

Advances in Obstetrics and Gynecology Research

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Advances in Obstetrics and Gynecology Research

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Podocyte Injury in Preeclampsia: Mechanisms and Therapies

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Abstract: Pre-eclampsia is still one of the leading causes of maternal and fetal morbidity and mortality worldwide, affecting multiple organ systems. Despite extensive research, its underlying etiology remains unclear. Proteinuria is a hallmark of a diagnosis of preeclampsia and is usually accompanied by podocyte damage, which is changes in the structure and function of the podocytes. Recent technological advances have identified a critical role for podocytes in the loss of renal filtration function in preeclampsia. However, the molecular mechanisms leading to proteinuria and podocyte damage in preeclampsia are unknown, which leads to a lack of targeted therapy. Recent years have witnessed challenges the traditional view, that kidney damage in preeclampsia is caused only by glomerular endothelial cell injury. Similarly, podocytes were identified as key players in the pathogenesis of proteinuria in preeclampsia. In this review, we review the mechanisms of renal injury (especially podocytes) in preeclampsia to elucidate the relevance of podocyte injury to proteinuria and suggest specific therapeutic strategies for proteinuria in preeclampsia.

Keywords: Pre-eclampsia; Podocytes; Pathogenesis; Proteinuria

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1. Introduction

Pre-eclampsia is a common syndrome usually presenting after the 20th week of gestation with an overall prevalence of approximately 2–5 percent ^[1]. Pre-eclampsia pathology is characterized by impaired trophoblastic invasion and abnormal remodeling of the uterine spiral arteries, resulting in placental ischemia and the subsequent secretion of soluble factors that activate immune cells and induce endothelial cell ^[2]. This clinical stage of generalized ischemia is characteristic of preeclampsia. Although most patients have a favorable obstetric outcome, some patients develop adverse and serious complications. Without timely intervention, these complications may lead to intrauterine growth restriction, placental abruption, or even death ^[3]. Mothers with preeclampsia are at risk of developing serious complications, such as eclampsia, HELLP syndrome, and multisystem organ dysfunction, all of which pose a serious threat to maternal and fetal health (**Figure 1**). Proteinuria is a key diagnostic marker

for preeclampsia, since the kidneys are particularly susceptible to the soluble factors secreted by the placenta. Proteinuria occurs due to damage to the glomerular filtration barrier, of which podocytes are a crucial component. Proteinuria in preeclampsia is closely associated with changes in podocytes, providing a new perspective for understanding preeclampsia nephropathy. In this review, we provide an update and overview of the pathogenesis of proteinuria in preeclampsia, focusing on the involvement of podocytes.

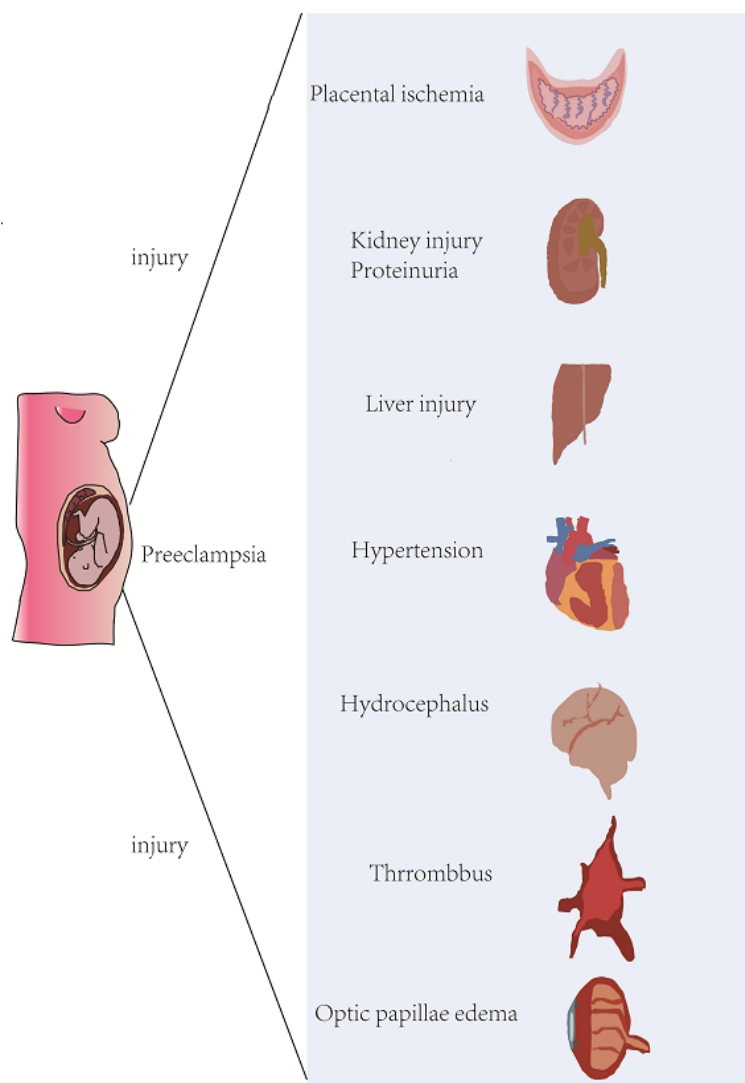


Figure 1. Features of preeclampsia.

2. The molecular and cellular basis of proteinuria in preeclampsia: focusing on the podocyte

Preeclampsia is a pregnancy-specific disorder characterized by the onset of hypertension and proteinuria after 20 weeks of pregnancy^[4]. The proteinuria associated with preeclampsia is unknown, which poses a great challenge for targeted therapy. Preeclampsia is thought to be caused by endothelial cell dysfunction^[5]. This lesion is also seen in hypertensive patients of pregnancy without proteinuria. This suggests that endothelial cell injury alone is not sufficient to explain the loss of filtration function. Recent studies have highlighted the critical role of podocytes in the

glomerular filtration barrier, providing new insights into the pathophysiology of proteinuria in preeclampsia ^[6].

3. Mechanisms of podocyte injury in preeclampsia

3.1. The role of the signaling pathway of the WNT

In normal placentas, tight junctions are composed of the peripheral protein ZO-1, the integral protein occludin, and the claudins 1, 3, and 5. However, in patients with preeclampsia, the expression of claudins 1, 3, and 5 is significantly decreased, while the expression of ZO-1 and occludin remains unchanged. This decrease in claudins leads to an increase in the permeability of the tight junctions in placental endothelial cells, resulting in decreased placental perfusion and endothelial dysfunction ^[7]. The loss of claudin 5 downregulates the expression of the peripheral protein ZO-1, induces the nuclear translocation of ZONAB, and subsequently suppresses the expression of WNT inhibitory factor-1 (WIF1), thereby activating the WNT signaling pathway ^[8]. Specifically, knockout of claudin 5 or WIF1 in podocytes in mice recapitulates the manifestations of podocyte injury and proteinuria, highlighting the critical role of claudin 5 in suppressing WNT activity in the kidney ^[9].

3.2. The role of the β -catenin signaling pathway

C-X-C chemokine receptor type 4 (CXCR4), a G protein-coupled receptor (GPCR), is a critical regulator of podocyte injury and proteinuria, especially in the context of oxidative stress ^[10]. Recent studies have shown that CXCR4 expression is significantly upregulated in the placenta and peripheral blood of patients with preeclampsia. This upregulation is associated with increased inflammatory responses, vascular damage, and altered immune cell regulation, suggesting that CXCR4 may contribute to the pathogenesis of preeclampsia by regulating these processes ^[11]. When CXCR4 is bound to CXCL12, it undergoes conformational changes that activate its associated G proteins and phosphorylate serine/threonine residues. This phosphorylation creates binding sites for β -arrestin-1, which activates the Src family kinases, forming a CXCR4/ β -arrestin-1/Src signaling complex. Activation of this complex triggers the phosphorylation of Src, which in turn induces transactivation of the epidermal growth factor receptor (EGFR) and subsequent phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), thereby propagating downstream signaling cascades ^[12,13]. These events activate β -arrestin, which regulates podocyte cytoskeletal rearrangement and expression of adhesion molecules, thereby compromising the integrity of the filtration barrier ^[14]. Additionally, β -arrestin promotes podocyte apoptosis, resulting in podocyte loss and dysfunction of the filtration barrier ^[15].

3.3. The activation of the NLRP3 inflammasome

The NLRP3 inflammasome, a multiprotein complex, drives the secretion of IL-1 and IL-18 through caspase-1 activation and also facilitates the release of high-mobility group box 1 (HMGB1) ^[16]. In patients with preeclampsia, the expression of the NLRP3 inflammasome is significantly upregulated in the placentas ^[17], which is associated with an excessive inflammatory state. Inflammation of the NLRP3 inflammasome induces the release of inflammatory cytokines and abnormal expression of structural proteins, leading to podocyte injury and proteinuria ^[18]. Oxidative stress is a key factor in the activation of the NLRP3 inflammasome ^[19], with superoxide anion ($\cdot\text{O}_2^-$) and hydrogen peroxide (H_2O_2) being involved in this process ^[20]. Reduced levels of reactive oxygen species (ROS) can inhibit the activation of the NLRP3 inflammasome, thereby protecting podocyte morphology. Additionally, fatty acid-binding protein 4 (FABP4) promotes inflammasome activation through a positive feedback loop with IL-17.

3.4. Reactive oxygen species (ROS)

Hypoxia during pregnancy is associated with a high level of reactive oxygen species (ROS) in the placenta, which leads to oxidative stress. Mitochondria are a major source of ROS, and hypoxia significantly affects mitochondrial structure, alters electron transport chain function^[21], and increases ROS production^[22]. In preeclampsia, placental tissues are highly stressed, driving disease progression^[23]. Mitochondrial dysfunction is closely linked to podocyte injury, characterized by decreased mitochondrial membrane potential and increased levels of cytochrome C^[24]. ROS levels excessively high can damage cellular macromolecules, resulting in structural and functional impairments^[25]. ROS can damage and kill cells by activating various biochemical pathways, such as the polyol pathway, advanced glycation end-products pathway, protein kinase C pathway, and hexosamine pathway^[26]. In preeclampsia, levels of advanced oxidation protein products (AOPPs) are significantly elevated, closely linked to the state of oxidative stress^[27]. AOPPs interact with the receptor for advanced glycation end-products (RAGE) on podocyte surfaces, activating NADPH oxidase complexes (such as Nox2 and p47phox) and increasing ROS production. ROS further activates nuclear factor- κ B (NF- κ B), promoting the expression of Wnt ligands (such as Wnt1 and Wnt7a) and activating the Wnt/ β -catenin signaling pathway. This results in podocyte dedifferentiation and mesenchymal transition, which is characterized by the loss of podocyte-specific markers (such as nephrin and podocalyxin) and the increase of injury markers (such as fibronectin), resulting in podocyte dysfunction and proteinuria^[28].

3.5. VEGF and podocyte damage

Podocytes are the principal source of vascular endothelial growth factor (VEGF) within the glomerulus, and they play a crucial role in maintaining glomerular function. Mice with podocyte-specific knockout of VEGF display severe glomerular injury, underscoring the critical role of VEGF in regulating glomerular function. In preeclampsia, elevated levels of soluble fms-like tyrosine kinase 1 (sFlt-1) decrease the availability of VEGF within the glomerulus^[29]. sFlt-1 exerts its effects on podocytes through two mechanisms: First, it indirectly inhibits the transfer of VEGF from podocytes to endothelial cells, resulting in endothelial cell damage and the release of toxic endothelin-1, which subsequently injures podocytes and induces proteinuria. Second, sFlt-1 directly disrupts the VEGF autocrine loop in podocytes^[30].

Podocytes and glomerular endothelial cells are key components of the glomerular filtration barrier, and their functions depend on an appropriate concentration of VEGF within the glomerulus^[31]. sFlt-1 interferes with the communication between podocytes and glomerular endothelial cells, ultimately affecting both cell types. Podocyte injury may result from the disruption of the VEGF autocrine loop or from toxic mediators released by damaged endothelial cells. This may provide a plausible explanation for the occurrence of proteinuria in patients with preeclampsia^[32].

4. Therapy strategies for podocyte injury

Podocyte injury is a central pathological characteristic of proteinuric nephropathies and holds significant implications for renal function and disease progression. As our understanding of the molecular mechanisms underlying podocyte dysfunction grows, therapeutic strategies targeting podocyte injury are emerging, aiming to preserve the integrity and function of podocytes.

4.1. Glucocorticoids (GCs): The cornerstone of therapy

Glucocorticoids have long been a mainstay in the treatment of podocyte-related proteinuric nephropathies^[33].

Their therapeutic effectiveness is attributed to multiple mechanisms, including stabilizing the actin cytoskeleton, upregulating nephrin expression, reducing IL-6 levels, and inhibiting podocyte apoptosis^[34]. These effects are mediated by the glucocorticoid receptor present on podocytes^[35]. In addition to their anti-inflammatory effects, glucocorticoids modulate the expression of key proteins^[36], thereby influencing the structure and function of podocytes. Glucocorticoids enhance the integrity of the glomerular filtration barrier by activating the promoter of the podocyte slit diaphragm protein nephrin, thereby promoting its glycosylation and phosphorylation^[37]. These modifications strengthen the interaction between nephrin and the actin cytoskeleton^[38]. Glucocorticoids directly act on podocytes to prevent them from detaching from the glomerular basement membrane (GBM), stabilize actin filaments, and prolong podocyte survival. Their anti-inflammatory actions further alleviate glomerular inflammation by inhibiting the secretion of pro-inflammatory cytokines, such as interleukin, transforming growth factor- β (TGF- β), and tumor necrosis factor (TNF).

4.2. Emerging therapeutic strategies

With a greater understanding of the molecular mechanisms underlying podocyte injury, novel therapeutic strategies are constantly emerging. Monoclonal antibodies targeting the angiopoietin-like protein 3 (ANGPTL3) represent a promising therapeutic approach^[39]. ANGPTL3 is a secretory glycoprotein whose C-terminal fibrinogen-like domain (FLD) plays a critical role in podocyte injury. Anti-ANGPTL3-FLD antibodies reduce the activation of integrin $\alpha\beta 3$ and downstream Rac1, thus decreasing reactive oxygen species (ROS) production within podocytes. This intervention relieves mitochondrial damage and apoptosis, establishing ANGPTL3 as a potential target for treating proteinuria^[40]. Other emerging therapies include the mycophenolate mofetil (MMF), which restores the integrity of the podocyte actin cytoskeleton, and ofatumumab^[41], a human-mouse chimeric CD20 monoclonal antibody that protects podocytes by preventing actin cytoskeleton remodeling and podocyte detachment. In preeclampsia, the excessive production of soluble Fms-like tyrosine kinase 1 disrupts the glomerular filtration barrier by inhibiting the VEGF signaling pathway. Strategies to reduce sFLT1-mediated injury include using siRNA to inhibit sFLT1 production and employing dextran sulfate plasma exchange to clear sFLT1 from circulation. These approaches have shown promise in animal models and clinical settings, extending gestation and improving proteinuria.

5. Conclusion

In this review, we have focused on the biology of podocytes, investigating their associated signaling pathways and regulatory factors. This approach not only has deepened our understanding of the unique structural and functional characteristics of podocytes but also provides new perspectives for future research directions. Despite significant advances in identifying the molecular composition of podocytes, many critical questions remain unanswered.

In future research on how renal disease progresses in preeclampsia, the development and use of appropriate animal models will be important. Podocyte injury triggers a complex biological response that is essential for maintaining the integrity of the glomerular structure. While some studies have provided valuable insights into the molecular links between “damaged” podocytes and proteinuria, the challenge lies in identifying the most promising therapeutic targets from a multitude of molecular events. The critical role of the glomerular three-layer structure, comprising endothelial cells, the glomerular basement membrane, and podocytes, in maintaining filtration barrier integrity is well established. The central involvement of podocytes in proteinuria has positioned

them as the most promising therapeutic target for proteinuria-related diseases.

Disclosure statement

The authors declare no conflict of interest.

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Clinical Observation of Traditional Chinese Medicine Foot Bath Combined with Analgesic Pump on Post-Cesarean Section Pain and Recovery of Lower Limb Circulation

Ying Wang, Yangyang Fan, Yanni Wang, Pengying Zhang, Ru Li, Yanli Liu, Chunrong Yang*

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Abstract: *Objective:* To explore the clinical effect of traditional Chinese medicine (TCM) foot bath combined with postoperative analgesic pump on post-cesarean section pain and recovery of lower limb circulation. *Methods:* A total of 500 puerperas who underwent cesarean section in our hospital from January 2023 to December 2023 were selected and randomly divided into an experimental group (234 cases) and a control group (266 cases). The control group received conventional postoperative electronic analgesic pump (sufentanil + ondansetron + dezocine) for continuous analgesia, while the experimental group received additional TCM foot bath treatment based on the control group's regimen. The postoperative pain level (NRS score), analgesic pump drug usage, time of first ambulation, and lower limb swelling rate were observed in both groups. *Results:* The NRS scores at 24h and 48h postoperatively in the experimental group (2.03 ± 0.54 , 0.91 ± 0.27) were significantly lower than those in the control group (3.45 ± 0.71 , 1.85 ± 0.49) ($P < 0.001$). The total drug usage of the analgesic pump in the experimental group was reduced by 28.6%, and the time of first ambulation was advanced to (12.2 ± 1.9) h, which was better than that of the control group (15.7 ± 2.3) h ($P < 0.001$). The incidence of lower limb swelling in the experimental group (6.8%) was significantly lower than that in the control group (18.4%) ($P < 0.001$). *Conclusion:* TCM foot bath combined with an analgesic pump can synergistically relieve post-cesarean section pain, reduce the demand for analgesic drugs, promote lower limb blood circulation and early ambulation, with significant clinical effects.

