



Review Article

Ovulation: A consequence of acute inflammation cultivated by E2-induced reactive oxygen species and triggered by progesterone withdrawal

Ying-Hsi Chen^{a,b}, Tang-Yuan Chu^{a,b,c*}

^aDepartment of Obstetrics and Gynecology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, ^bInstitute of Medical Sciences, Tzu Chi University, Hualien, Taiwan, ^cCenter for Prevention and Therapy of Gynecological Cancers, Department of Medical Research, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

Submission : 11-Apr-2025
Revision : 15-May-2025
Acceptance : 27-May-2025
Web Publication : 09-Oct-2025

ABSTRACT

Ovulation is a complex biological process essential for female fertility, driven by the luteinizing hormone (LH) surge and involving a cascade of molecular events that lead to follicular rupture and oocyte release. This process shares characteristics with acute inflammation, including the generation of reactive oxygen species (ROS) by E2, immune cell recruitment, and tissue remodeling. E2 enhances mitochondrial ROS production through integrin-dependent signaling, regulating key ovulatory events such as cumulus expansion and extracellular matrix breakdown. The LH surge triggers follicular luteinization and progesterone production, which are critical for preparing the endometrium for implantation and modulating inflammation. Progesterone, acting through its receptor (PGR), suppresses ROS-induced inflammation by inhibiting the NF- κ B pathway, ensuring controlled inflammatory responses. However, a transient decline in progesterone levels following the LH surge initiates acute inflammation, leading to follicle rupture and ovulation. This process involves the upregulation of matrix metalloproteinases and other proteases that degrade the follicular wall, facilitated by structural changes such as cumulus expansion and decellularization at the follicular apex. Post-ovulation, the remaining follicular cells undergo luteinization to form the corpus luteum, which produces progesterone to support early pregnancy. The ovulation wound heals rapidly through a process resembling ordinary wound healing where follicular fluid plays a dual role by promoting ovulation wound healing and when spilled into the pelvic cavity, potentially contributing to postoperative adhesions. Understanding the molecular mechanisms of ovulation provides valuable insights into fertility promotion, contraception development, and the prevention of reproductive disorders.

KEYWORDS: *Cumulus granulosa cells, Mural granulosa cell, Ovulation*

INTRODUCTION

Ovulation is a highly coordinated biological process essential for female fertility, driven by the luteinizing hormone (LH) surge and a cascade of molecular events that lead to follicular rupture and oocyte release. This process shares key characteristics, including the generation of reactive oxygen species (ROS) by estrogen (E2), acute inflammation responses, immune cell recruitment, and tissue remodeling. Among them, E2 increases ROS generation in mitochondria. ROS plays a crucial role in regulating ovulatory signaling, such as steroidogenesis, cumulus expansion, and extracellular matrix (ECM) breakdown. Both ROS and antioxidants as well as E2 and progesterone (P4) work together to regulate ovulation. Imbalances between them can contribute to the

development of female reproductive disorders and even cancers [1].

This review aims to explore two aspects of mechanisms involving ovulation, the mechanism of follicle maturation and rupture, and the mechanism of postovulatory wound repair, as well as the implications in the issues of fertility, sterility, and disease management. Understanding this dynamic molecular interplay before and after ovulation may offer new insights into fertility promotion, contraception, and

**Address for correspondence:* Dr. Tang-Yuan Chu,
Department of Obstetrics and Gynecology, Hualien Tzu Chi Hospital,
Buddhist Tzu Chi Medical Foundation, 707, Chung-Yang Road,
Hualien, Taiwan.
E-mail: hidrchu@gmail.com

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How to cite this article: Chen YH, Chu TY. Ovulation: A consequence of acute inflammation cultivated by E2-induced reactive oxygen species and triggered by progesterone withdrawal. Tzu Chi Med J 2025;37(4):351-9.

Access this article online	
Quick Response Code: 	Website: www.tcmjmed.com
	DOI: 10.4103/tcmj.tcmj_127_25

the prevention and management of diseases and sequelae related to ovulation.

THE ROLE OF LUTEINIZING HORMONE SURGE IN FOLLICULAR LUTEINIZATION AND STEROIDOGENESIS

The LH surge is a pivotal event in the ovulatory process, initiating the transformation of the ovarian follicle into the corpus luteum (CL) and modulating steroid hormone production. Prior to the LH surge, granulosa cells within the follicle are primarily responsible for estrogen (E2) synthesis. These cells convert androgens, produced by theca cells, into E2s through the enzyme aromatase. Theca cells, located in the outer layer of the follicle, synthesize androgens under the influence of LH, providing the necessary substrates for estrogen production. However, the LH surge induces profound changes in both cell types. Granulosa cells undergo luteinization, differentiate into luteal cells, and shift their steroidogenic output from E2 to progesterone. Concurrently, theca cells contribute to the formation of the CL and continue to supply androgens, which support ongoing steroidogenesis. This transition is essential for the establishment of the CL, which plays a critical role in maintaining early pregnancy through the secretion of progesterone [2-4].

