miRNAs, which are important mediators of intercellular communication within the follicular microenvironment [26]. miRNAs regulate multiple cellular processes associated with folliculogenesis, oocyte maturation, and embryogenesis, while extracellular vesicles and exosomes present in FF may facilitate the transfer of regulatory miRNAs, proteins, and other bioactive cargo between follicular cells and the oocyte [27, 28].

Altered miRNA profiles have been observed in FF of women with Hodgkin lymphoma (HL). Thirteen miRNAs were significantly deregulated in comparison to healthy controls, including altered expression of let-7b-5p, miR-423-5p, miR-503-5p, miR-574-5p, and miR-1303, which have been previously associated with ovarian response and oocyte quality [29]. These miRNAs are involved in several key signaling pathways associated with gametogenesis, embryogenesis, and cancer, including the p53, PI3K-Akt, MAPK, TGF-β, Hippo, Wnt, and FoxO signaling pathways, as well as oocyte meiosis [29].

Several of the deregulated miRNAs identified in HL patients have also been associated with reproductive disorders and impaired follicular function. Their summarization can be found in Table 1. let-7b-5p downregulation was observed in FF and plasma of patients with endometriosis and PCOS, possibly reflecting abnormal folliculogenesis [30, 31]. It has also been demonstrated that expression of let-7b-5p was decreased in fair-quality blastocysts and was found to be negatively correlated with the number of metaphase II oocytes, two-pronuclei cells, and embryo development grade [32]. Similarly, downregulation of miR-423-5p expression has been observed in the granulosa cells of patients with a high ovarian response to exogenous gonadotropins, suggesting a possible role in the regulation of ovarian response [33]. Higher levels of miR-1303 were reported in the FF of poor-quality oocytes, and have also been associated with ovarian aging in mice [34, 35]. Altered expression of miR-574-5p was observed in FF samples and granulosa cells from IVF patients, with expression patterns varying according to oocyte maturation stage, while miR-503-5p could be directly involved in the formation and maintenance of the corpus luteum [36, 37]. let-7b-5p was identified as the central node in the regulatory network, showing the highest number of shared target genes involved in follicle development and oocyte maturation with other deregulated miRNAs. Such alterations in the FF miRNA profile suggest that HL can affect the signaling pathways essential for follicular homeostasis and oocyte maturation, potentially compromising their competence and reproductive outcomes [29]. It has been shown that cancer-associated alterations in

FF extend beyond changes in metabolic composition and involve interconnected oxidative, inflammatory, and regulatory signaling pathways capable of disrupting follicular homeostasis, as summarized in Table 2 [8, 26]. Since FF contains high concentrations of bioactive molecules, including ROS, cytokines, growth factors, metabolites, and miRNAs, alterations in its composition may also influence the surrounding epithelial tissues exposed to ovulatory FF [38]. These observations support the hypothesis that FF may also act as an active participant in ovarian carcinogenesis.

FF as a tumor initiator/promoter

As FF is a biologically rich fluid, its composition can also have a negative effect on the morphology of the surrounding cells. The incessant ovulation theory proposes that repeated ovulation over a woman's lifetime increases the risk of epithelial ovarian cancer [14]. Ovulation is a hormone-induced physiologic process that causes rupture of the dominant follicle and injury to the ovarian surface or surrounding tissue, followed by repair [14, 39]. Each ovulatory cycle creates a localized inflammatory environment, with the release of ROS and other factors capable of causing DNA damage. Although standard cellular repair mechanisms usually correct this damage, repeated cycles of injury and repair increase the chance of accumulated genetic mutations. Over time, this cumulative damage may lead to malignant transformation of epithelial cells, contributing to the development of ovarian cancer [14]. Importantly, the biological effects of FF appear to depend on both the duration and context of exposure. Acute oxidative and inflammatory responses during ovulation are necessary for follicular rupture and tissue repair, whereas chronic and repeated exposure of the fimbrial epithelium to ROS-rich FF may progressively impair antioxidant defenses and promote accumulation of DNA damage, particularly in cells harboring TP53 dysfunction [15, 17, 39].