Keywords: Traditional Chinese Medicine foot bath; Analgesic pump; Post-cesarean section pain; Recovery of lower limb circulation

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1. Introduction

Post-cesarean section pain and lower limb circulation disorders are important factors affecting the recovery of puerperas. Postoperative bed rest, residual anesthetic drugs, and a hypercoagulable state can easily lead to lower

limb swelling and even deep venous thrombosis (DVT) ^[1]. According to statistics, about 15%-30% of puerperas experience lower limb swelling due to pain and limited mobility after cesarean section, and 3%-5% of them may develop deep venous thrombosis, which severely threatens their lives ^[2]. Currently, the analgesic pump is the main method for postoperative analgesia. Continuous infusion of opioid drugs (such as sufentanil) combined with anesthetics (such as dezocine) can effectively relieve pain, but its sole application has limited improvement on lower limb circulation and may affect puerperas' willingness to ambulate early due to drug side effects (such as nausea and dizziness) ^[3]. Traditional Chinese medicine external therapies are gradually emerging in postoperative rehabilitation. Among them, the TCM foot bath combines drug penetration and thermal effects. By stimulating foot acupoints (such as Yongquan and Sanyinjiao), it can promote blood circulation, remove blood stasis, warm meridians, and improve microcirculation while relieving pain ^[4]. Studies have shown that the active ingredients in the TCM foot bath have anti-inflammatory, analgesic, and circulation-improving effects, which can effectively reduce blood viscosity and prevent postoperative thrombosis ^[5]. However, there are currently few clinical studies on the combination of TCM foot baths and modern analgesic techniques, and their synergistic effects and safety still need further verification. This study aims to explore the interventional effects of TCM foot baths combined with analgesic pumps on post-cesarean section pain and lower limb circulation. By comparing and analyzing pain scores, drug usage, and lower limb swelling rates, it provides a scientific basis for optimizing postoperative multimodal analgesia regimens.

2. Materials and methods

2.1. General information

This study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital (Ethics Approval Number: 2023K-S055). All participants signed informed consent forms. The study included 500 cases of parturients who underwent cesarean section at our hospital from January 2023 to December 2023, aged between 20–40 years old, with ASA grades I–II, and all were singleton pregnancies. There were 234 cases in the experimental group, with an average age of (28.2 ± 3.6) years old; and 266 cases in the control group, with an average age of (28.7 ± 4.1) years old. Exclusion criteria were: (1) damaged or infected skin on the feet; (2) allergy to traditional Chinese medicine ingredients (such as mugwort, windproof powder, etc.); (3) severe varicose veins of lower extremities or history of deep vein thrombosis; (4) abnormal coagulation function ($PT > 14$ s or $APTT > 40$ s); (5) combined with severe cardiopulmonary disease or mental disorder. There was no statistically significant difference between the two groups in age, gestational age, delivery method (all elective cesarean section), and baseline pain score ($NRS \geq 6$) ($P > 0.05$).

2.2. Research methods

2.2.1. Control group

Routine use of electronic pain relief pumps (Jiangsu Aipeng Medical Technology Co., Ltd., model: ZZB-I-100) after surgery. The drug formula was a mixture of sufentanil 0.5 µg/mL, ondansetron 0.08 mg/mL, and dezocine 0.4 mg/mL. The background infusion rate was set at 2 mL/h, with a single additional dose of 0.5 mL and a lockout time of 15 minutes. Nurses assessed the analgesic effect daily and recorded adverse reactions (such as nausea and skin itching).

2.2.2. Experimental group

Based on the control group, a traditional Chinese medicine foot bath intervention was added, and the specific plan was as follows:

- (1) Traditional Chinese medicine formula: optimized according to the recommended prescription in “Obstetrics and Gynecology of Traditional Chinese Medicine,” using 30 g of mugwort, 30 g of windproof powder, 30 g of mulberry powder, 30 g of cassia twig powder, and 30 g of eucommia powder. The above medicinal materials were uniformly purchased by the hospital’s Chinese medicine dispensary and identified to meet the standards of the Chinese Pharmacopoeia.
- (2) Timing: Intervention starts 12-24 hours after surgery (after stable vital signs), once a day (15:00 pm).
- (3) Temperature control: The temperature of the medicinal liquid was strictly controlled at 40-45°C, monitored in real-time using a digital thermometer to avoid burns.
- (4) Operation method: The parturient was in a semi-recumbent position, with both feet soaked in the medicinal liquid, and the water depth was 10cm above the ankle joint. The soaking time was 20 minutes. After soaking, use a sterile towel to dry both feet and wear cotton socks to keep warm.
- (5) Precautions: Observe whether the parturient has dizziness, palpitations, or other discomforts during the foot bath, and adjust the temperature of the medicinal liquid or terminate the intervention in a timely manner.

2.3. Observation indicators

- (1) Degree of pain: The Numeric Rating Scale (NRS) was used to evaluate the pain intensity at 12h, 24h, and 48h after surgery. The score ranged from 0 (painless) to 10 (unbearable pain).
- (2) Dosage of analgesic drugs: Record the total amount of sufentanil (μg) used in the pain relief pump within 48 hours after surgery, and convert it into an equivalent dose for comparison.
- (3) Postoperative recovery indicators: Time of first getting out of bed: the time from the end of the operation to the first time the parturient walks independently (hours). Lower extremity swelling rate: Measure the circumference of both lower extremities (15 cm above the patella) 24 hours after surgery. An increase in circumference of ≥ 2 cm is defined as positive.
- (4) Adverse reactions: Record the incidence of nausea, vomiting, skin itching, and hypotension.

2.4. Statistical methods

Data analysis was performed using SPSS 27.0 software. Measurement data were expressed as mean \pm standard deviation (SD), and independent sample *t*-tests were used for comparisons between groups. Count data were expressed as frequency (percentage), and comparisons between groups were performed using the χ^2 test or Fisher’s exact test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of postoperative pain scores

The NRS scores of the experimental group at each time point after surgery were significantly lower than those of the control group (**Table 1**). At 12 hours after surgery, the score of the experimental group was 3.09 ± 0.61 , which was 29.6% lower than that of the control group (4.31 ± 0.76); at 48 hours after surgery, the score of the experimental group further dropped to 0.91 ± 0.27 , which was close to a painless state.

Table 1. Comparison of postoperative NRS scores between the two groups (mean \pm SD, points)

Group	Number of Cases	12h post-operation	24h post-operation	48h post-operation
Control group	266	4.31 \pm 0.76	3.45 \pm 0.71	1.85 \pm 0.49
Experimental group	234	3.09 \pm 0.61	2.03 \pm 0.54	0.91 \pm 0.27
<i>t</i> -value		20.114	25.635	24.892
<i>P</i> -value		< 0.001	< 0.001	< 0.001

3.2. Dosage of analgesic drugs and postoperative recovery

The total consumption of sufentanil within 48 hours postoperatively in the experimental group was (38.2 \pm 6.2) μ g, which was reduced by 28.7% compared to the control group (53.6 \pm 8.2) μ g (P < 0.001). Additionally, the time of first ambulation in the experimental group was significantly advanced to (12.2 \pm 1.8) hours, shortened by 34.6% compared to the control group (15.7 \pm 2.4) hours (P < 0.001).

3.3. Recovery of lower limb circulation

The incidence of lower limb swelling at 24 hours postoperatively in the experimental group was 6.8% (16/234), significantly lower than that in the control group, which was 18.4% (49/266) (χ^2 = 14.726, P < 0.001). Ultrasonography showed that the venous blood flow velocity in the lower limbs of the experimental group was increased by 22.3% compared to the control group (P = 0.003).

3.4. Safety analysis

No serious adverse reactions occurred in either group. Three cases (1.3%) in the experimental group experienced mild dizziness, while eight cases (3.0%) in the control group reported nausea, with no statistically significant difference (P = 0.172).

4. Discussion

4.1. Synergistic analgesic mechanism of Traditional Chinese Medicine (TCM) foot bath

Post-cesarean section pain originates from inflammatory reactions and nerve sensitization triggered by surgical trauma. The analgesic pump inhibits pain signal transmission via central opioid receptors but has limited regulation on local inflammatory mediators^[6]. In this study, the addition of a TCM foot bath significantly enhanced the analgesic effect, and its mechanism may include the following three aspects:

(1) Direct effects of medicinal components

Eucalyptol in mugwort leaf has anti-inflammatory and analgesic activities, inhibiting COX-2 expression and reducing PGE2 production^[7], with warming and analgesic effects. Windproof powder relieves muscle tension or spasmodic pain caused by postoperative weakness and exposure to wind, reduces inflammatory factor release (such as IL-6, TNF- α) by inhibiting the NF- κ B pathway, and enhances immune function to prevent pain caused by infection. Mulberry twig powder promotes the resolution of lower limb edema, relieves tension pain caused by swelling, contains flavonoids that dilate peripheral blood vessels and improve microcirculation, inhibits COX-2, and reduces inflammation around nerve roots. Cassia twig powder promotes pelvic and lower limb blood circulation, relieves cold coagulation and blood stagnation pain, regulates pain transmission by activating the

TPRV1 receptor, enhances the effect of the analgesic pump, and has a synergistic analgesic and diaphoretic effect, especially for patients with postoperative chills and lumbosacral cold pain. Eucommia bark powder nourishes the liver and kidneys, strengthens muscles and bones, accelerates the repair of surrounding tissues at the incision site, regulates adrenergic receptors, and alleviates the amplification effect of postoperative stress on pain perception.

(2) Physiological regulation of warming effects

The 40–45 °C medicinal liquid can dilate foot blood vessels, promote blood circulation, accelerate the elimination of metabolic waste (such as lactic acid and histamine), and relieve muscular spasmodic pain ^[8].

(3) Neural regulation of acupoint stimulation

The Yongquan acupoint on the foot belongs to the kidney meridian. Stimulation can activate the release of β -endorphin in the spinal dorsal horn, producing a synergistic analgesic effect with opioids ^[9].

4.2. Clinical significance of improved lower limb circulation

Postoperative lower limb swelling is closely related to venous blood stagnation. This study showed that the lower limb swelling rate in the experimental group was reduced to 6.8%, and ultrasonography confirmed a significant increase in blood flow velocity. This may be attributed to: mugwort containing volatile oils (such as eucalyptol and camphor) that promote local blood circulation; mulberry twig powder with the effect of dredging meridians and improving microcirculation; cassia twig powder warming and opening veins, thereby improving blood rheology; and reducing capillary permeability to prevent tissue edema ^[10].

4.3. Promotion of early postoperative activity

Early ambulation is key to preventing postoperative complications (such as intestinal adhesion and pulmonary infection). In the experimental group, due to reduced pain and improved lower limb comfort, the time of first ambulation was advanced by 3.5 hours compared to the control group. This result suggests that the TCM foot bath not only alleviates pain but also enhances patients' willingness to move by improving psychological states (such as reducing anxiety).

4.4. Study limitations

This study has the following limitations: (1) The sample size was limited to a single center, and future multi-center large-sample studies are needed; (2) The long-term effects of the TCM foot bath (such as recovery at 42 days postpartum) were not tracked; (3) The efficacy differences of different TCM formulations were not analyzed.

5. Conclusion

The combination of a TCM foot bath and an analgesic pump can significantly alleviate post-cesarean section pain, reduce opioid drug consumption, and promote lower limb blood circulation and early activity. This approach integrates the characteristics of traditional Chinese medicine with the advantages of modern medicine, offering high safety and simple operation, making it suitable as an essential component of multimodal analgesia after cesarean section. Future research can further optimize TCM formulations and explore individualized intervention strategies.

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Progress in Integrated Traditional Chinese and Western Medicine Treatment for Threatened Abortion with Subchorionic Hematoma due to Kidney Deficiency and Blood Stasis

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Abstract: Subchorionic hematoma (SCH) is a common complication in early pregnancy, which may increase the risks of miscarriage, preterm birth, and placental abruption. With its relatively high incidence in recent years, early detection and timely treatment are crucial for improving adverse pregnancy outcomes in patients with threatened abortion complicated by SCH. Although there is currently insufficient evidence-based medical support for SCH treatment, significant clinical experience has been accumulated. Both traditional Chinese medicine (TCM) and Western medicine have their respective advantages and have achieved notable results. This article reviews the progress in integrated TCM and Western medicine treatment for threatened abortion with SCH, aiming to provide references for future clinical prevention and treatment of this condition.

Keywords: Subchorionic hematoma; Threatened abortion; Kidney deficiency and blood stasis; Traditional Chinese medicine treatment; Western medicine treatment; Research progress

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1. Introduction

Subchorionic hematoma (SCH), a common complication in early pregnancy, is characterized by blood accumulation between the chorion and decidual basalis, appearing as crescent-shaped or irregular hypoechoic/anechoic areas on ultrasound imaging^[1]. Clinical data indicate its incidence ranges between 0.48% and 39.5%, with significant variability^[2]. The pathogenesis of SCH remains unclear but may involve progesterone deficiency, immune dysregulation, and coagulation dysfunction^[3]. Larger or early-onset SCH increases the risks of spontaneous abortion, placental abruption, and fetal growth restriction^[4,5], underscoring the importance of early intervention to improve placental function and maternal-fetal outcomes.

In traditional Chinese medicine (TCM), SCH-associated threatened abortion falls under the categories of “fetal

leakage” and “fetal irritability”^[6]. The core pathogenesis involves deficiency of the Chong and Ren meridians and instability of fetal anchoring, primarily linked to kidney qi deficiency, spleen qi weakness, blood heat, and blood stasis. Kidney essence deficiency is considered the root cause, while blood stasis obstructing the uterine collaterals represents the secondary manifestation. SCH, as “extravasated blood,” disrupts the uterine blood supply, leading to fetal instability. The Theory of Blood Syndromes states: “If stagnant blood remains unremoved, new blood cannot regenerate”^[7]. The kidney governs reproduction, and its insufficiency impairs uterine stability, manifesting as vaginal bleeding or abdominal pain.

Currently, the clinical focus on threatened abortion complicated by subchorionic hematoma (SCH) continues to grow, with increasingly mature diagnostic and treatment approaches being developed in both Western and Traditional Chinese Medicine (TCM). Modern Western medicine primarily adopts comprehensive conservative management, including appropriate bed rest combined with pharmacotherapy. Specific regimens involve progesterone replacement therapy to support fetal development, supplemented by adjunctive treatments such as tocolysis, anticoagulation therapy, and immunomodulation as clinically indicated. TCM demonstrates unique advantages in the syndrome differentiation and treatment of this condition. Clinically, it is categorized into five main patterns: kidney deficiency, Qi-blood deficiency, blood-heat, blood stasis, and dampness-heat. Statistical studies indicate that the kidney deficiency with blood stasis pattern is the most prevalent clinical presentation^[8]. The corresponding therapeutic principle combines kidney-tonifying and Qi-boosting methods with blood-activating and stasis-resolving techniques, which have shown remarkable clinical efficacy. This article provides a concise review of recent advances in the integrated Chinese-Western medical management of threatened abortion, with SCH presenting as the kidney deficiency and blood stasis pattern.