BEFORE LUTEINIZING HORMONE SURGE, E2 INDUCES A REDOX BALANCE THROUGH REACTIVE OXYGEN SPECIES AND ANTIOXIDANT RESPONSES

Before the LH surge, estrogen (E2) primes the ovarian follicle for ovulation by establishing a delicate balance between oxidative signaling and antioxidant defense [5,6].

E2 activates NOX4 to induce reactive oxygen species production

E2 promotes the generation of mitochondrial ROS, particularly hydrogen peroxide (H_2O_2), which acts as a critical signaling molecule for ovulatory events such as cumulus expansion and steroidogenesis [7]. This ROS production is mediated by NADPH oxidase 4 (NOX4) and is triggered through anchorage- and integrin-dependent signaling, independent of classical estrogen receptor (ER) activation [5,8]. ROS accumulation is enhanced when antioxidant systems like catalase are inhibited [5,9], supporting its role as a regulated signaling mediator. However, excessive ROS can induce oxidative damage, necessitating tight control mechanisms.

E2 simultaneously enhances antioxidant defenses to maintain redox homeostasis

To counteract potential oxidative stress, E2 concurrently activates ER-dependent transcription of antioxidant enzymes, including catalase (CAT), extracellular superoxide dismutase, and manganese superoxide dismutase [10-15]. These enzymes function to neutralize superoxide and convert H_2O_2 into water, thus preserving cellular integrity. In breast cancer cells, E2 was shown to increase superoxide dismutase (SOD) 1 expression, preventing ROS-induced protein damage [15].

Moreover, E2 directly protects mitochondria, which express ERs and respond to E2 by upregulating antioxidant pathways [12,16,17].

Proteomic studies of human follicular fluid confirm the enrichment of antioxidant enzymes such as CAT, SOD1, SOD3, glutathione transferase, and protein disulfide isomerase [18-20]. These systems work in concert to regulate ROS levels and protect granulosa cells during follicular maturation. Thus, E2 establishes a physiological redox balance that supports both signaling and cellular protection in preparation for ovulation [Figure 1].

AFTER LUTEINIZING HORMONE SURGE, E2 CONTINUOUSLY BUILDS REACTIVE OXYGEN SPECIES TO PREPARE THE FOLLICLE FOR OVULATION

Following the LH surge, ROS production in granulosa cells intensifies and becomes essential for the structural and functional changes required for ovulation [7,21]. E2 rapidly increases intracellular ROS levels, predominantly H_2O_2 , through integrin-dependent mitochondrial signaling. These ROS promote cumulus expansion and oocyte release. Experimental studies show that antioxidants suppress LH-induced cumulus mucification and progesterone production, indicating the necessity of ROS for follicular remodeling [7]. Moreover, H_2O_2 can mimic LH activity by inducing cumulus-oocyte complex expansion and activating key ovulatory signaling pathways, including EGFR phosphorylation and the p42/44 MAPK cascade, which contributes to the proliferation, differentiation, and enzyme synthesis in the granulosa cells and is necessary for the resumption of meiosis in oocytes [7,22,23]. Notably, this ROS generation occurs independently of ER activation and is mediated through anchorage-dependent mitochondrial mechanisms [5].

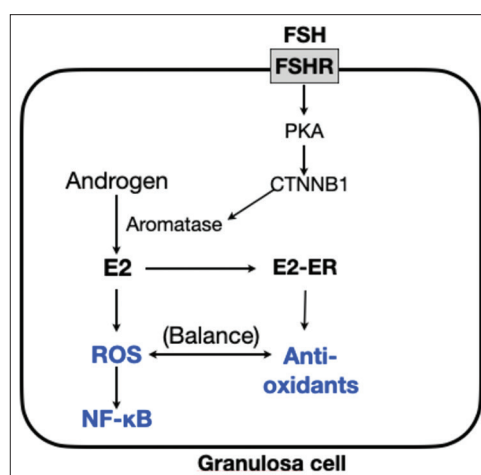


Figure 1: Before LH surge, Follicle-stimulating hormone (FSH) regulates E2 and antioxidants with a redox balance in granulosa cells. FSH binds to its receptor (FSHR) in granulosa cells, activating the protein kinase A signaling pathway, which subsequently regulates β -catenin (CTNNB1). CTNNB1 promotes the expression of aromatase, an enzyme responsible for converting androgens into E2. E2 directly promotes ROS production, leading to the activation of the NF- κ B signaling pathway. Concurrently, by binding to ER, E2 enhances antioxidant capacity, thus maintaining a balance between ROS and antioxidants to ensure cellular stability and function