FF can induce gene expression changes that mimic early carcinogenic events in oviductal cells. Exposure of bovine oviductal epithelial cells to FF from women undergoing IVF resulted in an increase of IL-8 and PTGS2, essentially creating a transient pro-inflammatory environment. Decreased expression of NR3C1, an anti-inflammatory receptor, suggested reduced sensitivity to anti-inflammatory signaling, potentially maintaining the cells in a prolonged pro-inflammatory state. The decreased expression of DAB2, a tumor suppressor involved in growth factor signaling regulation, could disrupt growth factor signaling and potentially facilitate tumor cell metastasis. Reduced expression of antioxidant enzymes SOD2 and GPX3 suggests that exposure to FF may impair the cells' ability to neutralize ROS, therefore contributing to possible DNA damage. Although the observed inflammatory response was transient, repeated exposure to FF over hundreds of ovulatory cycles in a woman's lifetime could cumulatively "wear down" the cellular defense

mechanisms, contributing to the initiation of HGSC as proposed by the incessant ovulation theory [15].

Similarly, exposure of human fallopian tube epithelial cells isolated from benign surgical specimens to FF altered the expression of genes involved in proliferation, cell cycle regulation, inflammation, and DNA repair. The strongest response occurred at the 4-hour mark, suggesting that FF can trigger an acute biological reaction. A slight but significant increase in proliferation without cytotoxicity was observed, indicating that FF does not necessarily induce uncontrolled growth, but instead supports a proliferative environment. Increased expression of the pro-inflammatory cytokine IL-8 further linked ovulation-related inflammation to early neoplastic changes. Increased levels of a double-stranded break marker γH2AX indicated that FF possesses genotoxic properties likely due to the presence of ROS and other damaging agents. Accumulation of TP53 protein resembling the "p53 signature", an early precursor lesion associated with serous carcinoma, was also observed. These findings support the hypothesis that repeated exposure to ovulatory FF may contribute to DNA damage accumulation and early fimbrial transformation [16].

Recent studies further support the notion that FF-induced oxidative stress leads to DNA damage and carcinogenesis. ROS-rich FF was shown to induce oxidative DNA damage and epithelial injury in fallopian tube epithelial cells, reinforcing the concept that FF acts as a mutagenic microenvironment during ovulation [40]. FF exposure has also been shown to induce DNA damage and lipid peroxidation in fallopian tube epithelial cells in an age-dependent manner while simultaneously promoting cell adhesion, spreading, and proliferation [41]. This suggests that FF contributes not only to the initiation of DNA damage but also to microenvironmental alterations that favor tumor progression and dissemination.

The identification of serous tubal intraepithelial carcinoma (STIC) as a likely precursor lesion of HGSC has highlighted the importance of early TP53 dysfunction in initiating malignant transformation within the fallopian tube epithelium. As HGSC is one of the most common gynecological cancers characterized by early TP53 loss-of-function mutations, increasing attention has been directed toward understanding how ovulatory FF may promote carcinogenesis in the context of impaired DNA damage responses [17]. FF shows a variability in ROS concentrations, with ROS-rich FF being capable of inducing intracellular ROS accumulation, oxidative DNA damage, and apoptosis in fimbrial epithelial cells. The fimbrial epithelium appears particularly susceptible to these effects, consistent with its greater physiological exposure to ovulatory FF during ovulation. However, in the absence of functional p53, cells fail to undergo apoptosis despite persistent DNA damage, allowing the survival and proliferation of genetically compromised cells. Therefore, repeated exposure to ROS-rich FF may progressively overwhelm antioxidant defenses and DNA repair mechanisms, promoting the accumulation of oncogenic alterations in TP53-

deficient cells. Experimental evidence further suggests that ROS-rich FF possesses both tumor-initiating and tumor-promoting properties, supporting the hypothesis that chronic exposure of the fimbrial epithelium to ovulatory FF contributes to HGSC development [17].