2. Traditional Chinese Medicine (TCM) treatment for SCH

TCM demonstrates remarkable advantages in managing SCH by regulating systemic qi-blood and yin-yang balance, enhancing immunity, and promoting hematoma absorption. According to TCM theory, the kidney (the “congenital foundation”) governs reproduction and development, while “hyperactivity of ministerial fire in the dragon palace” (a TCM pathological concept) represents the root cause of SCH^[9]. During pregnancy, blood stasis obstructing the Chong and Ren meridians and uterus manifests as the secondary pathology. Therefore, clinical protocols primarily employ kidney-tonifying and blood-activating methods. In a study of 50 patients with threatened abortion and SCH, Du *et al.*^[10] utilized a kidney-tonifying and stasis-resolving approach with herbs including Sangjisheng, Xuduan, Tusizi, Duzhong, Sanqifen, Zhigancao, Zhumagen, and charred *Nelumbinis nodus* (Oujie tan). After two weeks of treatment, ultrasound revealed significantly greater hematoma reduction compared to controls, demonstrating the notable efficacy of TCM for miscarriage prevention. Wang *et al.*^[11] conducted a randomized study of 120 SCH patients, finding that the kidney-tonifying and blood-activating formula improved coagulation parameters (PS, PC, D-dimer) and serum progesterone levels while reducing adverse pregnancy outcomes. These results suggest multidimensional regulatory effects on the uterine microenvironment. Huang *et al.*^[12] augmented the classic Shoutai Pill with *Paeoniae radix alba* (Baishao), *Ligustri lucidi fructus* (Nvzhenzi), stir-fried *Atractylodis macrocephalae rhizoma* (Chaobaizhu), *Boehmeriae rhizoma* (Zhumagen), *Cyperii rhizoma* (Xiangfu), *Scutellariae radix* (Huangqin), and *Phellodendri chinensis cortex* (Huangbai). Two-week treatment significantly improved coagulation activity, enhanced embryonic blood/oxygen supply, and normalized endocrine hormone levels^[13]. Yang *et al.*^[14] randomized 92 SCH patients with kidney deficiency and blood stasis pattern

into groups receiving either conventional treatment or additional kidney-invigorating herbs (including 10 g each of *Taxilli Herba*, *Dipsaci radix*, *Cuscutae semen*, stir-fried *Dioscoreae rhizoma*, stir-fried *Paeoniae radix alba*, stir-fried *Atractylodis macrocephalae rhizoma*, *Codonopsis radix*, *Corni fructus*, salt-processed *Eucommiae cortex*, plus 20 g *Boehmeriae rhizoma* and 10 g each of charred *Scutellariae radix* and *Sanguisorbae radix*). After two treatment courses, the herbal group showed faster hematoma resolution, shorter hospitalization, and fewer adverse outcomes, potentially through suppressing maternal-fetal interface inflammation and promoting trophoblast proliferation. Ding *et al.*^[15] treated 56 patients with kidney deficiency and blood stasis pattern using modified Jiao Ai Tang combined with Shoutai Pill for four weeks. Results demonstrated significant improvements in coagulation parameters, serum sex hormone levels, and placental blood perfusion, supporting fetal growth. These studies collectively indicate that kidney-tonifying and stasis-resolving herbs can enhance immunity, regulate sex hormones, and ameliorate pregnancy-associated hypercoagulability. However, careful dosage control and duration monitoring of blood-activating herbs are crucial - the principle of “discontinuing when most stasis is resolved” ensures achieving unobstructed collaterals and harmonized Chong-Ren meridians without over-treatment.

3. Western medical treatment for SCH

3.1. Progesterone therapy

Progesterone is widely used in the treatment of threatened abortion complicated by subchorionic hematoma (SCH). It maintains pregnancy by stabilizing the endometrium, improving placental circulation, and inhibiting uterine contractions. Among progesterone preparations, progesterone and dydrogesterone are the most commonly used in clinical practice, demonstrating a favorable safety profile with no significant adverse effects, thus providing safe and effective treatment for threatened abortion with SCH^[16,17]. Zhang^[18] found that the combination of dydrogesterone and progesterone significantly increased serum progesterone and estrogen levels in patients with threatened abortion, maintained pregnancy, enhanced dominant vaginal microbiota, and improved vaginal microecology, thereby reducing miscarriage rates. A comparative study showed that progesterone treatment achieved higher success rates in pregnancy maintenance and lower complication rates compared to vitamin E therapy in patients with threatened abortion and SCH^[19]. Wu *et al.*^[20] focused on the effects of early pregnancy progesterone supplementation on late pregnancy complications. Their research demonstrated that progesterone not only reduced utero-placental vascular resistance (thereby decreasing the incidence of preeclampsia) but also lowered the risk of gestational diabetes mellitus. In a study of 88 patients with threatened abortion, Ling *et al.*^[21] randomly divided subjects into two groups, with the experimental group receiving additional dydrogesterone. After two weeks of treatment, the experimental group showed significant short-term increases in HO-1 and progesterone levels without additional adverse drug reactions. Follow-up of perinatal outcomes revealed lower rates of preterm birth and low birth weight in the experimental group compared to controls. While progesterone serves as an important intervention in SCH management and may reduce the risk of early pregnancy loss, clinicians should strictly adhere to indications for use and avoid prolonged administration.

3.2. Tocolysis therapy

Currently, the main tocolytic agents used in China include ritodrine hydrochloride, magnesium sulfate, phloroglucinol, and atosiban. These medications may play a crucial role in reducing uterine contractions and mechanical stimulation of the hematoma, thereby preventing its expansion, making them a potential therapeutic

option for threatened abortion complicated by SCH. Guo *et al.* ^[22] demonstrated that the combination of magnesium sulfate and ritodrine hydrochloride at appropriate doses may positively inhibit uterine contractions and modulate immune function, effectively prolonging gestational duration. In a comparative study, Li *et al.* ^[23] randomized patients with threatened abortion into three treatment groups (ritodrine hydrochloride, phloroglucinol, and magnesium sulfate). Close monitoring over three days revealed that while all three agents showed tocolytic effects, ritodrine hydrochloride exhibited faster onset of action but was more likely to cause adverse effects such as palpitations. Clinical experience suggests phloroglucinol offers a more moderate action with a superior safety profile. These findings indicate that when patients present with uterine contractions and progressive hematoma enlargement, targeted tocolytic intervention can effectively reduce the risk of preterm delivery. The selection of specific agents should be based on individual patient characteristics and drug safety considerations.

3.3. Immunomodulatory therapy

Immune dysfunction represents a significant pathogenic factor in SCH, primarily manifested as Th1/Th2 cytokine imbalance. The administration of intravenous immunoglobulin (IVIG) in treating threatened abortion with SCH can replenish protective cells and factors, restore disordered immune mechanisms, and create favorable conditions for hematoma absorption and pregnancy maintenance ^[24]. Li *et al.* ^[25] demonstrated that weekly IVIG infusions in SCH patients until clinical improvement significantly reduced IgA, IgG, IgM, and TNF- α levels while markedly increasing complement C3, C4 and IL-10 levels. These findings indicate that IVIG can rebalance immune function and enhance defensive capacity in SCH patients, thereby reducing pregnancy failure risk. Clinical observations revealed that combined IVIG and low molecular weight heparin therapy effectively restored Th1/Th2 cytokine balance and elevated progesterone levels, achieving favorable pregnancy outcomes ^[26]. Qian ^[27] further confirmed that IVIG infusion in SCH patients with threatened abortion risk significantly improved Th1/Th2 expression profiles and reestablished immune homeostasis, representing a valuable adjunct to optimal clinical management strategies. The integration of Traditional Chinese Medicine (TCM) and Western medicine represents a significant trend in future medical development. In clinical practice, this combined approach has become an important therapeutic strategy for managing threatened abortion with SCH in patients presenting with kidney deficiency and blood stasis syndrome. By synergizing these two medical systems, clinicians can achieve enhanced therapeutic effects through complementary mechanisms while overcoming the limitations of each approach.

3.4. Anticoagulant therapy

The occurrence of SCH is also associated with hypercoagulability during pregnancy. Therefore, low-dose low-molecular-weight heparin (LMWH) has emerged as an effective approach for preventing and treating SCH through its anticoagulant and antithrombotic effects. Wang *et al.* ^[28] conducted a study of 100 patients with threatened abortion and SCH, demonstrating that adding LMWH to standard care significantly accelerated the resolution of clinical symptoms and reduced rates of inevitable abortion and fetal demise compared to placebo. Their research further revealed that LMWH not only exerts anticoagulant effects but also promotes trophoblast growth, highlighting its clinical value. In another clinical trial, Li *et al.* ^[29] treated 36 SCH patients with subcutaneous LMWH sodium (5000 IU daily) combined with oral dydrogesterone for one week. The results showed this regimen enhanced antithrombin III activity, effectively improving pregnancy-associated hypercoagulability with a favorable safety profile. Liu *et al.* ^[30] observed additional benefits in 30 SCH patients treated with LMWH, including increased progesterone levels and improved uterine perfusion, without significant adverse effects.

Current evidence suggests that while LMWH demonstrates remarkable efficacy in anticoagulation, immune modulation, and progesterone enhancement, clinicians must carefully balance thrombotic and hemorrhagic risks. Strict monitoring for bleeding complications is essential during treatment. These findings provide valuable reference for clinical decision-making, though individualized therapeutic strategies remain paramount.

4. Integrated Chinese-Western therapy

The integration of Traditional Chinese Medicine (TCM) and Western medicine represents a significant trend in future medical development. In clinical practice, this combined approach has become an important therapeutic strategy for managing threatened abortion with SCH in patients presenting with kidney deficiency and blood stasis syndrome. By synergizing these two medical systems, clinicians can achieve enhanced therapeutic effects through complementary mechanisms while overcoming the limitations of each approach. Zhang *et al.* ^[31] demonstrated that the combination of Zishen Yutai Pill and drotaverine hydrochloride significantly promoted hematoma absorption, improved clinical symptoms, and reduced both miscarriage and preterm birth rates, providing novel insights into integrated treatment protocols. Fang *et al.* ^[32] reported superior outcomes in patients with kidney deficiency and blood stasis SCH treated for two weeks with low molecular weight heparin plus modified Shoutai Pill, showing faster resolution of vaginal bleeding and abdominal pain along with better serological markers compared to heparin monotherapy, establishing this as a safe and highly effective regimen. In a clinical study of Baotailing Capsule combined with dydrogesterone, Zhang *et al.* ^[33] observed more pronounced hematoma size reduction, significantly higher serum progesterone and estradiol (E2) levels, and enhanced immune function compared to progesterone-only treatment. A meta-analysis ^[34] confirmed that adding kidney-tonifying and blood-activating herbal formulas to conventional Western medication better promotes uterine and placental circulation while maintaining intrauterine homeostasis. Kong *et al.* ^[35] achieved markedly reduced uterine artery resistance and a higher pregnancy maintenance rate (90.0% vs 72.5% in controls) in 40 patients treated with kidney-invigorating herbs plus dydrogesterone, providing compelling evidence for the efficacy of this integrated approach. Current clinical practice increasingly adopts this combined therapeutic model, leveraging the strengths of both medical systems to achieve multi-target regulation and reduce pregnancy loss rates. Further exploration in future clinical practice is warranted to optimize these integrated treatment protocols.

5. Conclusion and future perspectives

In summary, subchorionic hematoma (SCH) is a pregnancy-related disorder with complex etiology, incompletely understood pathogenesis, and limited evidence-based treatment options. It may contribute to adverse pregnancy outcomes through multiple pathological mechanisms, including disruption of the maternal-fetal interface microenvironment, increased risks of fetal growth restriction and threatened abortion, and psychological burdens such as anxiety and depression in pregnant women, creating a dual “pathological-psychological” impact. Therefore, early detection, comprehensive evaluation, close monitoring, and personalized treatment strategies are essential for optimal management. Currently, therapeutic approaches differ between medical systems: Traditional Chinese Medicine (TCM) follows the principle of “tonifying the kidneys to stabilize the fetus and resolving stasis to stop bleeding,” employing herbs to strengthen the spleen and kidneys, dissolve stasis, and secure the Chong meridian. These formulations modulate immunity, enhance intrauterine circulation, and promote hematoma absorption. Western medicine focuses on progesterone supplementation, tocolytics, and low-molecular-weight

heparin (LMWH) to maintain decidual stability, correct hypercoagulability, and improve perfusion. While Western drugs provide rapid symptomatic relief, TCM offers systemic regulation with long-term efficacy and high safety. The integrated Chinese-Western approach, combining the strengths of both paradigms, is poised to become a cornerstone in future SCH management. However, current limitations, such as small sample sizes and methodological variability in studies, highlight the need for large-scale, high-quality, multicenter clinical trials to refine precision treatment protocols for SCH with kidney deficiency and blood stasis patterns.

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Progress in Clinical Research of Early Pregnancy Chorionic Hematoma in Traditional Chinese and Western Medicine

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Abstract: Subchorionic hematoma is a common cause of vaginal bleeding in early pregnancy, closely related to adverse outcomes such as miscarriage and premature birth. Its onset involves multiple factors such as coagulation abnormalities, immune imbalance, and placental vascular rupture. Western medicine mainly relies on progesterone supplementation and anticoagulant therapy, while traditional Chinese medicine improves the maternal and fetal environment through overall adjustments such as tonifying the kidneys, promoting blood circulation, and stabilizing the fetus. The combination of the two has synergistic advantages in regulating immunity, improving coagulation, and increasing the success rate of fetal preservation. However, existing research has problems such as a lack of standardization in syndrome differentiation and insufficient evidence of long-term efficacy, and there is an urgent need to integrate clinical evidence to optimize diagnosis and treatment strategies. This article provides a systematic review of the etiology, mechanism, and treatment progress of SCH in both traditional Chinese and Western medicine. It emphasizes the potential of combining traditional Chinese and Western medicine to reduce hematoma and improve pregnancy outcomes through the synergistic use of compounds such as progesterone, low molecular weight heparin, and Shou Tai Wan. The purpose of writing this review is to sort out controversies, propose the need for future multi-center large sample studies, establish a biomarker-based syndrome differentiation system, and develop targeted anticoagulant and immunomodulatory Chinese and Western compound preparations. Through interdisciplinary collaboration and technological innovation, we aim to promote the standardized and precise development of SCH diagnosis and treatment, ultimately improving the health level of mothers and infants.

Keywords: Subcystic hematoma; Integrated Traditional Chinese and Western Medicine Therapy; Etiology and pathogenesis; Review

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1. Introduction

Subchorionic hematoma refers to bleeding caused by the separation between the chorionic plate and the decidua, which appears as a dark area between the uterine wall and the gestational sac on ultrasound. The incidence rate of

SCH varies greatly in various studies. Threatened miscarriage refers to a clinical and pathological condition that occurs in early pregnancy, characterized by paroxysmal lower abdominal pain, slight irregular vaginal bleeding, and discomfort in the lumbar and sacral regions. Its clinical features include cervical canal closure and absence of embryonic tissue discharge ^[1]. Vaginal bleeding in early pregnancy is a common complication of pregnancy, with an incidence rate of 16% to 25% ^[2]. SCH is the most common cause of vaginal bleeding in patients between 10 and 20 weeks of pregnancy, accounting for approximately 11% of cases ^[3]. SCH is mainly characterized by vaginal bleeding and lower abdominal pain, and is closely related to adverse pregnancy outcomes such as placental abruption, premature birth, early and late miscarriage ^[4]. Other studies have shown that the widespread use of assisted reproductive technology has become an important risk factor for SCH ^[5]. At the same time, the clinical promotion of early pregnancy ultrasound technology has led to a significant increase in the detection rate of asymptomatic SCH. Pan *et al.* found that the presence, size, and duration of asymptomatic submucosal hematoma in early pregnancy may be related to adverse pregnancy outcomes in later pregnancy ^[6]. Asymptomatic SCH in the first trimester of singleton pregnancy is significantly associated with a variety of pregnancy complications. Its adverse outcomes include diabetes in pregnancy, thrombocytopenia in pregnancy, placental adhesion, fetal growth restriction, macrosomia, and preterm delivery in the third trimester. The presence, size, and duration of asymptomatic submucosal hematoma detected at 5–10 weeks of gestational age are associated with several adverse obstetric outcomes, such as full-term premature rupture of membranes and fetal growth restriction. Although the diagnosis of SCH relies on ultrasound examination, its etiology is complex, involving multiple factors such as maternal coagulation dysfunction, immune imbalance, and rupture of placental chorionic blood vessels ^[7].

The clinical treatment of SCH is mainly based on Western medicine intervention, including progesterone supplementation and hemostasis and anticoagulation. Progesterone, such as progesterone, can reduce the risk of miscarriage by maintaining endometrial receptivity. Chen *et al.* found that progesterone has clinical value in patients with early pregnancy complicated with SCH by regulating progesterone levels and adjusting Th1/Th2 cytokine balance ^[8]. Its mechanism of action may involve the reconstruction of the immune microenvironment, significantly improving the effectiveness of fetal protection treatment and maternal-fetal prognosis. Low molecular weight heparin is suitable for patients with SCH and a hypercoagulable state ^[9], but the long-term thrombotic risk of its use still needs to be carefully evaluated. Traditional Chinese medicine treatment emphasizes overall adjustment, such as Fang *et al.* using modified Shou Tai Wan formulas to improve the maternal fetal microenvironment by tonifying the kidneys, consolidating the Chong, nourishing Qi and blood, while tonifying the kidneys, promoting blood circulation, and stabilizing the fetus formula can regulate coagulation function and improve pregnancy success rate ^[10,11]. Luo focuses on tonifying the kidneys and strengthening the spleen, and uses the method of promoting blood circulation and removing blood stasis to eliminate pathological factors. He has selected Pinghe products to promote blood circulation and remove blood stasis, which have shown significant therapeutic effects in treating SCH ^[12].