THE ANTI-INFLAMMATORY ROLE OF PROGESTERONE AFTER LUTEINIZING HORMONE SURGE

The LH surge plays a critical role in inducing luteinization and progesterone (P4) synthesis within the ovarian follicle. On binding to its G protein-coupled receptors on granulosa and theca cells, LH activates intracellular signaling pathways that elevate cyclic AMP (cAMP) levels. This, in turn, stimulates protein kinase A (PKA), leading to the upregulation of key steroidogenic enzymes, including cholesterol side-chain cleavage enzyme (CYP11A1) and 3 β -hydroxysteroid dehydrogenase (3 β -HSD). These enzymatic changes drive the conversion of cholesterol into progesterone, marking the transition of theca and granulosa cells into luteal cells. The resulting increase in progesterone production is essential for preparing the endometrium for potential embryo implantation as well as the rupture of the follicle.

P4 serves a vital anti-inflammatory role by mitigating the oxidative stress induced by E2-ROS accumulation within the follicle. By modulating redox-sensitive signaling cascades, P4 establishes a transiently controlled inflammatory milieu that supports ovulation without tissue damage. This dual role of LH-induced progesterone production – supporting both reproductive readiness and follicular homeostasis – highlights the hormone's importance in ovarian physiology.

PROGESTERONE RECEPTOR-MEDIATED REPRESSION OF NUCLEAR FACTOR KAPPA B SIGNALING RESTORES POST-OVULATORY HOMEOSTASIS

Progesterone exerts its anti-inflammatory effect through the progesterone receptor (PGR), whose expression is upregulated by LH and E2/ER in mural granulosa cells. Activated PGR suppresses prostaglandin synthesis by downregulating prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX-2), and restores I κ B levels, thereby inhibiting NF- κ B activation [24-28]. This antagonizes E2/ROS-driven NF- κ B signaling, ensuring that ovulatory inflammation is limited to a brief and controlled duration, preventing excessive tissue damage [Figure 2].

This transient “pre-destructive inflammation control” mechanism is not unique to ovulation – it also occurs before menstruation and parturition, highlighting the P4/PGR axis as a universal gatekeeper that curtails inflammation before reproductive tissue breakdown [2,3,24-28].

FALLING OF PROGESTERONE

Studies in various animal models have demonstrated that progesterone levels exhibit a transient decline following the initial rise after the LH surge, reflecting the dynamic process of luteinization. In rabbit ovarian follicles, P4 levels rise within 1–3 h after human chorionic gonadotropin (hCG) administration, peaking around 3 h after mating and then decreasing [29,30]. In ruminants such as cattle and sheep, progesterone levels have been observed to fluctuate within 24–48 h postovulation. This transient decline is associated

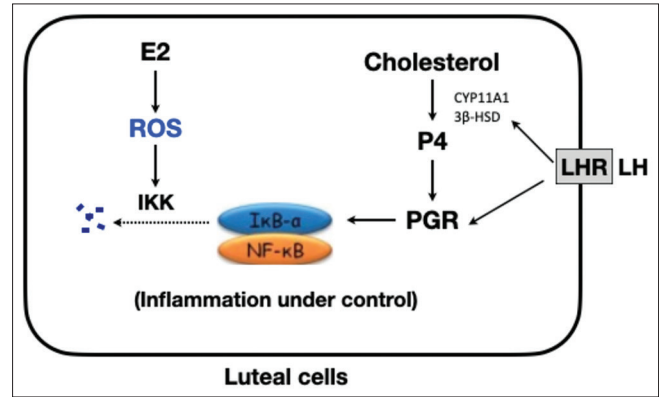


Figure 2: After the LH surge, E2-induced ROS activates the IKK/NF- κ B pathway by promoting I κ B degradation. Simultaneously, LH stimulates progesterone (P4) synthesis through the LHR-cAMP-PKA-CYP11A1/3 β -HSD axis. P4, via PGR activation, restores I κ B and suppresses NF- κ B signaling, thereby resolving inflammation and preserving luteal cell function. This E2-ROS-NF- κ B and P4-PGR-I κ B balance ensures a tightly regulated inflammatory transition during ovulation

with the incomplete vascularization and steroidogenic enzyme activity during the early stages of luteal formation [31,32]. In rodent models, similar patterns have been reported, where progesterone secretion initially rises in response to the LH surge but temporarily decreases as luteal cells undergo functional maturation [3]. *In vitro* studies using granulosa cell cultures further support this phenomenon, showing that progesterone production may fluctuate due to the gradual upregulation of steroidogenic enzymes (e.g., 3 β -HSD) and the dependence on sustained LH stimulation [33]. These findings highlight the transitional nature of luteinization, where the temporary decline in progesterone is a physiological response to the incomplete functional and structural maturation of the CL.