Further evidence suggests that ovulatory FF provides extensive transformation activity throughout the entire process of HGSC development. FF exposure enhanced proliferation, epithelial-to-mesenchymal transition (EMT), anoikis resistance, anchorage-independent growth, and metastatic "seeding" capacity across cell lines representing different stages of HGSC development. Activation of the IGF-1R/AKT and AKT pathway, not mediated by IGF-1R, promoted the survival and invasive properties of transformed cells. FF also enhanced peritoneal attachment and EMT, facilitating invasion into surrounding tissues. *In vivo* experiments demonstrated increased tumor formation in mice injected with intermediate- and advanced-stage HGSC cells together with FF, whereas no tumor growth was observed in mice injected with early precursor cells alone. These findings suggest that ovulatory FF acts as a potent "booster" throughout multiple stages of HGSC development, from early precursor lesions to invasive carcinoma [18].

Nevertheless, interpretation of current findings requires caution. The experimental studies investigating FF-induced carcinogenic mechanisms utilize FF collected from women undergoing IVF, where hormonal ovarian stimulation may alter hormone concentrations, inflammatory mediators, and ROS levels compared with spontaneous ovulation [38, 39]. Furthermore, ROS concentrations vary among FF samples, and *in vitro* exposure models may not fully replicate the intermittent and dynamic nature of physiological ovulatory exposure *in vivo*. Despite this, current evidence supports the concept that FF is a biologically active microenvironment capable of promoting inflammation, oxidative stress, DNA damage, survival signaling, and tumor progression associated with HGSC initiation and development.

Offspring health and live birth outcomes

Beyond their potential role in ovarian carcinogenesis, alterations in FF composition may also have important implications for reproductive competence and fertility outcomes. Increasing evidence suggests that disturbances in oxidative balance, metabolic homeostasis, and intercellular signaling within FF may negatively affect oocyte quality, fertilization potential, and subsequent embryo development [26, 38]. Therefore, understanding how cancer-associated FF alterations influence reproductive outcomes may be important not only for fertility preservation strategies but also for long-term assessment of reproductive and offspring health.

Current evidence suggests that fertility preservation using cryopreserved oocytes or embryos collected before gonadotoxic cancer treatment can result in successful pregnancies and live births [10, 42]. Live births of children conceived from cryopreserved oocytes harvested prior to cancer

treatment have been reported, showing that fertility preservation can successfully preserve reproductive potential in oncology patients [42]. Long-term follow-up studies have further shown successful pregnancies and apparently normal child development following oocyte cryopreservation in female cancer patients undergoing fertility preservation over a 25-year period [10]. However, reproductive outcomes following fertility preservation remain variable and appear to be influenced by several factors, including patient age, ovarian reserve, cancer type, and the quality of retrieved oocytes [11]. Cancer-associated alterations within FF may contribute to impaired reproductive outcomes by disrupting the signaling pathways necessary for normal folliculogenesis and oocyte maturation. Oxidative stress within the follicular microenvironment has been associated with mitochondrial dysfunction, impaired spindle formation, DNA damage, and reduced embryo developmental potential [8, 25].

Importantly, current clinical evidence does not suggest major increases in congenital abnormalities or impaired early childhood development among children born following fertility preservation procedures in cancer patients [10]. However, long-term follow-up data remain limited, and the potential effects of cancer-associated alterations in the follicular microenvironment on offspring health are still poorly understood.

Conclusion and recommendations

Accumulating evidence shows that cancer profoundly alters the composition of FF, affecting metabolites, lipids, antioxidants, inflammatory mediators, and regulatory miRNAs that are critical for oocyte competence and follicular homeostasis [9, 20, 29]. So far, studies have been examining the FF composition of BC and lymphoma patients, which is a relatively narrow and insufficiently representative range of malignancies. Expanding this research to include other malignancies could significantly enhance the understanding of the reproductive and oncogenic implications of cancer-associated alterations in FF, therefore improving the quality of reproductive care provided to women undergoing cryopreservation and possibly helping to identify specific biomarkers for early detection.

FF itself also exhibits tumor-initiating and tumor-promoting properties, particularly through inflammatory mediators, ROS, and growth factor signaling. Studies demonstrate that repeated exposure to ovulatory FF can induce DNA damage, enhance proliferation, suppress apoptosis, and promote malignant transformation, especially in the context of p53 dysfunction. These findings support the incessant ovulation theory and position FF as an active participant in the initiation and progression of HGSC [15-18]. Further research should focus on identifying the specific components of FF that drive DNA damage, proliferation, and tumor-promoting effects, as well as how FF interacts with cells harboring cancer-relevant mutations. This could help improve understanding of

early ovarian carcinogenesis, identify potential risk biomarkers, and develop preventive or therapeutic strategies.