Based on the theory of traditional Chinese medicine pathogenesis of “Yin deficiency, blood heat, and insufficient flushing and retention,” Li added raw *Rehmannia glutinosa* to nourish Yin and cool blood, Di Gu Pi to clear and eliminate deficiency heat, Xuan Shen to nourish Yin and detoxify, Fu Ling to invigorate the spleen and calm the heart, Huang Qin to clear heat and stabilize the fetus, Di Yu Charcoal to converge and stop bleeding, and ramie root to solidify flushing and stop leakage to nourish kidney Yin, clear blood heat, and solidify fetal essence ^[13]. In addition, extensive clinical studies have been conducted on the treatment of SCH during pregnancy using a combination of traditional Chinese medicine and Western medicine. Wang *et al.* found that the combination of

traditional Chinese medicine tonifying the kidney, activating blood circulation, and stabilizing the fetus formula with progesterone significantly increased the levels of Ps and Pc in early pregnancy SCH patients, while the levels of coagulation factor VIII, D-dimer (D-D), and NK cells were significantly reduced ^[11]. The improvement in coagulation function in the integrated Chinese and Western medicine treatment group was significantly better than that in the single Chinese medicine/Western medicine intervention group, indicating that the combination of Chinese and Western medicine has a synergistic effect in correcting coagulation dysfunction, inhibiting pathological thrombosis, and promoting fibrinolysis activation. However, there are still controversies surrounding existing treatment plans, such as a lack of standardization in syndrome differentiation and insufficient evidence of long-term efficacy ^[14].

This article systematically reviews the pathogenesis, treatment progress, and synergistic effects of traditional Chinese and Western medicine in SCH in recent years, aiming to integrate clinical evidence and explore potential directions for optimizing treatment strategies. By analyzing the limitations of existing research, this article further proposes that future research should focus on standardized diagnosis and treatment guidelines, interdisciplinary collaboration, and the development of new compound formulations, in order to provide the scientific basis for improving pregnancy success rates and maternal and infant safety.

2. Traditional Chinese Medicine's understanding of submucosal hematoma

2.1. Etiology and pathogenesis of submucosal hematoma

There is no SCH related disease name in ancient Chinese medicine books, but according to the clinical manifestations of “a small amount of vaginal bleeding and fetal movement and descent” as described in classics such as “The Treatise on the Origins of Various Diseases” ^[15], the core pathogenesis belongs to the categories of “fetal leakage” and “fetal movement instability”. The disease is located in the Chong and Ren meridians, and is essentially caused by Chong and Ren damage and fetal instability. Its etiology and pathogenesis are closely related to kidney deficiency, blood stasis, and Qi and blood imbalance. Traditional Chinese Medicine believes that the kidneys are the foundation of innate nature and play a major role in reproduction. If the kidney Qi is insufficient, it will not be able to maintain a stable circulation of the meridians, and blood will not return to the meridians. This can lead to blood stasis and stagnation in the uterus, resulting in the formation of hematomas ^[16]. The Blood Syndrome Theory ^[17] states: “Although it is the blood that deviates from the classics, it is clear blood and fresh blood, but it is also stagnant blood.” The article points out that the “blood that deviates from the classics” is called “stasis blood.” If the stasis blood does not disappear, new blood will not be born, and the fetus will lose its nourishment, causing fetal instability. The book “Yi Lin Gai Cuo” ^[18] states: “Without knowing that there is blood stasis occupying the uterus, the fetus grows to three months and there is no place to live inside. Fetal diseases rely on compression, and blood cannot enter the placenta, flowing down from the side. Therefore, blood appears first. Since blood does not enter the placenta, the fetus lacks blood nourishment, resulting in miscarriage. Blood stasis blocks the uterus, causing blood to not follow the meridians and resulting in fetal leakage. Blood stasis blocks the fetus, leading to loss of nourishment and disturbance of the fetal element, resulting in unstable fetal movement and ultimately leading to miscarriage. Modern research has also shown that traditional Chinese medicine's “blood stasis” is associated with microcirculatory disorders and immune disorders ^[19], which may increase the risk of adverse pregnancy outcomes. In addition, the “Annotation on Women's Good Prescriptions” ^[20] states: “If the wife strengthens her stomach Qi and promotes harmony, then the fetus will find a place like a fish

in the abyss.” In the “Gezhi Yulun”^[21], it is said: “Qi and blood deficiency and lack of nourishment will cause the fetus to fall naturally.” It can be seen that Qi and blood deficiency are also the main pathological factors leading to fetal leakage. The kidneys are responsible for reproduction and play an important role in it. The abundance of kidney essence depends on the microdistribution of water and grain essence in the middle energizer. The spleen and stomach are the source of blood biochemistry in the postnatal stage. When the spleen and stomach are healthy, blood and Qi are abundant. The spleen and the kidney are always closely related, and the process of reproduction can only proceed smoothly if they complement each other. The spleen and stomach are the organs of the granary, and abnormal transport and transformation of the middle energizer can lead to a lack of sources of kidney essence, which in turn can cause reproductive dysfunction. If the deficiency of spleen and kidney leads to insufficient transformation of essence and blood, and the operation of qi is obstructed, it will form stasis, causing dysfunction of the Chong Ren meridians, leading to the loss of fetal essence, and ultimately causing symptoms such as fetal leakage and fetal instability.

2.2. Traditional Chinese Medicine syndrome differentiation and prescription selection for Subcystic hematoma

In summary, the etiology and pathogenesis of SCH are centered around “deficiency and blood stasis.” Fang *et al.*^[22] found through clinical investigation that SCH is mainly divided into five syndrome types: kidney deficiency syndrome, spleen kidney deficiency syndrome, blood heat syndrome, kidney deficiency and blood stasis syndrome, and Qi and blood deficiency syndrome. Among them, kidney deficiency and blood stasis are the most common syndrome types. For kidney deficiency and kidney deficiency blood stasis types, the classic formula is Shoutai Wan. Modern pharmacological research has confirmed that Shoutai Wan simulates endogenous hormone function through estrogen-like biological effects, while inhibiting abnormal contractions of uterine smooth muscle, thereby maintaining pregnancy stability and playing a role in preventing miscarriage and leakage^[23]. The deficiency of qi and blood can be treated by nourishing Qi and blood. Huangqi, *Codonopsis pilosula*, and other herbs can be used to nourish qi and blood, while *Artemisia argyi* can be used to stop bleeding and improve the patient’s deficiency of Qi and blood^[24]. For the spleen and kidney deficiency combined with blood stasis type, the formula of tonifying the kidney, promoting blood circulation, and stabilizing the fetus can strengthen the spleen and kidney, remove blood stasis, and generate new blood. Clinical studies have shown that it can significantly reduce D-dimer levels and improve the pregnancy success rate^[11]. The treatment of blood heat syndrome should mainly focus on tonifying the kidney and spleen, supplemented with heat-clearing products such as *Scutellaria baicalensis* and *Eclipta alba*, which can nourish the liver and kidneys, cool blood and stop bleeding. Another doctor used modified Huanglian ass hide glue Decoction to treat SCH, and the results showed that the efficacy was significantly better than that of the group only taking didroxyprogesterone^[25].

3. Western medicine’s understanding of subdural hematoma

3.1. Pathogenesis and risk factors of submucosal hematoma

The etiology of SCH has not yet reached a consensus. Although its incidence rate is on the rise, the specific pathophysiological process and its impact on pregnancy outcomes are still controversial. At present, the research on the etiology of SCH mainly focuses on several aspects, such as coagulation system dysfunction, autoimmune factors, the widespread use of assisted reproduction, medication during pregnancy and vaginal flora imbalance^[26]. A study found that anti cardiolipin antibodies can induce abnormal platelet aggregation on one hand, and on the other hand,

they can bind to phospholipid dependent antigens on the surface of trophoblast cells, interfere with the normal differentiation process of trophoblast cells, and ultimately lead to insufficient synthesis of key hormones that maintain pregnancy in the placenta, thereby significantly increasing the risk of embryonic arrest and spontaneous abortion ^[27]. Homocysteine is a non-protein-derived sulfur-containing amino acid, and its abnormal increase in serum concentration has been confirmed as an independent risk factor for adverse pregnancy outcomes. It can activate coagulation factors VII and V in the body, promote thromboxane formation, and inhibit the activity of coagulation factors III and IV. This dual effect breaks the dynamic balance between human coagulation and anticoagulation mechanisms, causing blood to show a hypercoagulable tendency and significantly increasing the risk of intravascular thrombosis ^[28]. Assisted reproductive technology (IVF-ET) is an important risk factor for SCH. A survey of IVF-ET patients showed that the incidence of SCH in pregnant women after IVF-ET was significantly higher than that in the non-IVF group, with an incidence rate of about 22.4% ^[29]. Yue *et al.* found that there is a correlation between the risk of developing SCH and previous pregnancy history, with patients who undergo IVF-ET having a higher number of pregnancies having a higher incidence of SCH. Other risk factors include advanced pregnancy (≥ 35 years old), multiple pregnancies, and a history of miscarriage ^[30,31].

3.2. Western medicine treatment of subdural hematoma

In recent years, the strategies for treating SCH in Western medicine have tended towards individualization and multidisciplinary collaboration. Progesterone supplementation is still the preferred option. Dexmedetomidine is a progesterone drug similar to progesterone, which can ensure pregnancy homeostasis through multidimensional mechanisms of action, without male or female hormones or adverse reactions, and has high safety ^[32]. For SCH patients with a pre-thrombotic state, low molecular weight heparin calcium has strong antithrombotic effects, counteracts the pre-thrombotic state, regulates immunity, maintains maternal-fetal immune tolerance, improves endometrial receptivity, and improves microcirculation at the maternal-fetal interface ^[33]. Sun used a combination therapy of tranexamic acid and progesterone to treat patients with threatened miscarriage and SCH in early pregnancy, which showed significant therapeutic effects ^[34]. Studies have shown that tranexamic acid can inhibit inflammatory reactions, reduce vascular permeability, inhibit prostaglandin synthesis, inhibit platelet activation, reduce cervical relaxation and uterine contractions, and have analgesic effects. Therefore, it can be used to treat SCH, alleviate SCH symptoms, reduce related risks, and improve patients' quality of life. Phloroglucinol is a myophilic smooth muscle antispasmodic drug with pharmacological properties that specifically acts on smooth muscle tissue in a spastic state. Its mechanism of action does not rely on the anticholinergic pathway and does not significantly interfere with normal physiological smooth muscle function. It can effectively improve abdominal pain symptoms in patients with threatened miscarriage by accurately relieving abnormal uterine contractions, while significantly reducing the risk of bleeding caused by pathological uterine contractions, inhibiting premature rupture of membranes, and having no effect on embryo and fetal development ^[35]. Scholars have proposed that the combination of immunoglobulin and low molecular weight heparin has a synergistic effect on the quality of SCH ^[36]. Through the unique antibody effect exerted by immunoglobulin, the degree of immune disorder in patients is reduced. Then, low molecular weight heparin is used to improve the receptivity of the endometrium and the intrauterine environment in patients, thereby maintaining normal pregnancy. The combined use of low molecular weight heparin and immunoglobulin in the treatment of SCH patients can effectively improve the success rate of fetal preservation while significantly improving the serum progesterone levels of patients, thereby reducing the risk of adverse pregnancy outcomes such as premature birth and miscarriage ^[37]. Low molecular weight heparin combined with immunoglobulin

therapy can significantly improve the efficacy of treating SCH in pregnancy. While effectively reducing the incidence of adverse events, miscarriage, and premature birth during pregnancy, it can also promote the recovery of pregnancy-related hormones and cytokine expression levels such as IFN- γ and IL-10 in patients ^[38].

4. The synergistic advantages of combining traditional Chinese and Western medicine in the treatment of subdural hematoma

The combination of traditional Chinese medicine and Western medicine in the treatment of SCH demonstrates a significant synergistic effect by integrating the holistic treatment of traditional Chinese medicine with precise intervention of Western medicine. Traditional Chinese medicine treatment focuses on syndrome differentiation and treatment, while Western medicine directly improves placental blood flow and maternal-fetal immune microenvironment through methods such as supplementing progesterone and low molecular weight heparin anticoagulation. Clinical studies have shown that the combination of traditional Chinese and Western medicine significantly improves coagulation factors, sex hormones, pregnancy outcomes, and pregnancy complications compared to high single Western medicine and single traditional Chinese medicine treatments ^[11]. Clinical studies by Xu *et al.* have shown that for early stage SCH patients with cold coagulation and blood stasis type, the use of a combination of traditional Chinese and Western medicine treatment with modified Jiaoai Tang and progesterone can not only significantly promote hematoma absorption, effectively improve cold coagulation and blood stasis symptoms such as lower abdominal pain and vaginal bleeding, but also reduce the incidence of adverse pregnancy outcomes ^[39]. This therapy has been clinically validated to have high safety and no significant side effects have been observed. According to literature records, the classic formula Shou Tai Wan, as recorded in the “Medical Zhong Zhong Shen Xi Lu,” is mainly used in the clinical practice of traditional Chinese medicine for the treatment of pregnancy-related diseases such as kidney deficiency type fetal restlessness, fetal leakage, and fetal developmental delay. Through modern pharmacological research, it has been found that Shoutai Pill can promote the secretion of Th2 cytokines, inhibit the secretion of Th1 cytokines, restore the balance between the two, reduce immune rejection, enhance resistance, and restore normal serum levels ^[40]. Some studies have shown that for patients with early pregnancy complicated with SCH, the application of a combination of traditional Chinese and Western medicine treatment with modified Shou Tai Wan and Western medicine can achieve multiple therapeutic effects simultaneously. While significantly improving the clinical manifestations of patients, it can effectively reduce the hematoma area, shorten the course of the disease, and significantly improve the stability of embryonic development and pregnancy maintenance rate ^[9,41,42]. In addition, traditional Chinese medicine characteristics external treatment methods such as ear acupuncture combined with dexamethasone, can improve the clinical efficacy of treating early threatened miscarriage with SCH. In terms of regulating immune factors and promoting hematoma absorption, it has a good synergistic effect with dexamethasone, reducing the risk of Western medicine dose dependence and side effects ^[43].

5. Summary and prospect

Although the current combination of traditional Chinese and Western medicine has achieved phased results in the treatment of SCH, the following key issues still need to be addressed: firstly, existing studies are mostly single-center small sample sizes, and it is urgent to conduct multi-center randomized controlled trials to verify the efficacy. Secondly, there is a lack of unified standards for TCM syndrome differentiation and classification, and

an objective evaluation system based on biomarkers needs to be established. Thirdly, the mechanism of action of traditional Chinese medicine formulas has not been fully elucidated, and the molecular pathways regulating the maternal-fetal interface can be analyzed using network pharmacology and metabolomics techniques. Future research should also explore new treatment strategies, such as developing targeted anticoagulation and immune regulation Chinese Western compound preparations, or using scoring to construct SCH risk prediction. In addition, long-term follow-up studies are crucial for evaluating the safety of treatment and the impact on offspring health. Through a systematic review of existing literature, the combination of traditional Chinese and Western medicine therapy is expected to provide more accurate and personalized diagnosis and treatment plans for SCH, to reduce the risk of adverse pregnancy and improve the fetal survival rate.

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Disclosure statement

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Research Progress on Subchorionic Hematoma in Clinical Studies

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Abstract: Subchorionic hematoma (SCH), a common cause of vaginal bleeding in early pregnancy, is frequently associated with threatened abortion and preterm labor. It is primarily detected via ultrasonography, with reported incidence rates varying widely across studies (approximately 0.46–48%). With the accelerated pace of modern life, increasing stress, occupational factors, and emotional influences, the prevalence of SCH has risen significantly. The widespread adoption of ultrasound technology has also led to a growing number of asymptomatic SCH cases. Furthermore, the implementation of China's three-child policy and the rising proportion of pregnancies at advanced maternal age pose additional challenges for the clinical management of SCH. This article systematically reviews the etiology, pathogenesis, diagnostic criteria, pregnancy outcomes, and therapeutic advances in SCH from both traditional Chinese medicine (TCM) and Western medicine perspectives, aiming to provide evidence-based insights for clinical research and personalized treatment strategies.

Keywords: Subchorionic hematoma; Etiology; Pathogenesis; Diagnosis; Pregnancy outcomes; Integrated Chinese-Western medicine therapy

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1. Introduction

Subchorionic hematoma (SCH), also referred to as subchorionic hemorrhage, is a common complication in early pregnancy characterized clinically by vaginal bleeding, lumbar soreness, and lower back pain. It arises from blood accumulation between the chorionic plate and decidua basalis. The pathogenesis of SCH is closely linked to abnormal angiogenesis at the maternal-fetal interface, coagulation-fibrinolysis imbalance, and immune microenvironment dysregulation. Modern medical management primarily involves progesterone supplementation, anticoagulation therapy, and symptomatic treatment, though efficacy varies among individuals and carries risks of pharmacological side effects. TCM, guided by holistic principles and syndrome differentiation, employs kidney-tonifying, Chong Mai-stabilizing, blood-activating, and stasis-resolving methods. Integrating TCM with Western therapies has shown significant improvements in pregnancy outcomes. This review synthesizes recent clinical

studies to provide insights for advancing SCH research and personalized therapeutic approaches.