PROGESTERONE WITHDRAWAL TRIGGERS ACUTE INFLAMMATION AND SUBSEQUENT OVULATION

The temporary decline in P4 following its LH-induced rise removes its anti-inflammatory restraint on the preovulatory follicle, thereby initiating a localized, acute inflammatory response. Progesterone, acting via its nuclear receptor (PGR), typically suppresses oxidative and inflammatory stress by upregulating antioxidant enzymes such as SOD and glutathione peroxidase, and by stabilizing I κ B to inhibit NF- κ B activation [28,34]. Withdrawal of P4 relieves this inhibition, enabling E2-induced ROS to activate the IKK/NF- κ B pathway, which in turn promotes transcription of matrix metalloproteinases (MMPs), ADAMTS proteases, and COX-2 – all essential for follicular wall breakdown and oocyte release [Figure 3].

This controlled inflammatory switch is a recurring mechanism in female reproduction, governing the tissue breakdown required for ovulation, menstruation, and parturition. In each of these contexts, the P4/PGR axis ensures timely and spatially restricted inflammation, reinforcing its central role in reproductive homeostasis and success [28,35]. In summary, this PGR-mediated regulation limits ovulatory inflammation to a short, controlled period [36].

STRUCTURAL CHANGES LEADING TO FOLLICLE RUPTURE AND OVULATION

The remodeling of the ovarian follicle ECM is a critical process for both ovulation and the subsequent vascularization of the CL. Under the influence of follicle-stimulating hormone (FSH), preovulatory follicles undergo rapid growth, forming a fluid-filled antral cavity. These follicles then become highly responsive to the mid-cycle LH surge, which coordinates oocyte maturation, cumulus expansion, follicular rupture, and luteinization [37].

Remodeling of the extracellular matrix

At the LH surge peak, LH receptor activation on granulosa cells stimulates adenylyl cyclase, initiating the breakdown of the ECM across multiple follicular layers, including the theca interna, theca externa, and tunica albuginea. The degradation process is tightly regulated by MMPs and their inhibitors (TIMPs), produced by cumulus cells, oocytes, and surrounding stromal cells. These proteolytic activities are essential for weakening the follicular wall and enabling oocyte extrusion [38-41].

Cumulus mucification and follicle expansion

A key event in ovulation is the LH-induced mucification of the cumulus-oocyte complex (COC). In response to the LH surge, cumulus cells secrete large amounts of hyaluronic acid (HA), a glycosaminoglycan that forms a hydrated matrix around the oocyte. This process, known as cumulus expansion, is mediated by the upregulation of HA synthase 2 and the downregulation of HA-degrading enzymes. The accumulation of HA increases the hydrostatic pressure within the follicle, promoting rapid tissue remodeling and creating a pathway for the oocyte to exit the follicle. Cumulus expansion also ensures that the oocyte is surrounded by a protective matrix, which is crucial for its survival and fertilization [2,26].