Although the available evidence remains limited, current data suggest that children born to cancer patients who decided to conceive using their cryopreserved oocytes are healthy and go through normal development. Moreover, live birth rates, miscarriage rates, and oocyte survival rates of cancer patients are comparable to those observed in non-cancer patients undergoing IVF [10]. However, due to the limited number of studies and the lack of long-term follow-up, the question of whether the health and development of these children may be affected later in life remains. Further studies focusing on the overall health, development, and genome of the children should be carried out to properly assess the safety of using cryopreserved oocytes of cancer patients for conception.

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References

[1] PAN Y, PAN C, ZHANG C. Unraveling the complexity of follicular fluid: insights into its composition, function, and clinical implications. J Ovarian Res 2024; 17: 237. https://doi.org/10.1186/s13048-024-01551-9

[2] BASUINO L, SILVEIRA CF. Human follicular fluid and effects on reproduction. JBRA Assist Reprod 2016; 20: 38-40. https://doi.org/10.5935/1518-0557.20160009

[3] LI J, ZHANG Z, WEI Y, ZHU P, YIN T et al. Metabonomic analysis of follicular fluid in patients with diminished ovarian reserve. Front Endocrinol (Lausanne) 2023; 14: 1132621. https://doi.org/10.3389/fendo.2023.1132621

[4] WANG W, CRAIG ZR, BASAVARAJAPPA MS, HAFNER, KS, FLAWS JA. Mono-(2-Ethylhexyl) Phthalate Induces Oxidative Stress and Inhibits Growth of Mouse Ovarian Antral Follicles. Biol Reprod 2012, 87: 152. https://doi.org/10.1095/biolreprod.112.102467

[5] CHANDLER C, LIU T, BUCKANOVICH R, COFFMAN LG. The double edge sword of fibrosis in cancer. Transl Res 2019; 209, 55-67. https://doi.org/10.1016/j.trsl.2019.02.006

[6] SUN Z, CHANG HM, WANG A, SONG J, ZHANG X et al. Identification of potential metabolic biomarkers of polycystic ovary syndrome in follicular fluid by SWATH mass spectrometry. Reprod Biol Endocrinol 2019; 17: 45. https://doi.org/10.1186/s12958-019-0490-y

[7] KARAER A, TUNCAY G, MUMCU A, DOGAN B. Metabolomics analysis of follicular fluid in women with ovarian endometriosis undergoing in vitro fertilization. Syst Biol Reprod Med 2018; 65: 39-47. https://doi.org/10.1080/19396368.2018.1478469

[8] ORISAKA M, MIZUTANI T, MIYAZAKI Y, SHIRAFUJI A, TAMAMURA C et al. Chronic low-grade inflammation and ovarian dysfunction in women with polycystic ovarian syndrome, endometriosis, and aging. Front Endocrinol 2019; 14: 1324429. https://doi.org/10.3389/fendo.2023.1324429

[9] CASTIGLIONE MORELLI MA, IULIANO A, SCHETTINI SCA, PETRUZZI D, FERRI A et al. NMR metabolomics study of follicular fluid in women with cancer resorting to fertility preservation. J Assist Reprod Genet 2018; 35: 2063-2070. doi:https://doi.org/10.1007/s10815-018-1281-7

[10] PORCU E, CIPRIANI L, DIRODI M, DE IACO P, PERRONE AM et al. Successful Pregnancies, Births, and Children Development Following Oocyte Cryostorage in Female

Cancer Patients During 25 Years of Fertility Preservation. Cancers 2022; 14: 1429. https://doi.org/10.3390/cancers14061429

[11] FRAISON E, HUBERLANT S, LABRUNE E, CAVALIERI M, MONTAGUT M et al. Live birth rate after female fertility preservation for cancer or haematopoietic stem cell transplantation: a systematic review and meta-analysis of the three main techniques; embryo, oocyte and ovarian tissue cryopreservation. Hum Reprod 2022; 38: 489-502. https://doi.org/10.1093/humrep/deac249