2. Etiology of SCH

2.1. Western medical etiology and pathogenesis

The precise etiology and pathogenesis of SCH remain incompletely understood. Studies suggest that SCH may arise from abnormal placental formation or implantation, immune dysfunction, coagulation disorders, and assisted reproductive technology (ART) pregnancies ^[1]. For instance, reduced protein S (PS) activity, decreased antithrombin III levels, elevated homocysteine (HCY) levels, and positive autoantibodies are associated with SCH susceptibility.

Medawara proposed the concept of “allogeneic fetal transplantation,” positing that the fetus, as a semi-allogeneic entity, triggers a protective maternal immune response during pregnancy ^[2]. Immune tolerance to fetal antigens ensures safe gestation, with T lymphocytes and their subsets, particularly Th1/Th2 imbalance, playing a central role in SCH development ^[3].

SCH has been linked to hypercoagulability, positive autoantibodies (e.g., antiphospholipid antibodies, anti- β 2 glycoprotein I antibodies, antinuclear antibodies), ART pregnancies, genital infections, unhealthy lifestyles, and trauma ^[4]. Wang *et al.* reported that advanced maternal age, a hematoma-to-gestational sac volume ratio $\geq 25\%$, prior intrauterine procedures, SCH detection before 8 weeks of gestation, and hematoma volume $> 30 \text{ cm}^3$ correlate with higher risks of miscarriage, preterm birth, and placental adhesion ^[5]. Many studies investigating *in vitro* fertilization-embryo transfer (IVF-ET) assisted conception and SCH have shown that the incidence of SCH in IVF-ET pregnancies is higher than in natural pregnancies, and the reasons for this may be related to the following: firstly, the patients who undergo this technique have their reproductive endocrine abnormalities, and poor technique during IVF-ET may lead to shallow implantation or the use of medications that may affect the embryo to a greater or lesser extent, which may cause SCH. Firstly, the patients undergoing IVF-ET have their reproductive endocrine abnormalities, and poor technique during IVF-ET, resulting in shallow implantation, or the use of medications that affect the embryo to a greater or lesser extent, may cause SCH. In addition, coagulation abnormalities, immunologic abnormalities, infertility factors, history of miscarriage, history of menstrual cycles, and inflammation of the uterus or tubes of the pregnant women conceiving in IVF-ET may be related to the occurrence of SCH, and among them, coagulation abnormalities and immunologic abnormalities are closely related to the occurrence of SCH ^[6].

2.2. Summary of etiological categories

- (1) Maternal factors: Coagulation dysfunction, immune dysregulation (e.g., antiphospholipid antibody syndrome), autoimmune diseases, thrombophilia.
- (2) Placental abnormalities: Placental abruption, abnormal implantation, or developmental defects.
- (3) External factors: Abdominal trauma, vigorous physical activity, or mechanical irritation from intercourse.
- (4) Infection/Inflammation: Genital tract infections or vaginal dysbiosis.
- (5) High-risk factors: Advanced maternal age, ART (e.g., IVF), polycystic ovary syndrome (PCOS), and smoking.

2.3. Pathogenetic mechanisms

- (1) Aberrant angiogenesis: Downregulated placental angiogenesis factors (e.g., VEGF) impair decidual

vascular remodeling, precipitating hemorrhage^[7].

- (2) Coagulation-fibrinolysis imbalance: Hypercoagulable states (e.g., thrombophilia, antiphospholipid syndrome) promote hematoma formation at the maternal-fetal interface.
- (3) Immune microenvironment dysregulation: Th1/Th2 imbalance and elevated proinflammatory cytokines exacerbate fetomaternal immune rejection.

2.4. TCM etiology and pathogenesis

Traditional Chinese Medicine believes that SCH belongs to the categories of “fetal leakage” and “fetal restlessness.” Its pathogenesis centers on kidney deficiency and blood stasis, often complicated by spleen deficiency, liver Qi stagnation, and blood heat. The kidneys govern reproduction; insufficient kidney qi weakens the Chong and Ren meridians, depriving the fetus of nourishment. Blood stasis obstructs the uterus, diverting blood from its normal pathways and exacerbating hemorrhage risks. Modern TCM scholars emphasize the theory that “extravasated blood transforms into pathological stasis,” highlighting the hematoma’s dynamic pathological nature^[8].

In “Fu Qing’s Gynecology,” the description of fetal leakage and fetal restlessness explains that the kidneys (the “root of innate vitality”) and spleen (the “root of acquired vitality”) are interdependent. Kidney essence relies on the spleen’s transformative function to extract nutrients from food. If Qi deficiency cannot absorb blood, the fetus will leak. Blood heat causes movement, and movement causes fetal leakage. Treatment principles include tonifying the spleen and kidneys, replenishing Qi to control bleeding, and clearing heat to stabilize the fetus.

The descriptions of fetal leakage and fetal restlessness in “The Zhu Bing Yuan Hou Lun” and “Nv Ke Mi Zhi” indicate that the external causes of SCH patients include excessive work and rest, improper diet, invasion by the six exogenous pathogenic factors, environmental factors, and emotional factors, etc. The internal causes can be divided into “mother’s illness,” “unstable fetus,” and “damage to the internal organs of the fetus.” The Chong and Ren meridians are weakened and unable to collect blood and nourish the fetus, resulting in an unstable fetal origin.

3. Diagnosis of SCH: Western and TCM perspectives

3.1. Western medical diagnosis

The diagnosis of SCH primarily relies on ultrasonography, which visualizes the hematoma’s size, location, morphology (crescent-shaped, triangular, annular, or irregular configurations), and involvement with the placenta or fetus. Ultrasound also distinguishes between acute, subacute, and chronic phases of SCH and rules out other causes of vaginal bleeding, such as ectopic pregnancy or hydatidiform mole, thereby guiding clinical management^[9].

Hormonal biomarkers further aid in predicting adverse pregnancy outcomes during early gestation: Progesterone (P) and β -human chorionic gonadotropin (β -hCG) are critical for early fetal development. Declining progesterone levels or suboptimal β -hCG rise signal indicate abnormal pregnancy progression. Serum estradiol (E2) reflects placental and fetal growth. A slow or inadequate increase in E2 with advancing gestational age indicates potential pregnancy complications.

Emerging evidence highlights the role of Th1/Th2 cytokine imbalance in SCH prognosis. Elevated serum TNF- α and IL-2 (pro-inflammatory Th1 cytokines) coupled with reduced IL-4 and IL-10 (anti-inflammatory Th2 cytokines) correlate with higher risks of adverse outcomes^[10].

3.1.1. Classification criteria for SCH

- (1) By hematoma severity:
 - Mild: Hematoma-to-gestational sac area (or volume) ratio $< 1/3$
 - Moderate: Ratio $1/3-1/2$
 - Severe: Ratio $> 1/2$
- (2) Alternative grading based on maximum diameter:
 - Small: $< 25\%$ of gestational sac diameter
 - Medium: $25-50\%$
 - Large: $> 50\%$
- (3) By gestational timing:
 - Early SCH: Diagnosed before 12 weeks of gestation
 - Mid-term SCH: Diagnosed between 12–20 weeks
 - Late SCH: Diagnosed after 20 weeks (less common) ^[11]

3.2. TCM diagnosis

The Tai Chan Xin Fa distinguishes fetal leakage from fetal restlessness:

- (1) Fetal leakage: Slight vaginal bleeding during pregnancy without lumbar soreness, abdominal pain, or pelvic pressure.
- (2) Fetal restlessness: Vaginal bleeding accompanied by lumbar soreness, abdominal cramping, or a sensation of lower abdominal distension.
- (3) Contemporary TCM practitioners classify SCH into seven primary syndrome patterns based on clinical manifestations ^[12]: Blood stasis pattern; Damp-heat pattern; Blood-heat pattern; Spleen-kidney deficiency pattern; Qi-blood deficiency pattern; Kidney Qi deficiency pattern; Traumatic injury pattern.

Statistical analyses indicate that kidney Qi deficiency is the most prevalent pattern, forming the basis for the therapeutic principle of “tonifying the kidneys to stabilize the fetus” in clinical practice.

3.3. Pregnancy outcomes of SCH

Studies indicate that SCH diagnosed before 12 weeks may either resolve or enlarge in later gestation, whereas those diagnosed after 12 weeks are more likely to expand, increasing risks of adverse outcomes ^[13]. SCH is associated with complications such as oligohydramnios, preterm premature rupture of membranes (PPROM), and fetal distress, ultimately leading to miscarriage, preterm birth, or low birth weight ^[14].

Shi *et al.* reported that SCH with threatened miscarriage significantly elevates risks of placental abnormalities (e.g., placenta previa, placental abruption), gestational diabetes, placenta accreta, and postpartum hemorrhage ^[15]. Chen observed that patients diagnosed with SCH during early gestation (4–13 weeks) exhibited less severe adverse outcomes compared to those diagnosed in mid- (14–27 weeks) or late gestation (28–34 weeks) ^[11]. Wang *et al.* concluded that the ratio of hematoma to the maximum diameter of the gestational sac was divided into large, medium and small hematomas, and that pregnant women with large and medium hematomas in the middle stage of pregnancy were more likely to have miscarriages and preterm deliveries ^[16]. The duration of hematoma was more than 4 weeks and more likely to be miscarried than less than 4 weeks; and pregnant women with subchorionic hematomas in the middle stage of pregnancy who were accompanied by vaginal bleeding were more likely to have miscarriages and preterm deliveries. Therefore, the earlier the SCH appears, the longer it lasts, the larger it is, and

the more likely it is to lead to an adverse pregnancy outcome if it is not diagnosed and treated in a timely manner.

4. Treatment of SCH

Given the adverse pregnancy outcomes associated with subchorionic hematoma (SCH), early detection through regular prenatal care and timely intervention are critical to mitigate risks. Current therapeutic strategies emphasize bed rest, emotional stabilization, and pharmacotherapy.

4.1. Western medical approaches

- (1) Progesterone support: Dydrogesterone and progesterone are widely used to suppress uterine contractions and alleviate symptoms. International studies confirm dydrogesterone's efficacy in reducing miscarriage rates in threatened abortion ^[17].
- (2) Anticoagulation therapy: Low-molecular-weight heparin (LMWH) and immunoglobulins address hypercoagulability and immune dysregulation.
- (3) Adjunctive therapies: Magnesium sulfate, phloroglucinol, and α -lipoic acid are employed for symptom relief.

4.2. TCM medical approaches

TCM attributes SCH to kidney Qi deficiency, blood stasis, and spleen-stomach dysfunction, disrupting the Chong-Ren meridians' ability to nourish the fetus. Treatment focuses on: Tonifying the kidneys and spleen; Replenishing Qi and blood; Activating blood circulation and resolving stasis.

Huang *et al.* believe that the appearance of SCH in traditional Chinese medicine is related to insufficient kidney Qi, which leads to internal stagnation of blood stasis ^[18]. Kidney deficiency is the root cause, blood stasis is the standard, and the principle of taking the specimen into account is to nourish the kidney, promote blood circulation, remove blood stasis, stop bleeding, and stabilize the fetus. The combination of modified Shoutai Wan and Diqu progesterone has achieved good therapeutic effects in clinical practice. Wei *et al.* achieved good results in the treatment of threatened miscarriage combined with SCH with a combination of kidney tonifying, stasis resolving, and fetal stabilizing formula composed of 30 g yam and 15 g *Atractylodes macrocephala* ^[19]. Yang *et al.* found that in clinical patients with blood heat type SCH, the main symptoms are small abdominal pain or back pain, with a small amount of vaginal bleeding, bright red or deep red color, accompanied by dry mouth and throat ^[20]. Some people are accompanied by restlessness and lack of sleep, hot hands and feet, short and yellow urine, and constipation. The tongue is red in color, with thin yellow or greasy coating, and the veins are smooth or slippery. Didrogestrone combined with Huanglian Decoction granules is more effective than Didrogestrone alone. Some people have achieved good therapeutic effects on SCH by adding spleen tonifying drugs on the basis of kidney tonifying, combined with progesterone and vitamin E ^[21]. Sun achieved good results in the treatment of patients with Qi and blood deficiency by self-formulating Shoutai Wan modified Yiqi Yangxue Formula combined with progesterone injection when treating SCH ^[22]. The combination of Zishen Yutai Pill and Dexmedetomidine has achieved good results in the treatment of threatened miscarriage with spleen and kidney deficiency type SCH ^[23]. Guo found that the combination of kidney tonifying, blood activating, and fetal stabilizing formula and dexamethasone can effectively alleviate symptoms and improve treatment efficacy in the treatment of threatened miscarriage with SCH in early pregnancy ^[24].

Zhu *et al.* found that Jiaoai Hutai Tang can increase serum HCG and P levels in patients, help promote corpus luteum growth, synthesize pregnancy related hormones, reduce uterine contractions to prolong pregnancy, promote hematoma regression, reduce vaginal bleeding, and greatly increase the rate of full-term pregnancy ^[25]. Its combination with progesterone injection can achieve good therapeutic effects in the treatment of threatened miscarriage with SCH. Shoutai Pill is composed of *Cuscuta chinensis*, *Dipsacus aspera*, and Sangshi. It can strengthen the effect of convergence and hemostasis when used together with Puhuang charcoal. The combination of didroxyprogesterone has achieved good results in the treatment of kidney deficiency type SCH, can promote the absorption of hematoma, improve hormone levels, effectively shorten the time of hematoma and vaginal bleeding, and reduce the occurrence of adverse pregnancy outcomes ^[26]. Jiang *et al.* also adopted a combination of traditional Chinese and Western medicine treatment methods ^[27]. Western medicine used progesterone injection and dexamethasone, while traditional Chinese medicine gave oral administration of kidney tonifying and fetal stabilizing Chinese medicine and hemostatic moxibustion. In the treatment of SCH, the efficacy was better than using Western medicine alone or Western medicine combined with oral Chinese medicine. Intramuscular injection of progesterone injection combined with modified Jiaoai Tang has significant therapeutic effects on early SCH treatment ^[28].

In addition to traditional Chinese medicine decoction combined with hormone to treat SCH, there are other treatment schemes combined with treatment, such as traditional Chinese patent medicines and simple preparations combined treatment: Baotieling capsule combined with Diqu Progesterone has a good effect in treating SCH of kidney deficiency and blood stasis type ^[29]. Western medicine combined treatment: Tranexamic acid injection inhibits fibrinolysis by suppressing the activity of fibrinolytic enzymes, thereby exerting hemostatic effects. It is combined with HCG injection and progesterone injection to supplement hormones needed for pregnancy, inhibit uterine contractions, improve the success rate of fetal protection, and reduce the occurrence of adverse reactions ^[30]. Low molecular weight heparin has anticoagulant, antithrombotic, and immunomodulatory functions, and has a good effect on protecting the fetus. When combined with levonorgestrel, it has a better effect on treating subdural hematoma than levonorgestrel alone. It can effectively shorten the disappearance time of vaginal bleeding, abdominal pain, and subdural hematoma, reduce the incidence of adverse pregnancy outcomes, improve the secretion levels of E2, P, and β - HCG hormones, and increase the success rate of protecting the fetus ^[31].

Other combination therapies include research by Li *et al.* has shown that the combination of Jiawei Shoutai Pill and Metoprolol Injection can improve clinical efficacy in treating patients with threatened miscarriage and SCH accompanied by paroxysmal pain in the lower back or abdomen ^[32]. The combination of ear acupuncture and dexamethasone has also achieved good results in the treatment of SCH ^[33]. Acupoint application combined with HCG injection and progesterone injection has also achieved significant results in the treatment of SCH ^[34]. Zhang *et al.* believe that immune disorders are one of the causes of SCH. In the treatment of SCH, individualized treatment can be given to patients with abnormal levels of autoantibodies and cellular immune status. Suitable immune regulatory drugs such as immunoglobulin, low molecular weight heparin, alpha lipoic acid, progesterone preparations, and some traditional Chinese medicine formulas can be selected to achieve good results in the treatment of SCH. However, further research is needed in this area ^[35].

The above research indicates that the combination of traditional Chinese and Western medicine is the best choice for treating SCH, and individualized treatment plans should be adopted for specific clinical situations. The best clinical prescription should be selected for each patient based on syndrome differentiation and treatment.

5. Summary and prospect

In summary, SCH can lead to many adverse pregnancy outcomes, which hurt both the family and the pregnant woman herself. Therefore, clinical doctors should pay attention to every SCH patient, instruct them to undergo timely prenatal check-ups, and detect and treat SCH promptly. SCH patients can be diagnosed through examinations such as ultrasound, hormone levels, and influencing factors, and provide personalized treatment plans for each patient. The combination of traditional Chinese and Western medicine can be the first choice for treating SCH patients. However, there are many causes of SCH, and the pathogenesis of SCH is not yet clear. Further study is needed to clarify the factors that cause SCH and the pathogenesis of SCH, so that targeted treatment can be carried out. Clinical doctors should also improve their own diagnosis and treatment level to improve the success rate of the cure.