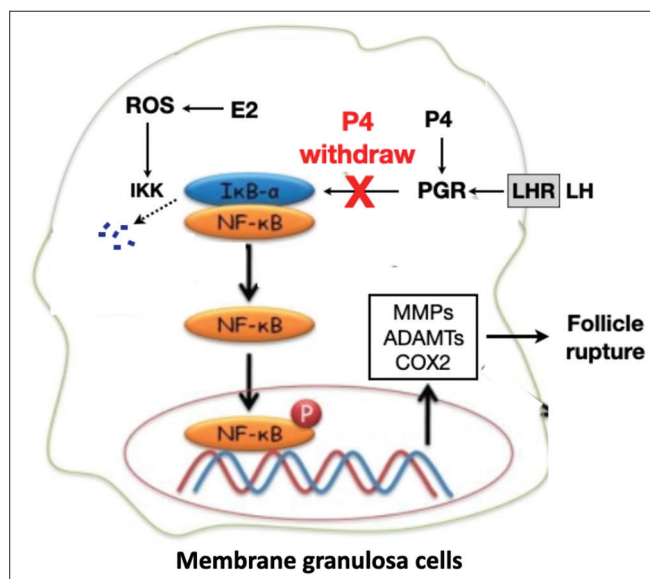


Figure 3: Mechanism of follicle rupture following LH surge, driven by transient progesterone withdrawal. In granulosa cells, E2-induced ROS activate the IκB kinase (IKK), promoting IκB degradation and NF-κB translocation. LH stimulates P4 synthesis, which normally suppresses NF-κB via PGR-mediated IκB stabilization. However, P4 withdrawal lifts this inhibition, resulting in the upregulation of MMPs, ADAMTs, and COX-2, facilitating follicular rupture

Decellularization at the apex of the follicle

Successful ovulation requires precise structural changes at the follicular apex, where a localized breach, known as the stigma, forms to release the oocyte. This process involves the breakdown of the ECM within the granulosa, theca, and ovarian surface epithelium (OSE) layers [Figure 4]. The LH surge upregulates proteases such as MMPs and ADAMTS-1, which degrade collagen and other ECM components, weakening the follicular wall [40,41]. Concurrently, apoptosis of the OSE, mediated by pro-apoptotic factors like Fas ligand and caspase-3, creates a focal point for rupture [42].

The stigma formation is further supported by localized inflammation and vascular changes. Leukocytes infiltrate the apical region, releasing additional proteases and ROS that enhance ECM degradation [43]. Increased hydrostatic pressure from peri-follicular vessel leakage, combined with enzymatic activity, culminates in follicular rupture [26]. These coordinated mechanisms ensure the precise and timely release of the oocyte during ovulation. Follicular rupture is initiated at the apex through coordinated ECM degradation and targeted cell death. The LH surge upregulates MMPs and ADAMTS-1, which disassemble collagen and proteoglycans at the granulosa, theca, and ovarian surface epithelium (OSE). Simultaneously, apoptosis of OSE cells – mediated by Fas ligand and caspase-3 – thins the apical region, forming a localized rupture site known as the stigma [40-42].

Leukocyte infiltration into this area amplifies inflammation by releasing cytokines, ROS, and proteolytic enzymes. This promotes further ECM breakdown and increases vascular permeability, which raises intrafollicular hydrostatic pressure. These combined processes culminate in the timely and site-specific rupture of the follicle, ensuring precise oocyte release [26,43].

Luteinization of the mural follicle

While decellularization facilitates the formation of the apical rupture site, the remaining mural granulosa and theca

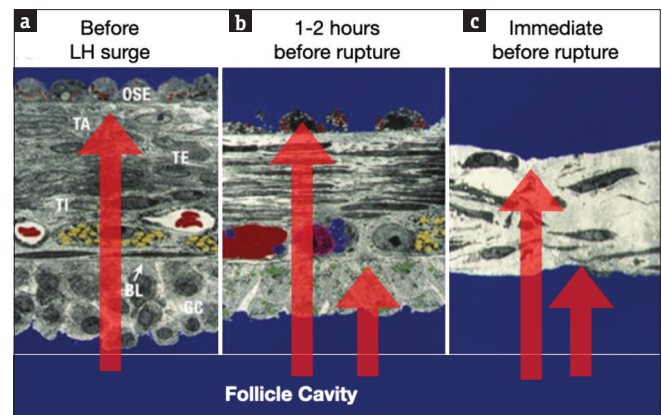


Figure 4: Follicle apex decellularization and stigma formation before rupture (Modified from [44]). Representative electron micrographs showing the progressive changes in the follicle wall structure leading up to ovulation. (a) Before the LH surge: the follicle wall remains intact, with the ovarian surface epithelium (OSE), tunica albuginea (TA), theca externa (TE), theca interna (TI), basement lamina (BL), and granulosa cells (GC) visible. (b) 1-2 h before rupture: wall thinning and OSE disruption begin. (c) Just before rupture: ECM fragmentation facilitates oocyte release. Red arrows indicate rupture direction

cells undergo luteinization to form the CL. Granulosa cells cease proliferating, enlarge (hypertrophy), and accumulate lipid droplets that supply cholesterol for steroid hormone synthesis. The disruption of the granulosa cell basal lamina, coupled with the production of prostaglandins and vascular growth factors by granulosa and theca cells, attracts new capillary growth into the previously avascular granulosa cell layer. Stromal vessels invade the granulosa layer, forming an extensive capillary network that ensures the delivery of nutrients and hormones to the developing CL [2,3].