[12] KURMAN RJ, SHIH IM. The Origin and Pathogenesis of Epithelial Ovarian Cancer: A Proposed Unifying Theory. Am J Surg Pathol 2010; 34: 433-443. https://doi.org/10.1097/PAS.0b013e3181cf3d79

[13] LABIDI-GALY SI, PAPP E, HALLBERG D, NIKNAFS N, ADLEFF V et al High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun 2017; 8: 1093. https://doi.org/10.1038/s41467-017-00962-1

[14] FATHALLA MF. Incessant ovulation and ovarian cancer - a hypothesis re-visited. Facts Views Vis Obgyn 2013; 5: 292-297.

[15] LAU A, KOLLARA A, JOHN ES, TONE AA, VIRTANEN C et al. Altered expression of inflammation-associated genes in oviductal cells following follicular fluid exposure: Implications for ovarian carcinogenesis. Exp Biol Med (Maywood) 2014; 239: 24-32. https://doi.org/10.1177/1535370213508216

[16] BAHAR-SHANY K, BRAND H, SAPOZNIK S, JACOB-HIRSCH J, YUNG Y et al. Exposure of fallopian tube epithelium to follicular fluid mimics carcinogenic changes in precursor lesions of serous papillary carcinoma. Gynecol Oncol 2014; 132: 322-327. https://doi.org/10.1016/j.ygyno.2013.12.015

[17] HUANG HS, CHU SC, HSU CF, CHEN PC, DING DC et al. Mutagenic, surviving and tumorigenic effects of follicular fluid in the context of p53 loss: initiation of fimbria carcinogenesis. Carcinogenesis 2015; 36: 1419-1428. https://doi.org/10.1093/carcin/bgv132

[18] HSU CF, CHEN PC, SEENAN V, DING DC, CHU TY. Ovulatory Follicular Fluid Facilitates the Full Transformation Process for the Development of High-Grade Serous Carcinoma. Cancers 2021; 13: 468-468. https://doi.org/10.3390/cancers13030468

[19] HANAHAN D. Hallmarks of Cancer: New Dimensions. Cancer Discov 2022; 12: 31-46. https://doi.org/10.1158/2159-8290.CD-21-1059

[20] CASTIGLIONE MORELLI M, IULIANO A, MATERA I, VIGGIANI L, SCHETTINI S et al. A Pilot Study on Biochemical Profile of Follicular Fluid in Breast Cancer Patients. Metabolites 2023; 13: 441. https://doi.org/10.3390/metabo13030441

[21] MURANAKA H, AKINSOLA R, BILLET S, PANDOL SJ, HENDIFAR AE et al. Glutamine Supplementation as an Anticancer Strategy: A Potential Therapeutic Alternative to the Convention. Cancers 2024; 16: 1057. https://doi.org/10.3390/cancers16051057

[22] EL ANSARI R, MCINTYRE A, CRAZE ML, ELLIS IO, RAKHA EA et al. Altered glutamine metabolism in breast cancer; subtype dependencies and alternative adaptations. Histopathology 2017; 72: 183-190. https://doi.org/10.1111/his.13334

[23] ZHANG CH, LIU XY, WANG J. Essential Role of Granulosa Cell Glucose and Lipid Metabolism on Oocytes and the Potential Metabolic Imbalance in Polycystic Ovary Syndrome. Int J Mol Sci 2023; 24: 16247. https://doi.org/10.3390/ijms242216247

[24] KOBAYASHI H, YOSHIMOTO C, MATSUBARA S, SHIGETOMI H, IMANAKA S. Altered Energy Metabolism, Mitochondrial Dysfunction, and Redox Imbalance Influencing Reproductive Performance in Granulosa Cells and Oocyte During Aging. Reproductive sciences 2023; 31: 906-916. https://doi.org/10.1007/s43032-023-01394-7

[25] KHAN R, JIANG X, HAMEED U, SHI Q. Role of Lipid Metabolism and Signaling in Mammalian Oocyte Maturation, Quality, and Acquisition of Competence. Front Cell Dev Biol 2021; 9: 639704. https://doi.org/10.3389/fcell.2021.639704