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Disclosure statement

The authors declare no conflict of interest.

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Progress in the Induction of Gestational Diabetes Mellitus by Environmental Exposure to the Novel Flame Retardant Triphenyl Phosphate during Pregnancy

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Abstract: *Introduction:* Triphenyl phosphates, or TPhPs, are a family of newly discovered pollutants that contaminate the environment, endanger ecological safety, and cause health problems for people when they linger in the surrounding environment for extended periods of time. *Methods:* This study reviewed the state of the research on the environmental distribution of TPhP, the amount of TPhP exposure during pregnancy, the relationship between TPhP exposure and gestational diabetes mellitus, the possible mechanism by which TPhP exposure during pregnancy causes gestational diabetes mellitus, and the risk of gestational diabetes mellitus in the offspring as a result of TPhP exposure during pregnancy. *Results:* During pregnancy, if pregnant women come into contact with TPhP, which is widely used in various industrial products, it is highly likely to disrupt the body's metabolic balance, have a significant impact on the induction of gestational diabetes mellitus, and increase the health risks for both the mother and the baby. *Discussion:* These findings provide critical insights for risk assessment and prevention strategies targeting gestational TPhP exposure. *Significance:* What is already known about this subject? TPhP is a pollutant that is harmful to people's health. When pregnant women are exposed to TPhP for a long time, their chances of developing gestational diabetes increase. What does this study add? This paper systematically summarizes the effects and mechanisms of TPhP on pregnant women, and provides theoretical support and factual basis for the research on TPhP and gestational diabetes.

Keywords: Organophosphorus flame retardants; Triphenyl phosphate; Pregnancy exposure; Gestational diabetes mellitus

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1. Introduction

As the most widely used organophosphorus flame retardant (OPFR), triphenyl phosphate (TPhP) is extensively added to plastics, textiles, and consumer products as both a flame retardant and plasticizer^[1]. OPFRs, as persistent pollutants,

enter the environment via abrasion and volatilization, posing risks to ecosystems and human health ^[2–5]. Human exposure to these pollutants can occur through multiple pathways, including skin contact, ingestion of dust, and inhalation ^[6]. Human urine, placenta, and blood are examples of biological samples that include OPFRs or their metabolites ^[7]. Studies have shown that TPhP is a common endocrine disruptor, and long-term exposure is closely related to insulin resistance and glucose metabolism disorders ^[8]. Many epidemiologic studies show that pregnant women are exposed to OPFRs on a wide scale ^[3–7]. Therefore, to offer a scientific foundation for elucidating the health risks of TPhP to pregnant women, safeguarding the health of mothers and newborns, and preventing TPhP exposure, this study analyzes the effects of TPhP exposure during pregnancy on the induction of gestational diabetes mellitus. Given the widespread environmental presence of TPhP, it is critical to first characterize its distribution across different media to understand potential exposure pathways during pregnancy.

2. Grouping of TPhP environmental presence levels and OPFRs

This paper focuses on triphenyl phosphate (TPhP), one of the most commonly used organophosphate flame retardants. As a phosphate ester flame retardant, TPhP serves as a primary alternative to polybrominated diphenyl ethers (PBDEs) in resin and PVC materials ^[1,9–11]. TPhP can be physically added to materials, and it persists in the environment due to its extreme volatility and lack of chemical bonding ^[12]. TPhP and its metabolites are frequently detected in human biological samples (e.g., urine, placenta), highlighting direct exposure risks ^[13–16], especially the potential threat to the health of mothers and infants in pregnant women.

The most commonly used OPFR, TPhP, is commonly found in hydraulic fluids, construction materials, polymers, and electrical equipment ^[17]. Due to the release of materials from indoor furniture, decorations, and electrical appliances, OPFRs are in dust from almost all indoor environments. The concentration and compositional characteristics of OPFRs in indoor dust vary greatly between countries and regions. The concentration of TPhP in indoor dust is generally higher in developed countries. The predominant OPFRs in indoor dust include TPhP and its structural analogues ^[6]. These regional disparities in OPFR concentrations likely reflect differences in regulatory frameworks and industrial practices, emphasizing the role of policy in shaping exposure patterns. Developed regions exhibit significantly higher TPhP concentrations in indoor dust than developing countries ^[18]. This suggests that electronic equipment continually releases TPhP into the indoor air. Pregnant women and other special populations have occupational exposure to TPhP in these unique indoor situations that is significantly higher than the general population's daily exposure.

It is simpler to ignore OPFR contamination in water bodies, substrates, and soils than it is in the atmosphere and dust. Although precipitation and runoff into the water bodies, etc., all of which make the environmental water bodies, substrates, and soils important collection points for OPFRs, there are numerous ways for OPFRs to enter water bodies, substrates, and soils. These methods include industrial wastewater and domestic sewage discharges from flame retardant manufacturers, as well as OPFR residuals in the environmental media. Soil samples from urban areas show significant TPhP contamination via atmospheric deposition ^[19]. With the help of atmospheric movement, they can travel great distances before being deposited in their surroundings. The global dispersion of TPhP underscores the urgency to evaluate its toxicological impacts, particularly on vulnerable populations such as pregnant women.

3. Environmental toxicity study of TPhP exposure

Emerging evidence highlights TPhP's role as a metabolic disruptor, with studies linking its exposure to insulin resistance and glucose homeostasis impairment ^[20,21]. TPhP can accumulate in living things and may potentially harm human health at the highest trophic level through the food chain because it is primarily employed as an additive rather than being bonded by reaction with components. Since TPhP is widely dispersed throughout the environment, it is critical and essential to conduct a thorough toxicity study. The foundation for assessing TPhP's environmental risk is its toxicity data. epidemiological investigations have discovered that TPhP has an endocrine-disrupting effect that can cause metabolic disorders and other diseases ^[22]. According to cytotoxicity tests, TPhP disrupts the metabolism of cellular hormones ^[23–25]. Translating these toxicological findings to human health requires understanding real-world exposure levels, which have been increasingly documented in pregnant populations. Levels of TPhP exposure during pregnancy in domestic and international women.

Global studies confirm widespread OPFR exposure in pregnant populations ^[3–7,26,27]. Women in mid- and late-pregnancy had their urine examined by Kosarac *et al.*, who discovered that the majority of the OPFR metabolites in the urine were DPHP, a TPhP metabolite with an average concentration of 4.71 ng/mL and a detection rate of 97% ^[28]. The assays were comparable ^[29]. This information was gathered from nested case-control research. DPHP was found in the urine of mid-pregnant women by Feng *et al.* (100% detection rate, geometric mean 1.1 ng/mL) ^[30]. Multiple studies have demonstrated that the detection rate of DPHP, a TPhP metabolite, is detected in 79–100% of maternal urine samples ^[26,27,31]. While these toxic effects are concerning, their relevance to human health depends on the extent of real-world exposure, which has been increasingly documented in pregnant populations. The widespread detection of TPhP metabolites in pregnant women underscores the need to investigate its potential role in disrupting glucose metabolism during pregnancy.

4. Exposure to TPhP raises risk of glycemic disorders and insulin resistance

TPhP exposure has emerged as a novel risk factor for glucose metabolic disorders, independent of traditional contributors like obesity ^[8]. Among environmental pollutants, organophosphate flame retardants such as TPhP have emerged as potential diabetogens. Chinese cohort studies reveal widespread OPFR exposure due to industrial production, correlating with metabolic dysfunction ^[8,18]. People who are exposed to OPFRs may be more likely to develop type 2 diabetes, citing a previous study. According to earlier research, people who are exposed to OPFRs may have a higher chance of acquiring type 2 diabetes ^[18]. Ding *et al.* demonstrated that combined exposure to multiple OPFRs disrupts glucose homeostasis, with TPhP and DPHP being the primary contributors ^[8].

Exposure to TPhP has been shown to drastically decrease metabolic function in rodents, according to an animal investigation. Prenatal TPhP exposure in mice induced metabolic dysfunction in offspring, including insulin resistance ^[28]. PPAR γ -independent pathways may mediate TPhP-induced lipid accumulation. This suggests that PPAR γ is not the only pathway through which TPhP-induced adipogenesis is mediated ^[29]. An additional investigation on adult mice verified that exposure to TPhP led to increased levels of glucose and the HOMA-IR index ^[8]. To address this knowledge gap, emerging studies have begun to unravel the molecular pathways through which TPhP interferes with endocrine and metabolic functions in pregnancy.

5. Potential mechanisms by which exposure to TPhP during pregnancy causes endocrine disruption and gestational diabetes mellitus

The term “gestational diabetes mellitus” (GDM) describes the various degrees of problems in glucose metabolism found during pregnancy. In 2014, the prevalence of GDM in China reached a high of 18.9% ^[30]. Negative outcomes for mothers and babies (shoulder dystocia, cesarean delivery, suprapregnancy, preterm labor, and severe newborn abnormalities) are more likely in cases of GDM ^[32]. Research has demonstrated that the release of anti-insulin chemicals by the placenta during pregnancy causes aberrant glucose tolerance, which in turn causes a partial or total lack of insulin during pregnancy. A normal blood glucose level is maintained by compensating with an increase in insulin secretion, but an abnormal glucose tolerance occurs when there is a malfunctioning of the pancreatic β -cells in the secretion of insulin. As pregnancy goes on, the secretion of anti-insulin hormones such as placental prolactin, progesterone, and adrenocorticotrophic hormone gradually increases, and the anti-insulin effect gradually strengthens. Numerous OPFRs, including TPhP, have been demonstrated in recent *in vitro* experiments to exhibit binding-speak activity with the pregnane X receptor and to exhibit some endocrine-disrupting effects ^[32].

Fatty acid transport in placental trophoblast cells is regulated by PPAR γ -induced fatty acid transport protein (FATP), fatty acid binding protein (FABP), and cluster of differentiation 36 (CD36), according to *in vitro* mechanistic research ^[33]. To encourage trophoblast uptake of fatty acids, the PPAR γ agonist rosiglitazone works on placental trophoblast cells FATP and FABP ^[34]. It was discovered that rosiglitazone and TPhP target genes differently and activate PPAR γ phosphorylation sites inconsistently ^[35]. Studies on PPAR γ and its controlled lipid metabolism-related gene levels have confirmed in the literature that TPhP exposure significantly increases the quantity of CD36 protein in cells ^[36]. CD36, a transmembrane glycoprotein critical for fatty acid transport, is upregulated by TPhP exposure ^[36,37]. CD36 facilitates fatty acid uptake and esterification across cell types ^[37]. In the meantime, CD36 is a crucial membrane receptor for the cytosolic route, which allows cells to absorb low-density lipoprotein (LDL), a lipoprotein high in cholesterol ^[38]. The literature has shown that TPhP increases progesterone levels *in vitro* models, demonstrating TPhP-induced progesterone synthesis via PPAR γ ^[39]. Steroid hormone synthesis and TPhP-induced fat deposition may be mediated by the PPAR γ downstream target gene CD36. It has been established that TPhP is an endocrine disruptor. It can build up in the placenta, deposit lipids in the placental cell membrane. While these mechanistic insights are critical, bridging experimental evidence to clinical prevention strategies remains a challenge, necessitating focused research on TPhP-induced gestational diabetes.

Placental endocrine disruption by TPhP exacerbates insulin resistance, a core feature of GDM ^[8,32]. TPhP disrupts diabetes-related pathways by altering key receptors (e.g., insulin receptor and glucose transporters) and downstream signaling ^[8]. However, there have been no studies on the mechanism by which it induces gestational diabetes mellitus. Furthermore, many metabolites, like DPHP, a common metabolite of TPhP, other TPhP metabolites may have non-specific effects ^[40]. Future research should prioritize longitudinal cohorts to track TPhP exposure across trimesters, combined with multi-omics approaches to identify biomarkers linking placental dysfunction to GDM pathogenesis.

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Mechanistic Study of Acupuncture in Polycystic Ovary Syndrome: Insights from the Gut-Ovary Axis

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Abstract: Gut microbiota dysbiosis can induce chronic low-grade inflammation and disrupt metabolic homeostasis and intestinal barrier function, thereby impairing ovarian function and contributing to the development of polycystic ovary syndrome (PCOS). Acupuncture, as a traditional Chinese medicine (TCM) therapy, has been shown to regulate the gut microbiota and modulate multiple cytokines, thereby exerting beneficial effects on ovarian function. Guided by the TCM theories of organs and meridian systems, this study integrates the physiological and pathological correlations between the spleen-stomach-intestine system and the ovary, along with the interconnections of meridians. Taking the gut-ovary axis mediated by intestinal microbiota as a key link, we aim to elucidate the mechanism by which acupuncture improves PCOS by modulating gut microbiota. Acupoint selection is based on the principle of restoring intestinal microbial balance, providing a novel approach to the clinical diagnosis and treatment of PCOS from the perspective of the gut-ovary axis.

Keywords: Polycystic ovary syndrome; Gut microbiota; Gut-ovary axis; Acupuncture; Immune inflammation

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1. Introduction

Polycystic ovary syndrome (PCOS) is a complex and highly heterogeneous endocrine-metabolic disorder characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology. It is commonly accompanied by insulin resistance, chronic low-grade inflammation, obesity, and disturbances in glucose and lipid metabolism ^[1]. With a global prevalence of approximately 5–10%, PCOS not only impairs female fertility but also substantially increases the risk of type 2 diabetes and cardiovascular diseases ^[2,3]. Its pathogenesis involves a multifactorial interplay of dietary habits, obesity, inflammation, and dysregulation of the hypothalamic–pituitary–ovarian (HPO) axis, all of which contribute to immune-metabolic imbalances ^[4]. Recent studies have

indicated that alterations in the gut microbiota (GM) can disrupt downstream metabolic homeostasis, increase intestinal permeability, and elevate circulating levels of lipopolysaccharide (LPS), thereby inducing systemic chronic inflammation and promoting the onset and progression of PCOS^[5]. The maintenance of gut microbiota homeostasis plays a crucial role in overall health and shares therapeutic relevance with acupuncture. As a traditional Chinese medical approach, acupuncture has shown promising effects in managing PCOS, particularly through its anti-inflammatory properties, modulation of gut microbiota, and improvement of metabolic regulation^[6]. This review, grounded in the theory of the “gut–ovary axis,” aims to elucidate the relationship between PCOS and gut microbiota and to explore the underlying mechanisms by which acupuncture may alleviate metabolic and inflammatory disturbances in PCOS. These insights may offer novel perspectives for clinical intervention and provide a theoretical basis for further mechanistic studies.

2. Theoretical connotations of the gut–ovary axis

2.1. Traditional Chinese medicine perspective

In Traditional Chinese Medicine (TCM), the concept of the gut–ovary axis is rooted in the holistic interplay between the organs, meridians, and reproductive function. According to TCM, “the spleen and stomach are the foundation of acquired constitution,” emphasizing their central role in generating Qi and blood. As described in the *Su Wen- Discourse on the Divergent Meridians*, “Food enters the stomach, its essence diffuses and ascends to the spleen, and the spleen disperses it to nourish the organs via the meridians.” Through the processes of digestion and transformation, the spleen and stomach extract nutrients (essence of food and water), which are transported via meridians to the kidneys, replenishing kidney essence. This essence then nourishes the Chong and Ren vessels and, in turn, supports the uterus (*bao gong*), establishing a physiological link between the intestines, spleen, kidneys, and female reproductive system.

In this process, the gut microbiota serves as a microscopic manifestation of the spleen’s transportation and transformation functions, contributing to the digestion and absorption of nutrients. Microbial metabolites influence ovarian hormone regulation, reflecting the TCM principle that “postnatal essence nourishes prenatal essence.” The *Furen Gui* further states, “Menstrual blood is derived from the essence of food and water generated by the spleen and released by the kidneys.” Therefore, impaired spleen function can disturb the distribution of Qi and blood, reduce the nourishment of kidney essence, and disrupt the Chong and Ren meridians’ support of the uterus, ultimately leading to menstrual irregularities and ovulatory dysfunction. On the other hand, the intestines and ovaries are closely connected through the meridian system.

According to the *Su Wen–Golden Chamber’s Canonical Teachings*, “The north corresponds to the color black, enters and connects with the kidneys, and opens into the two Yin orifices,” indicating that the kidneys, uterus (*bao gong*), and large intestine are linked via the Foot Taiyang Bladder Meridian. The Chong meridian is described as “penetrating Yin and Yang and serving as the sea of the twelve meridians,” while the Ren meridian “governs the uterus and fetus.” Both originate in the uterus and connect directly with intestinal meridians, thereby forming a meridian system that reflects the physiological integration of the intestines, Chong and Ren vessels, and the reproductive organs. As recorded in the *Lingshu–Divergent Meridians*, “The Foot Taiyang branches into the anus, connects to the bladder, and disperses into the kidneys,” suggesting that the intestines and kidney meridian communicate through meridians, enabling the transmission of essential Qi and blood to the uterus. The Chong and Ren meridians, serving as hubs of Qi and blood convergence, convey the refined essence derived from the

spleen and stomach via the intestines to the uterus, thus laying the foundation for menstrual cyclicity and fetal development. As articulated in the Treatise on Blood Disorders-Fetal Qi, “The arrival of menstruation relies on the heavenly water (Tian Gui) reaching the uterus, and the blood of the Chong and Ren meridians responding accordingly.” This statement encapsulates the core of the gut–ovary axis: the essence and blood generated by the spleen and stomach nourish the uterus via the Chong and Ren meridians, thereby sustaining reproductive homeostasis.