PROTEASES INVOLVED IN FOLLICLE RUPTURE

Proteolytic enzymes are central to the controlled degradation of the ECM during ovulation. These enzymes coordinate with hormonal signals and local inflammatory cues to facilitate follicular rupture. Multiple classes of proteases – including MMPs, ADAMTS, and plasminogen activators – converge in a tightly regulated cascade.

MMP-2 and MT2-MMP in ECM degradation

MMPs, including collagenases, gelatinases, stromelysins, and membrane-type MMPs, play a critical role in degrading specific components of the ECM during follicle rupture. These enzymes are regulated by tissue inhibitors of metalloproteinases (TIMPs) and are present throughout follicular development, influencing follicular expansion and atresia [40,41]. In medaka, a small freshwater fish, MMP-2 (gelatinase A) hydrolyzes type IV collagen in the basement membrane between the granulosa and theca layers, whereas membrane-type 2 MMP (MT2-MMP and MMP15) degrades type I collagen in the theca cell layer. In addition, MT1-MMP and TIMP-2 regulate the activity of MMP-2, highlighting the complex interplay of proteases in follicular wall degradation [45].

ADAMTS-1 in ECM remodeling

ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin-like repeats-1) is a key metalloprotease regulated by LH and PGR signaling. It localizes to the ovulatory stigma and degrades proteoglycans such as versican and brevican, which are induced by the LH surge in granulosa and theca cells. ADAMTS-1 also activates epidermal growth factor-like peptides (EGFp), promoting cumulus expansion and follicular wall remodeling [41,43]. In ADAMTS-1 knockout mice, the invagination of the thecal layer and associated blood vessels does not occur, and versican – a key ECM component – fails to be cleaved. These defects highlight the essential role of ADAMTS-1 in remodeling follicular structure and enabling successful ovulation [43].

Urokinase-type plasminogen activator-1 (Plau1)/plasmin system

In species such as medaka (a small freshwater fish), follicular rupture involves a two-step proteolytic process: urokinase-type plasminogen activator-1 (Plau1) activates plasmin, which initiates ECM proteolysis and also activates MMPs. This cascade is tightly controlled by inhibitors including plasminogen activator inhibitor-1 and tissue inhibitor of metalloproteinase-2b, ensuring controlled and precise ECM degradation [45,46].

Source of lytic enzymes for follicle rupture

Leukocytes are a major source of proteases and other factors involved in ECM remodeling and angiogenesis during ovulation. Single-cell RNA sequencing has identified leukocyte-derived factors such as cytokines, MMPs, ADAMs, ADAMTSs, and TIMPs, which influence granulosa cell function and promote follicular rupture. For example, leukocyte-specific expression of CD68, IL1B, and MMP9 has been confirmed in human follicle tissues collected before and after hCG administration. These findings highlight the critical role of immune cells in regulating ovulation and luteinization [26,43]. Together, these protease systems create a temporally coordinated enzymatic environment that weakens the follicular wall, promotes cumulus dissociation, and facilitates oocyte release. Their spatial regulation by hormonal and immune inputs underscores the complexity – and potential therapeutic manipulability – of ovulatory tissue remodeling.

POST-OVULATORY REORGANIZATION AND TISSUE REPAIR

Ovulation concludes with follicular rupture, which is immediately followed by a tightly orchestrated process of tissue reorganization and wound healing. These events ensure not only the restoration of ovarian integrity but also the endocrine transition toward luteal function.

Luteinization: Formation of the Corpus Luteum (CL)

Following follicle rupture, the remaining follicular cells undergo a highly regulated process of tissue reorganization, marked by the activation of promatrix factors such as pro-MMPs and the differentiation of granulosa and theca cells into luteal cells. This transformation leads to the formation of the CL, a transient endocrine structure essential for progesterone production. Progesterone is critical for maintaining endometrial receptivity and supporting early pregnancy. The CL also regulates pituitary gonadotropin secretion through negative feedback, ensuring hormonal balance during the luteal phase [3,43]. If implantation does not occur, the CL undergoes luteolysis, a process characterized by the infiltration of immune cells, increased oxidative stress, and the downregulation of steroidogenic enzymes. This regression allows the ovarian cycle to reset, enabling the development of a new cohort of follicles [3,43].

Wound healing at the ovulatory rupture site

The healing of the ovulation wound resembles the process of ordinary wound healing, consisting of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling [47]. The ovulatory follicular fluid (FF) is rich in coagulation cascade proteins and inflammatory cytokines which facilitate hemostasis and the inflammatory phase of wound healing [48]. In addition, FF contains abundant growth factors, such as hepatocyte growth factor (HGF), insulin-like growth factor 2 (IGF2), and platelet-derived growth factor (PDGF), which promote re-epithelialization during the proliferation phase [47].