[26] WANG Z, ZHANG Y, YANG A, REN Y, ZHANG Q et al. Extracellular microRNAs in follicular fluids: implication for reproductive diseases. ExRNA 2021; 3: 4-4. https://doi.org/10.21037/exrna-20-3
[27] ZHANG Q, SU J, KONG W, FANG Z, LI Y et al. Roles of miR-10a-5p and miR-103a-3p, Regulators of BDNF Expression in Follicular Fluid, in the Outcomes of IVF-ET. Front Endocrinol (Lausanne) 2021; 12: 637384. https://doi.org/10.3389/fendo.2021.637384
[28] DI PIETRO C. Exosome-mediated communication in the ovarian follicle. Journal of Assisted Reproduction and Genetics 2016; 33: 303-311. https://doi.org/10.1007/s10815-016-0657-9
[29] CAPONNETTO A, BATTAGLIA R, RAGUSA R, BARBAGALLO D, LUNELIO F et al. Molecular profiling of follicular fluid microRNAs in young women affected by Hodgkin lymphoma. Reprod Biomed Online 2021; 43: 1045-1056. https://doi.org/10.1016/j.rbmo.2021.08.007
[30] SCALICI E, TRAVER S, MULLET T, MOLINARI N, FERRIÈRES A et al. Circulating microRNAs in follicular fluid, powerful tools to explore in vitro fertilization process. Sci Rep 2016; 6: 24976. https://doi.org/10.1038/srep24976
[31] PAPARI E, NORUZINIA M, KASHANI L, FOSTER WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertil Steril 2020; 113: 1232-1241. https://doi.org/10.1016/j.fertnstert.2020.01.026
[32] TIMOFEEVA AV, CHAGOVETS VV, DRAPKINA YS, MAKAROVA NP, KALININA EA et al. Cell-Free, Embryo-Specific sncRNA as a Molecular Biological Bridge between Patient Fertility and IVF Efficiency. Int J Mol Sci 2019; 20: 2912-2912. https://doi.org/10.3390/ijms20122912
[33] XIE S, BATNASAN E, ZHANG Q, LI, Y. MicroRNA Expression is Altered in Granulosa Cells of Ovarian Hyperresponders. Reprod Sci 2016; 23: 1001-1010. https://doi.org/10.1177/1933719115625849
[34] ZHANG D, LV J, TANG R, FENG Y, ZHAO Y et al. Association of exosomal microRNAs in human ovarian follicular fluid with oocyte quality. Biochem Biophys Res Commun 2020; 534: 468-473. https://doi.org/10.1016/j.bbrc.2020.11.058
[35] LI J, ZHOU F, ZHENG T, PAN Z, LIANG X et al. Ovarian Germline Stem Cells (OGSCs) and the Hippo Signaling Pathway Association with Physiological and Pathological Ovarian Aging in Mice. Cell Physiol Biochem 2015; 36: 1712-1724. https://doi.org/10.1159/000430144
[36] MORENO JM, NÚÑEZ MJ, QUIÑONERO A, MARTÍNEZ S, DE LA ORDEN M et al. Follicular fluid and mural granulosa cells microRNA profiles vary in in vitro fertilization patients depending on their age and oocyte maturation stage. Fertil Steril 2015; 104: 1037-1046.e1. https://doi.org/10.1016/j.fertnstert.2015.07.001
[37] REZA AMMT, CHOI YJ, HAN SG, SONG H, PARK C et al. Roles of microRNAs in mammalian reproduction: from the commitment of germ cells to peri-implantation embryos. Biol Rev Camb Philos Soc 2019; 94: 415-438. https://doi.org/10.1111/brv.12459
[38] DA BROI MG, GIORGI VSI, WANG F, KEEFE DL, ALBERTINI D et al. Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications. J Assist Reprod Genet 2018; 35: 735-751. https://doi.org/10.1007/s10815-018-1143-3
[39] HOLESH JE, BASS AN, LORD M. Physiology, Ovulation. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available at: https://www.ncbi.nlm.nih.gov/books/NBK441996/
[40] ZHU M, WANG N, WANG S, WANG Y, YANG X et al. Chen Y. Effects of Follicular Fluid on Physiological Characteristics and Differentiation of Fallopian Tube Epithelial Cells Implicating for Ovarian Cancer Pathogenesis. Int J Mol Sci 2023; 24: 10154. https://doi.org/10.3390/ijms241210154