2.2. Modern medical understanding of the gut–ovary axis

A close relationship exists between gut microbiota and ovarian function. Studies have demonstrated that the composition of gut microbiota in patients with PCOS differs significantly from that of healthy individuals, providing foundational evidence for the proposed concept of the “gut–ovary axis”^[7]. This theory posits that the intestine and ovaries form a regulatory network through metabolic, immune, and neuroendocrine pathways, in which disruptions in gut microbial homeostasis may impair ovarian function via systemic circulation. Accumulating evidence suggests that dysregulation of intestinal homeostasis, comprising gut microbiota, intestinal barrier integrity, and host metabolism, is closely associated with the onset and progression of PCOS^[8,9].

The integrity of the intestinal barrier is central to the gut–ovary axis. This barrier, consisting of epithelial cells, tight junction proteins, mucus layers, and commensal microbes, serves as a critical defense against endotoxin translocation and inflammatory responses^[10]. Under metabolic or oxidative stress, activation of hypoxia-inducible factor-1 α (HIF-1 α) and nuclear factor- κ B (NF- κ B) signaling pathways downregulates tight junction proteins such as claudin-1, leading to increased intestinal permeability or “leaky gut”^[11]. Consequently, LPS and other endotoxins may translocate into the ovarian microenvironment, triggering macrophage-mediated inflammation that disrupts follicular development and hormone secretion, thereby contributing to ovarian dysfunction^[12].

3. The intrinsic link between PCOS and the gut-ovary axis

3.1. Regulatory role of gut microbial metabolites

Dysregulation of gut microbial metabolites is a key driver in the pathogenesis of PCOS. Patients with PCOS commonly exhibit reduced microbial diversity and an altered Firmicutes-to-Bacteroidetes ratio, resulting in significantly decreased production of short-chain fatty acids (SCFAs)^[13]. SCFAs activate G protein-coupled receptors GPR41 and GPR43, playing critical roles in regulating host energy metabolism, suppressing fat accumulation, and improving insulin sensitivity. A deficiency of SCFAs exacerbates insulin resistance and ectopic lipid deposition^[14]. Gut dysbiosis often leads to elevated LPS levels, which trigger chronic low-grade inflammation and contribute to ovarian dysfunction in PCOS, as confirmed by animal studies^[15]. LPS activates the toll-like receptor 4 (TLR4) signaling pathway, stimulating macrophages to release pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukins (e.g., IL-6), thereby promoting both local and systemic low-grade inflammation^[16]. These inflammatory mediators impair the function of theca cells and downregulate estrogen receptor alpha (ER α), reducing the sensitivity of follicles to gonadotropins and disrupting folliculogenesis^[17]. Furthermore, reduced activity of bile salt hydrolase (bile salt hydrolase, BSH) impairs the synthesis of secondary bile acids, compromising the integrity of the intestinal mucus layer and further aggravating barrier dysfunction^[18].

3.2. Gut microbiota-associated signaling pathways in the pathogenesis of PCOS

Gut dysbiosis leads to alterations in proinflammatory mediators, involving several signaling pathways known to contribute to PCOS pathogenesis, such as the TLR and NF- κ B pathways. Wang et al. reported that ovarian tissue and peripheral blood from PCOS patients exhibit inflammatory infiltration by macrophages, T cell imbalance, and elevated levels of proinflammatory cytokines such as IL-17 and IL-6^[19]. In addition, overgrowth of *Bacteroides vulgatus* suppresses the synthesis of glycodeoxycholic acid, thereby impairing the secretion of IL-22 by group 3 innate lymphoid cells (ILC3), disrupting the intestinal mucosal barrier, and promoting LPS translocation into the systemic circulation^[20]. Circulating LPS binds to lipopolysaccharide-binding protein (LBP), myeloid differentiation factor 2, and CD14 to form a complex that activates TLR4 receptors on immune cells^[21], thereby triggering the MyD88-dependent NF- κ B signaling pathway and inducing the expression of proinflammatory cytokines, ultimately establishing a chronic inflammatory microenvironment^[22]. These inflammatory mediators impair pancreatic β -cell function, downregulate follicle-stimulating hormone receptor expression in granulosa cells, and activate the HPO axis. This results in aberrant pulsatile secretion of gonadotropin-releasing hormone (GnRH) and an increased follicle-stimulating hormone (FSH)/luteinizing hormone (LH) ratio, exacerbating hyperandrogenism and ovulatory dysfunction. This creates a vicious cycle involving intestinal barrier disruption, systemic inflammation, and hormonal dysregulation^[23]. Moreover, gut microbiota-derived metabolites such as serotonin may reach the hypothalamus via the vagus nerve, interfere with GnRH pulsatility, accelerate follicular atresia, and contribute to neuropsychiatric symptoms such as anxiety and depression in PCOS patients^[24]. These findings underscore the complex interplay between the gut, immune system, and ovarian function in PCOS, highlighting the integrative regulatory role of the gut–ovary axis in disease progression and providing a theoretical basis for targeted therapeutic interventions.

4. Acupuncture regulation of the gut–ovary axis in PCOS

4.1. Regulatory effects of acupuncture on the gut–ovary axis

4.1.1. Acupuncture improves gut microbiota composition

Acupuncture can modulate gastrointestinal motility and enhance intestinal mucosal barrier function, thereby promoting the restoration of gut homeostasis and improving both metabolic and reproductive functions in patients with PCOS. Studies have shown that acupuncture at acupoints such as Zusanli (ST36) and Tianshu (ST25) significantly promotes intestinal peristalsis, reduces intestinal permeability, and improves the ratio of Firmicutes to Bacteroidetes, contributing to the alleviation of energy metabolism disorders and insulin resistance^[25,26]. In addition, acupuncture has a regulatory effect on the gut microbiota, increasing microbial diversity and promoting the production of SCFAs. Li *et al.*^[27] reported that combined acupuncture and herbal medicine treatment significantly increased the abundance of *Bifidobacterium* and *Lactobacillus* in PCOS patients, while reducing the proportion of pro-inflammatory bacteria, potentially through modulation of glucose and lipid metabolism via the TLR4 signaling pathway. In the regulation of gut microbiota, acupuncture not only enhances the spleen and stomach functions responsible for digestion and absorption, thereby improving intestinal nutrient uptake and metabolic capacity, but also regulates the metabolic efficiency of microbial metabolites. These effects result in a coordinated therapeutic mechanism, integrating systemic regulation with local modulation, which contributes to the comprehensive treatment of PCOS.

4.1.2. Acupuncture suppresses inflammatory signaling along the gut–ovary axis

Acupuncture can inhibit inflammatory signaling pathways such as TLR4/NF- κ B, thereby interrupting the

pathological cascade in which intestinal barrier disruption leads to systemic inflammation and subsequent ovarian dysfunction. Zhang *et al.* [28] reported that electroacupuncture significantly reduced both visceral and subcutaneous fat accumulation in PCOS rats, while also improving their estrous cycles and reproductive function. Specifically, electroacupuncture restored the gut microbiota composition toward a more balanced state, an effect potentially associated with suppression of the LPS-mediated TLR4 signaling pathway [29].

Further research by Ding *et al.* [30] demonstrated that acupuncture at ST36 downregulated NF- κ B expression via the SCFA/FFAR3 pathway, thereby correcting dysbiosis, enhancing intestinal barrier integrity, and alleviating systemic low-grade inflammation. In this process, different acupoints may exert distinct anti-inflammatory effects. For instance, Daimai (GB26) may be more effective in regulating the flow of Qi and blood along the Chong and Ren meridians, while Zusanli focuses on modulating metabolic function in the middle jiao. Through its multi-targeted actions, acupuncture can simultaneously repair intestinal barrier dysfunction and attenuate local ovarian inflammation; however, its underlying molecular mechanisms warrant further investigation.

4.1.3. Acupuncture modulates systemic immune function

Acupuncture modulates systemic immune homeostasis by activating the HPO axis, thereby achieving neuro–endocrine–immune regulation through acupoint stimulation [31]. Studies have shown that warm acupuncture can regulate the secretion of GnRH, LH, and FSH, reduce inflammatory responses, and promote follicular development and endometrial growth [32]. Moreover, acupuncture facilitates the proliferation of beneficial gut microbiota such as *Lactobacillus* and *Bifidobacterium*, enhancing mucosal immune barrier function, suppressing LPS translocation and endotoxemia, and mitigating chronic low-grade inflammation associated with PCOS [33]. Jiang *et al.* [34] found that acupuncture at Zhongwan (CV12) and Sanyinjiao (SP6) significantly increased the proportion of regulatory T cells and inhibited the overactivation of Th17 cells, thereby improving the local ovarian immune microenvironment. Additionally, the meridian effects of acupuncture can alleviate abnormal visceral tension caused by functional dysregulation, thereby enhancing ovarian blood flow and optimizing the follicular development microenvironment [35]. Therefore, acupuncture exerts a bidirectional immunomodulatory effect—suppressing excessive inflammation while enhancing immune tolerance—which is particularly relevant in addressing the chronic inflammatory state of PCOS.

4.2. Acupoint selection

In acupuncture treatment for PCOS, acupoint selection is guided by the principles of strengthening the spleen and stomach to enhance transformation and transportation [5], and regulating local Qi and blood [36], with attention to local and systemic effects. Common local points include Guanyuan (CV4), Zhongji (CV3), Guilai (ST29), and Zigong (EX-CA1), which act directly on the uterus to improve Qi and blood flow in the ovaries and uterus. In addition, middle jiao points such as ST25, GB26, ST36, Shangjuxu (ST37), and Xiajuxu (ST39) are selected to restore the spleen and stomach's ascending and descending functions and help rebalance the gut microbiota.

ST25, the Front-Mu point of the Large Intestine on the Foot-Yangming Stomach Meridian, is also connected to the Chong and Ren vessels. According to the Qian Jin Fang, it is traditionally used to treat lower abdominal pain and prolonged or irregular menstruation caused by blood stasis and uterine dysfunction. Acupuncture at Tianshu helps to clear turbidity and promote the ascent of clear Qi, facilitating gut microbiota metabolism. It is also effective for irregular menstruation and pelvic pain. Studies have shown that acupuncture at Tianshu can indirectly reduce androgen levels, promote follicular development, and restore ovarian function, possibly through

downregulation of hypothalamic Kisspeptin expression and improvement of HPO axis function ^[37]. GB26 is a key acupoint for resolving phlegm, dampness, and blood stasis. Shen *et al.* ^[38] found that electroacupuncture at Daimai improved insulin resistance in PCOS rats, potentially by modulating NF-κB signaling protein expression in the hypothalamus. Furthermore, pairing ST36 with the lower he-sea points ST37 and ST39 enhances the spleen and stomach's transformative functions, promotes the descent of intestinal Qi, and clears damp-heat. Electroacupuncture at these points has been shown not only to regulate gut microbiota composition but also to alleviate ovarian dysfunction caused by chronic inflammation. The underlying mechanism may involve reduced serum levels of TNF-α and IL-1β ^[39,40]. These synergistic effects of multiple acupoints help improve the reproductive microenvironment and modulate metabolic and immune imbalances via the gut–ovary axis, reflecting the holistic and dual-target (root and symptom) approach of acupuncture therapy.

5. Conclusion

According to TCM, the spleen, stomach, and intestinal organs are functionally connected to the ovaries through meridians and the circulation of Qi and blood. Disruption of Qi dynamics in the six fu-organs can lead to gut microbiota imbalance, resulting in the downward flow of damp-heat and phlegm-turbidity along the Chong and Ren meridians, which impairs ovarian function. Modern research has revealed that dysbiosis of the gut microbiota interacts bidirectionally with ovarian function via microbial metabolites and compromised intestinal barrier integrity. This interaction promotes chronic inflammation and metabolic disturbances, both of which are recognized as key contributors to the pathogenesis of PCOS. Acupuncture exerts therapeutic effects by modulating the composition of gut microbiota, suppressing inflammatory pathways, and improving metabolic homeostasis, thereby regulating the gut–ovary axis. In clinical practice, acupoint selection should integrate both local and systemic approaches—combining points such as CV4 and EX-CA1 with middle-jiao points like ST36, ST37, and CV12—to help restore intestinal microbial balance. Despite growing interest in gut microbiota and acupuncture, studies focusing on their interaction in PCOS are still scarce. Future studies should further investigate the mechanisms by which acupuncture regulates the gut microbiota to treat PCOS to provide a solid theoretical foundation and practical guidance for precision therapy.

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Advances in Anticoagulation Therapy for Preeclampsia: A Systematic Review

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Abstract: Preeclampsia (PE) is a multisystem pregnancy disorder. Several pathological processes, such as vascular endothelial dysfunction, an imbalance between coagulation and anticoagulation, and changes in trophoblast characteristics, are involved in the development of PE. The article discusses the pathogenesis of PE. In the third trimester, a protective hypercoagulable state typically develops in normal pregnancies. However, in PE, this state is exacerbated, resulting in a thrombotic phenotype characterized by a systemic inflammatory response and activation of the clotting cascade. This article examines the potential mechanisms involved. The present treatment emphasizes the timely delivery of the fetus. The investigation of anticoagulant therapies is still ongoing, mainly focusing on aspirin and the use of low-molecular-weight heparin for drug-induced thrombosis prevention. In this review, we will summarize the recent findings of reported and ongoing anticoagulation therapy in the treatment of PE. This anticoagulant treatment strategy is essential for the improvement and prevention of PE.

Keyword: Preeclampsia; Therapy; Anticoagulation; Thrombosis; Coagulation monitoring; Heparin

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1. Overview of preeclampsia

Preeclampsia (PE) is a serious complication of pregnancy that affects 3-8% of pregnancies worldwide and is a major cause of fetus-maternal mortality and morbidity ^[1]. In PE, hypertension and proteinuria can lead to extensive end-organ damage ^[2]. This complex process involves multiple organ systems, including proteinuria, acute kidney injury, liver dysfunction, hemolysis, thrombocytopenia, and, less frequently, liver rupture, epilepsy, stroke, and death ^[3]. The pathogenesis of PE remains incompletely understood, but it is believed to involve multiple mechanisms, such as endothelial dysfunction, endovascular inflammation, syncytial trophoblast stress, immunomodulatory disorders, and microembolism ^[1,4-7].

PE manifests changes in procoagulant and anticoagulant pathways beyond the protective hypercoagulation experienced during pregnancy. There are intravascular agglutination, microvascular thrombosis and

hysteroplacental circulatory disturbance associated with the ischemic and oxidative damage of the placenta. The underlying mechanism of this prothrombotic state is influenced by endotheliopathy, achieved by inducing abnormal regulation of coagulation, platelets, and adhesion ligands ^[8].

Currently, the only treatment for PE is to terminate the pregnancy, but this is often associated with an iatrogenic preterm birth. Although the risk of immediate death decreases after birth, health risks for both mother and fetus increase after birth ^[3,6]. Therefore, research efforts are focused not only on the treatment of preeclampsia, but also on ways to prevent its occurrence ^[3]. Early identification and treatment of prothrombotic states can improve the maternal and maternal prognosis of PE. This makes anticoagulation therapy for PE a major research focus. Recent studies have demonstrated that anticoagulant drugs possess anti-inflammatory, anti-apoptotic, and promote the growth and development of trophoblast cells.

In this review, we provide an overview of the significance of anticoagulant therapy in the management of PE, examine the mechanism of action of current anticoagulant therapy and its application in PE.

2. Anticoagulation therapy in preeclampsia

Recent studies have begun to explore the potential of anticoagulation therapy as an innovative approach for managing PE ^[9]. This therapeutic strategy may not only mitigate the thrombotic risks associated with PE but also alleviate its symptoms and reduce the incidence of complications through mechanisms such as improving placental perfusion, reducing vascular endothelial inflammation, and inhibiting thrombus formation ^[10]. Anticoagulants commonly employed include aspirin and low-molecular-weight heparin.

Aspirin, particularly at doses exceeding 100 mg when initiated before 16 weeks of gestation, has demonstrated significant efficacy in reducing the incidence of preterm PE, with reductions exceeding 60%. A secondary analysis of data from the Aspirin Evidence-Based Prevention of PE trial further revealed that this intervention led to a 68% reduction in the length of stay in the neonatal intensive care unit, primarily due to fewer cases of PE occurring before 32 weeks of gestation ^[11].

The combination of low-dose aspirin (LDA) and low-molecular-weight heparin (LMWH) has emerged as a particularly effective regimen. Studies have shown that this combination not only enhances clinical efficacy but also reduces adverse reactions compared to LDA monotherapy ^[12]. This is especially important for pregnant women with comorbidities, as LDA alone may be insufficient to address the clotting disorders that can affect the placenta and fetus ^[13].