Research has identified LGR5⁺ epithelial stem cells distributed throughout the ovarian surface epithelium (OSE), which likely respond to stemness and growth-promoting

signals in FF to mediate postovulatory repair at the rupture site [49]. IGF2 has been demonstrated to promote stemness and clonal expansion (30852161), suggesting its importance in tissue regeneration. Following follicle rupture, thrombin activation converts the abundant HGF precursor into its active form [50], which may further stimulate re-epithelization at the wound site. Together, these stem cells and growth factors ensure efficient ovulation wound healing, maintaining ovarian integrity for subsequent reproductive cycles

CLINICAL IMPLICATIONS

Advances in the molecular mechanism of ovulation shown above have led to multiple clinical implications, ranging from fertility, sterility, postoperative adhesions, and cancer formation. In addition, recognition of the inflammatory and oxidative nature of follicular rupture has unveiled new challenges in contraceptives, ovulation induction, and cancer prevention.

Pharmacological control of ovulation

The detailed understanding of ovulation mechanisms has led to the development of pharmacological agents that either induce or suppress ovulation, depending on clinical needs. These agents target key pathways involved in follicular development, luteinization, and ovulation.

Ovulation Induction agents

Pharmacologic agents designed to induce ovulation are critical tools in the management of anovulatory infertility. Clomiphene citrate, a selective estrogen receptor modulator (SERM), acts by competitively inhibiting estrogen feedback at the hypothalamus and pituitary, thereby enhancing the release of FSH and LH to stimulate follicular growth. Alternatively, letrozole, an aromatase inhibitor, suppresses estrogen biosynthesis, resulting in a similar upregulation of endogenous gonadotropins that promotes follicular recruitment and maturation [51]. In addition, exogenous gonadotropins – typically recombinant FSH and LH – are administered directly to stimulate multifollicular development and trigger ovulation, often as part of assisted reproductive technology (ART) protocols [52]. These pharmacological strategies target distinct checkpoints within the hypothalamic–pituitary–ovarian axis to optimize folliculogenesis and oocyte release in women with ovulatory dysfunction.

Ovulation suppression agents

- (1) Combined oral contraceptives (COCs): COCs contain synthetic E2 and progestin. It is the progestin component in COCs that suppress ovulation by inhibiting the hypothalamic–pituitary–ovarian axis or P4 withdrawal. The former prevents follicular development and the LH surge, the latter prevents follicle rupture [53]
- (2) Morning-after pills: Progestins such as levonorgestrel and selective progesterone receptor modulators (SPRMs) such as ulipristal acetate are used to prevent pregnancy after unprotected intercourse. Progestins work primarily by avoiding progesterone withdrawal, thus delaying or inhibiting ovulation. Similarly, SPRMs block progesterone receptors, preventing progesterone effect after LH surge and follicular rupture [53]. These agents are most effective

when taken within 72 h (levonorgestrel) or 120 h (ulipristal acetate) after intercourse. The progesterone receptor antagonist (mifepristone or RU486) is even more effective by competitively binding to the progesterone receptors. When administered prior to ovulation, it delays or blocks the LH surge, preventing the follicular rupture. When taken in the peri-ovulatory or post-ovulatory phase, it inhibits the decidualization of the endometrium, thus reducing the receptivity of the embryo. Compared to other emergency contraceptives, such as levonorgestrel, mifepristone demonstrates a broader window of efficacy and a more potent suppression of ovulatory and endometrial processes, making it a valuable tool in reproductive medicine for post-coital pregnancy prevention

- (3) COX-2 inhibitors as an alternative emergent contraceptive and their fertility risk.

COX-2 enzymes play a critical role in ovulation by mediating prostaglandin synthesis, which is essential for follicular rupture. Studies have demonstrated that COX-2 inhibitors can serve as safe, nonhormonal emergency contraceptives, effectively delaying ovulation by up to 5 days [54-56]. However, their efficacy as regular oral contraceptives appears suboptimal. A clinical trial evaluating meloxicam (even at high doses of 30 mg daily) found that ovulation still occurred in over 20% of cycles, limiting its reliability for routine contraception [57].

Conversely, COX-2 inhibitors pose a significant fertility risk for women attempting to conceive. By disrupting prostaglandin-dependent ovulation, these drugs can cause delayed or failed follicular rupture, leading to luteinized unruptured follicle (LUF) syndrome – a condition in which the follicle fails to release the oocyte despite CL formation [58]. Given these effects, women planning pregnancy should avoid COX-2 inhibitors to prevent unintended ovulation suppression and maximize their chances of conception [59].