[41] SALVI A, LI W, DIPALI SS, COLOGNA SM, PAVONE ME. Follicular fluid aids cell adhesion, spreading in an age independent manner and shows an age-dependent effect on DNA damage in fallopian tube epithelial cells. Heliyon 2024; 10: e27336. https://doi.org/10.1016/j.heliyon.2024.e27336

[42] YANG D, BROWN SE, NGUYEN K, REDDY V, BRUBAKER C et al. Live birth after the transfer of human embryos developed from cryopreserved oocytes harvested before cancer treatment. Fertil Steril 2007; 87: 1469.el-1469.e4. https://doi.org/10.1016/j.fertnstert.2006.07.1546

Figure Legends

Figure 1. Reproductive and oncogenic consequences of cancer-associated alterations in FF. Cancer induces systemic metabolic, inflammatory, oxidative, and regulatory miRNA changes that modify FF composition. These alterations disrupt follicular homeostasis, impairing oocyte quality through mitochondrial dysfunction, oxidative stress, and altered signaling pathways. Repeated exposure of the fallopian tube epithelium to FF-enriched in ROS, cytokines, and growth-related factors promotes DNA damage, inflammatory signaling, and proliferative responses. In the presence of TP53 dysfunction, these processes may contribute to STIC formation and progression to HGSC.

Table 1. Overview of five miRNAs reported in human and animal studies associated with ovarian response and oocyte quality.

miRNA	Expression pattern	Association	Biological implication	References
let-7b-5p	Downregulated	Endometriosis	May reflect impaired folliculogenesis and reduced oocyte competence	[31]
		PCOS		[30]
		Fair-quality blastocysts; negative correlation with the number of metaphase II oocytes, two-pronuclei cells, and embryo development grade		[32]
miR-423-5p	Downregulated	High ovarian response to exogenous gonadotropins	Possible regulator of ovarian response	[33]
miR-1303	Upregulated	Poor-quality oocytes	Potential marker of reduced oocyte quality and ovarian aging	[34]
		Ovarian aging		[35]
miR-574-5p	Variable	Oocyte maturation stage	May participate in follicular maturation dynamics	[36]
miR-503-5p	Variable	Formation and maintenance of the corpus luteum	May contribute to luteal function	[37]

Note: The table summarizes the observed expression pattern, reported association, and proposed biological implication.

460 **Table 2.** Overview of cancer-associated alterations in FF and their potential clinical implications.

Cancer type	Alteration category	Changes in FF	Proposed mechanisms	Potential reproductive consequences	References
Breast cancer & Lymphoma	Metabolic alterations	Increased glucose citrate, creatine, glycerol, and glycerophosphocholine; decreased amino acids (asparagine, aspartate, proline), cholesterol, choline, lactate, lipids, glutamine, β-hydroxybutyrate, and TMAO	Cancer-associated metabolic reprogramming and altered energy metabolism may disrupt nutrient availability and mitochondrial homeostasis within the follicular microenvironment	Impaired oocyte energetic support, reduced oocyte competence, disrupted folliculogenesis, impaired embryo development	[9,20]
Breast cancer	Oxidative stress and antioxidant imbalance	Decreased antioxidant defense pathways, including reduced Nrf2 activity and altered SOD/NQO1 expression; increased pro-inflammatory chemokine CXCL10	Impaired antioxidant response disrupts redox homeostasis and promotes oxidative damage	Reduced developmental competence of oocytes	[20]
Hodgkin lymphoma	miRNA dysregulation	Deregulated expression of let-7b-5p, miR-423-5p, miR-503-5p, miR-574-5p, and miR-1303	Disrupted intercellular communication affecting PI3K/AKT, MAPK, TGF-β, Hippo, Wnt, FoxO, and p53-associated signaling pathways	Impaired oocyte maturation, altered ovarian response, abnormal folliculogenesis, reduced reproductive competence	[29]

461 Note: The table summarizes the observed alterations in the FF composition of cancer patients, the proposed mechanisms, and potential reproductive
462 consequences.

Fig. 1 [Download full resolution image]