Dual role of follicular fluid in wound healing, adhesions, and cancer formation

Follicular fluid, the microenvironment surrounding the oocyte, contains a high level of ROS and a rich array of growth factors, cytokines, and coagulation proteins that play critical roles in ovulation and wound healing. Notably, repeated ovulatory cycles expose ovarian tissues to chronic oxidative stress, which has been implicated in DNA damage, genomic instability, and malignant transformation – key factors in the pathogenesis of ovarian cancer [60,61]. Epidemiological studies suggest that incessant ovulation may increase ovarian cancer risk [62,63], further supporting the hypothesis that ROS-mediated ovulatory mechanisms could contribute to tumor initiation. Recent studies have also revealed that follicular fluid can also contribute to postoperative intra-abdominal adhesions when it comes into contact with surgical wounds [47].

Follicular fluid, the microenvironment surrounding the oocyte, contains a high level of ROS and a rich array of growth factors, cytokines, and coagulation proteins that play critical roles in ovulation and wound healing. Notably, repeated ovulatory cycles expose the fimbrial epithelium

of the fallopian tube to follicular fluid containing ROS, a known mutagen [64,65], and growth factor transforming agents such as IGF2, HGF, and EGF-like peptides [50,66-70]. The oncogenic potential of these ovulatory factors has been extensively demonstrated *in vitro* and *in vivo* in mouse and human systems [64,71-73]. Large-scale epidemiological studies have indicated that incessant ovulation increases ovarian cancer risk [62]. Consequently, ovulation suppression is considered one of the most effective strategies for ovarian cancer prevention [62,74].

Promotion of ovulation wound healing

FF is rich in coagulation cascade proteins (e.g., fibrinogen and thrombin) and growth factors (e.g., HGF [50], insulin-like growth factor 2 [IGF2] [66], and PDGF [75], which facilitate the four phases of wound healing: hemostasis, inflammation, proliferation, and remodeling [49]. For example, HGF promotes tissue repair and regeneration, whereas IGF2 supports stem cell proliferation and re-epithelialization. These factors ensure efficient healing of the ovulation wound, restoring ovarian surface integrity and preparing the tissue for subsequent cycles.

Contribution to postoperative adhesions

While FF promotes the efficient healing of ovulation wounds, its spillage into the pelvic cavity during ovulation can have detrimental effects on surgical wounds. A 2024 study published in *iScience* [47] demonstrated that FF contains excessive coagulation and HGF signals, which, when exposed to surgical wounds, can lead to intra-abdominal adhesions. These adhesions are fibrous bands of scar tissue that form between abdominal tissues and organs, often causing chronic pain, bowel obstruction, and infertility. The study found that FF-induced activation of coagulation pathways and excessive HGF signaling promote fibroblast proliferation and ECM deposition, key processes in adhesion formation. This dual role of FF highlights its context-dependent effects: while beneficial for ovulation wound healing, it can be harmful when interacting with surgical wounds.

CONCLUSION

Ovulation is a hormonally regulated, inflammation-like process that represents a finely tuned interplay between estrogen-induced oxidative signaling and progesterone-mediated anti-inflammatory control. The LH surge initiates a cascade involving E2-driven ROS production, ECM remodeling, cumulus expansion, and transient P4 withdrawal, which collectively culminate in follicular rupture. Subsequent luteinization and ovulatory wound healing demonstrate the remarkable capacity of ovarian tissue for rapid regeneration, mediated by the coagulants, growth factors reside in the follicular fluid, and epithelial progenitor cells reside in the ovarian surface epithelium.

Beyond its fundamental biological role, the ovulatory process carries significant clinical implications for reproductive medicine and women's health. Our growing molecular understanding of ovulation has already yielded targeted treatments for both infertility and contraception. However, emerging research reveals potential risks

associated with chronic ovulatory cycles – specifically, the cumulative effects of repeated inflammatory responses and tissue remodeling. These cyclical processes may contribute to several pathological conditions, including ovarian carcinogenesis, endometriosis development, and postoperative adhesion formation.

Data availability statement

The current study did not generate or analyze any data.

Financial support and sponsorship

This work was supported by the National Health Research Institute, Taiwan (NHRI-EX112-11216BI), the National Science and Technology Council (NSTC 2314-B-303-002), and the Buddhist Tzu Chi Medical Foundation (TCMMP114-02-03).

Conflicts of interest

There are no conflicts of interest.

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