Despite these promising findings, research on anticoagulation therapy for PE is still in its early stages. Further high-quality clinical trials and fundamental research are essential to validate these results and optimize treatment protocols. Future studies should focus on elucidating the mechanisms underlying the therapeutic effects of anticoagulants and identifying the most effective combinations and dosages for different patient populations.

3. Mechanisms of anticoagulant therapy

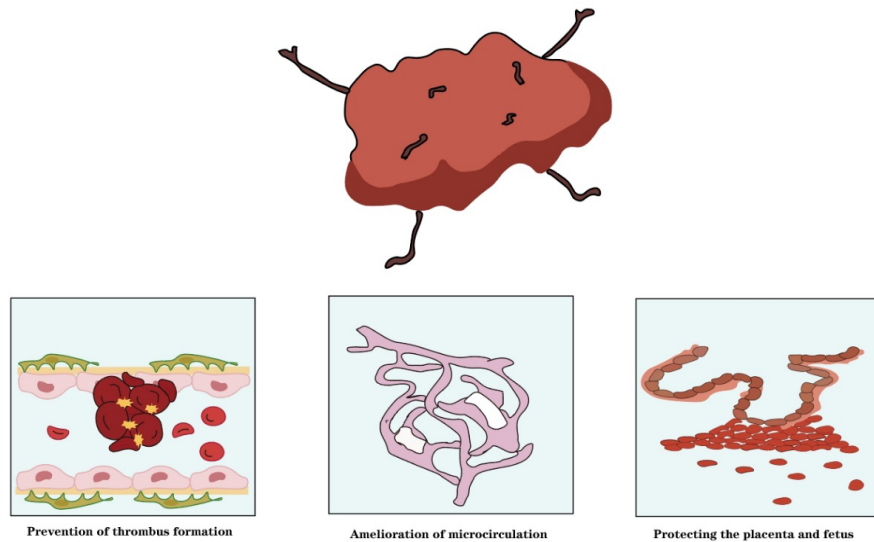


Figure 1. Schematic representation of anticoagulant therapy mechanisms.

3.1. Prevention of thrombus formation

Anticoagulant therapy is designed to prevent thrombus formation by inhibiting clotting factors or disrupting the coagulation cascade while preserving hemostasis.

FXa is central to both intrinsic and extrinsic coagulation pathways, driving prothrombin to thrombin conversion. Development of specific FXa inhibitors can halt thrombin production, achieving an anticoagulant effect while minimizing disruption to primary hemostasis. Thrombin inhibitors block the activation of factors V, VIII, XI, and XIII, inhibiting fibrinogen conversion and platelet activation, thereby preventing clot formation. Heparin activates antithrombin III (AT III) through a key pentasaccharide, inhibiting thrombin and other clotting factors (e.g., XIIa, IXa, XIa, Xa) ^[14]. Low-molecular-weight heparin (LMWH) preferentially targets FXa, reducing thrombin generation ^[15].

Inhibition of Factor XI (FXI) and Factor XII (FXII) reduces hemorrhagic complications while maintaining efficacy. FXI inhibition affects both intrinsic and extrinsic pathways, whereas FXII disruption impacts only the initial contact phase ^[16]. Platelet aggregation is crucial for thrombus formation. Synthetic compounds like 7-N-Acetyl-amino-4-methylcoumarin derivatives inhibit platelet aggregation by suppressing cyclooxygenase-1 (COX-1) and reducing thromboxane A₂ (TXA₂) levels ^[17]. APAC, a proteoglycan analog, blocks collagen- and thrombin-mediated platelet activation, reducing platelet and fibrin deposition in thrombosis models.

Endothelial cells synthesize antithrombin III (ATIII) and produce nitric oxide (NO), promoting vascular relaxation ^[18]. Heparin enhances endothelial NO availability and inhibits PECAM-1 function, reducing vascular inflammation ^[19]. Activated protein C (APC) inhibits intrinsic tenase and prothrombinase complexes, with additional anti-inflammatory and cytoprotective functions via interactions with EPCR and PAR-1. Tissue factor pathway inhibitor (TFPI) inhibits the tissue factor (TF)-VIIa complex, reducing thrombin generation ^[20]. These strategies highlight the multifaceted approach to preventing thrombus formation while balancing anticoagulation and hemostasis.

3.2. Amelioration of microcirculation

The microcirculation, a complex network of minuscule blood vessels, serves as the terminal vascular network of the systemic circulation, intricately delivering oxygenated blood to tissues and organs by their metabolic demands ^[21]. This network, comprising arterioles, capillaries, and venules, is the final destination of the cardiovascular system, where oxygen transfer from red blood cells to parenchymal cells occurs. The microcirculation is also essential for the regulation of solute exchange between the intravascular and tissular spaces, the distribution of hormones and nutrients to cells, the facilitation of immune responses, and the maintenance of hemostasis ^[22]. However, the presence of stable heteroaggregates in the absence of physiological shear stress can increase local blood viscosity and the likelihood of thrombus formation ^[23]. Excessive activation of the coagulation cascade, coupled with the deterioration of anticoagulant and fibrinolytic functions, exacerbates microcirculatory impairment, leading to tissue necrosis and thrombosis ^[23]. Such impairments are implicated in conditions like PE, highlighting the importance of developing methods to enhance microcirculatory function for maternal and fetal well-being.

Inflammation is a significant contributor to microcirculation dysfunction ^[24]. It can lead to endothelial dysfunction, increased vascular permeability, and leukocyte adhesion, all of which impair microcirculatory flow and contribute to tissue ischemia. Anticoagulants, such as heparin, can modulate the inflammatory process by affecting neutrophil migration, complement activation, and cytokine production ^[25]. Vitamin K antagonists (VKAs) also influence inflammatory pathways through the inhibition of growth-arrest-specific protein 6, which subsequently activates receptor tyrosine kinases, such as MER and AXL ^[26].

In the context of pregnancy, heightened inflammation and the initial demise of trophoblast cells can result in deficient placentation. This is characterized by compromised invasion, remodeling of blood vessels, and disruption of microcirculation within the placenta. Unfractionated heparin (UFH) has been shown to inhibit the migratory ability of extravillous trophoblasts induced by hepatocyte growth factor, thereby hindering their differentiation ^[27]. These findings underscore the intricate interplay between inflammation, microcirculation, and placental development and highlight the need for targeted therapeutic interventions to mitigate these effects.

Heparin exerts multifaceted effects on trophoblasts and microcirculation, which are particularly relevant in the context of PE. It has been demonstrated that heparin can prevent the consumption of trophoblasts by inhibiting matrix metalloproteinases (MMPs) and suppressing the activity of tissue inhibitors of metalloproteinases (TIMPs). Additionally, heparin prevents the activation of the complement system in trophoblasts, thereby mitigating inflammation and apoptosis ^[28].

During PE, elevated vascular tone in the maternal microvasculature is observed, primarily due to the vasoconstrictive effects of endothelin-1 and angiotensin II ^[29]. Experimental evidence indicates that women with PE exhibit reduced blood flow area in the choroidal capillaries compared to healthy pregnant individuals, a phenomenon likely mediated by vasospasm ^[30]. Notably, enoxaparin, a low-molecular-weight heparin, significantly reduces the rate of cerebral infarction linked to vasospasm and decreases the frequency of severe vasospasm ^[31].

PE impairs microcirculation through endothelial dysfunction and increased vascular resistance ^[32]. Brain microcirculatory dysfunction in preeclamptic patients is characterized by compromised autoregulation capacity, predisposing the microvasculature to harmful hyperperfusion. Anticoagulant therapy has been shown to reduce maternal vascular reactivity and mitigate vasoconstriction, thereby enhancing microcirculatory function. Therapies that enhance nitric oxide (NO) availability through endothelial nitric oxide synthase (eNOS) upregulation may potentially improve pregnancy outcomes. For instance, administration of unfractionated heparin (UFH) therapy enhances NO bioavailability, as evidenced by increased endothelium-dependent vasodilation mediated by flow-

mediated dilation and alterations in forearm blood flow in response to acetylcholine (ACh) ^[33]. Furthermore, the combination of LMWH, LDA, and pravastatin exerts a synergistic effect on eNOS, thereby enhancing placental blood flow and improving pregnancy outcomes ^[34].

3.3. Protecting the fetus

The immune landscape of pregnancy significantly shapes fetal growth and development ^[35]. LMWH therapy plays a pivotal role in modulating the maternal immune response, preserving fetal immune tolerance, and mitigating the risks associated with abnormal fetal development. By restoring Treg cell homeostasis and enhancing decidual IL-10 mRNA expression, LMWH therapy not only dampens caspase-3 activity but also improves pregnancy outcomes across diverse genetic backgrounds ^[36].

The therapeutic potential of LMWHs extends beyond mere anticoagulation. Their broad application in preventing early pregnancy loss is underpinned by their theoretical association with placental thrombosis and infarction. LMWHs may bolster placental function through various mechanisms, including modulating trophoblast cell apoptosis and influencing growth factors such as HB-EGF. Additionally, their interaction with molecules like Cyr61 suggests a multifaceted approach to placental health and fetal development ^[37].

4. Conclusion

A prothrombotic state is a critical pathological alteration. This prothrombotic tendency is associated with endothelial dysfunction, placental ischemia, and systemic inflammation, contributing to the clinical manifestations of PE.

Anticoagulation therapy has emerged as a vital strategy in managing preeclampsia, targeting the prothrombotic state to prevent thrombosis, improve microcirculation, lower blood pressure, protect the fetus, and enhance maternal prognosis. Low-dose aspirin has demonstrated efficacy in reducing the incidence and severity of preeclampsia and is widely recommended for high-risk pregnancies. Although the role of low molecular weight heparin in preeclampsia prevention remains inconclusive, evidence suggests potential benefits in specific high-risk scenarios.

While current anticoagulation therapies primarily focus on interventions within the coagulation system, future breakthroughs will likely require exploring new therapeutic frontiers. These include modulating endothelial cell function, optimizing immune regulatory mechanisms, and innovating interventions in the angiogenic process.

Disclosure statement

The authors declare no conflict of interest.

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Clinical Analysis of Premature Rupture of Membranes in Late Pregnancy and the Risk of Maternal and Neonatal Infections

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Abstract: *Objective:* To explore the relationship between premature rupture of membranes (PROM) in late pregnancy and diseases related to maternal and neonatal infections. *Methods:* A retrospective analysis was conducted on the clinical data of 300 cases of PROM puerperas (Group A) and 200 cases of normal delivery puerperas (Group B) who gave birth at Datong Fifth People's Hospital from January 2021 to December 2023. The amniotic fluid contamination, placental pathology, maternal and neonatal infection indicators, and the incidence of perinatal infectious diseases were compared between the two groups. *Results:* The degree of amniotic fluid contamination in the PROM group was lower than that in the control group ($P < 0.01$), but the incidence of bloody amniotic fluid was higher ($P < 0.05$). The infiltration rate of inflammatory cells in the placenta was significantly higher in Group A than in Group B ($P < 0.01$). In Group A, the white blood cell count, neutrophil percentage, and procalcitonin levels of the puerperas were significantly increased ($P < 0.05$). The incidence of intra-amniotic infection, neonatal respiratory distress syndrome, and meconium aspiration syndrome was higher in Group A ($P < 0.05$). The white blood cell count and neutrophil indicators of neonates were significantly elevated in Group A. *Conclusion:* Premature rupture of membranes in late pregnancy significantly increases the risk of maternal and neonatal infections. Joint monitoring of multiple laboratory indicators and rational use of antibiotics are important for improving outcomes.

Keywords: Premature rupture of membranes; Maternal and neonatal infections; Chorioamnionitis; Perinatal medicine

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1. Introduction

Premature rupture of membranes in late pregnancy refers to the rupture of the chorion and amnion due to various external factors from 28 weeks of gestation to before the onset of labor. PROM is a major cause of maternal and neonatal infections and deaths. PROM can easily induce puerperal infections, including uterine cavity infections, tubal and ovarian infections, thrombophlebitis, and septicemia, which can be life-threatening

to the mother in severe cases ^[1]. Simultaneously, PROM is highly correlated with the morbidity and mortality of perinatal infants, such as fetal distress, neonatal asphyxia, hypoxic-ischemic encephalopathy, infectious pneumonia, and sepsis. Therefore, it is very meaningful to pay sufficient attention to PROM, ensure the safety of mothers and children as much as possible, study the amniotic fluid situation, placental pathology, maternal infection indicators, perinatal infectious diseases, and neonatal infection indicators of puerperas with PROM in late pregnancy, and conduct a clinical analysis of the risk of maternal and neonatal infections. The research results are reported below.

2. Materials and methods

2.1. General information

The study subjects were parturient women in our hospital from January 2021 to December 2023, including 300 cases of premature rupture of membranes as the observation group (Group A) and 200 cases of normal pregnant women as the control group (Group B).

Inclusion criteria: those who meet the diagnostic criteria of the “Guidelines for the Diagnosis and Treatment of Premature Rupture of Membranes (2015)” issued by the Obstetrics Group of the Obstetrics and Gynecology Branch of the Chinese Medical Association ^[2], and whose first diagnosis upon admission is compatible with premature rupture of membranes, with complete clinical data.

Exclusion criteria: pregnancy complicated by respiratory infection; pregnancy complicated by acute appendicitis, cholecystitis, pancreatitis, or other systemic infectious diseases; pregnancy complicated by important organ diseases such as cardiovascular and cerebrovascular diseases; blood system diseases. The case data retrieved in this study for research analysis were obtained with the informed consent of the parturient’s family members, by medical ethics standards, and approved by the hospital ethics committee.

2.2. Diagnostic basis for premature rupture of membranes

- (1) The pregnant woman complains of vaginal fluid flow or wet underwear;
- (2) Vaginal examination reveals the formation of an amniotic fluid pool in the posterior fornix or amniotic fluid flowing out of the cervical os;
- (3) The amniotic fluid test paper or pad turns blue;
- (4) Microscopically, the fluid in the posterior fornix shows fern-like crystals ^[1].

2.3. Statistical methods

SPSS 22.0 was used for analysis. Measurement data were expressed as mean \pm standard deviation (SD). The *t*-test and chi-square test were used for comparison between groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Amniotic fluid and placenta conditions

In Group A, 86.00% of the amniotic fluid was clear, and the pollution level was significantly lower than that of Group B (69.00%, $P < 0.01$). However, the incidence of bloody amniotic fluid was higher (2.33% vs. 0.00%, $P < 0.05$). There was no significant difference in the incidence of no amniotic fluid between the groups. The infiltration rate of inflammatory cells in placental tissue was 48.33% in Group A, which was significantly

higher than the 14.5% in Group B ($P < 0.01$), indicating a higher incidence of intrauterine inflammation in the premature rupture of membranes group (**Table 1**).

Table 1. Comparison of amniotic fluid and placenta detection between the premature rupture of membranes group and the normal control group

Group	<i>n</i>	Amniotic fluid status [<i>n</i> (%)]						Placental pathology [<i>n</i> (%)]	
		Clear	I°	II°	III°	Blood-stained amniotic fluid	No amniotic fluid	Inflammatory cell infiltration	Non-inflammatory cell infiltration
Group A	300	258 (86.00%)	5 (1.67%)	14 (4.67%)	5 (1.67%)	7 (2.33%)	11 (3.67%)	145 (48.33%)	155 (51.67%)
Group B	200	138 (69.00%)	14 (7.00%)	27 (13.50%)	18 (9.00%)	0 (0.00%)	3 (1.50%)	29 (14.50%)	171 (85.50%)
χ^2		21.052	9.337	12.439	14.705	-	1.350	60.540	
<i>P</i>		0.000004	0.002	0.0004	0.0001	0.045	0.245	7.208E-15	

3.2. Infectious indicators of puerperas

The levels of WBC (10.64 ± 3.45), neutrophil percentage (76.08 ± 6.82), and PCT (0.50 ± 2.09) in Group A were higher than those in Group B (WBC: 7.63 ± 2.82 ; neutrophil percentage: 74.37 ± 9.00 ; PCT: 0.07 ± 0.13), and the differences were statistically significant ($P < 0.05$). There was no significant difference in NEUT# and CRP between the two groups, suggesting that WBC, neutrophil percentage, and PCT have more predictive value for intrauterine infection (**Table 2**).

Table 2. Comparison of laboratory indicators between the premature rupture of membranes group and the normal control group

Group	<i>n</i>	WBC count ($\times 10^9/L$)	Neutrophil count ($\times 10^9/L$)	Neutrophil percentage (%)	Procalcitonin (ng/mL)	CRP (mg/L)
Group A	300	10.636 ± 3.447	8.408 ± 4.402	76.079 ± 6.822	0.502 ± 2.089	15.728 ± 26.105
Group B	200	7.628 ± 2.823	10.070 ± 3.056	74.369 ± 9.000	0.073 ± 0.134	16.090 ± 27.241
<i>t</i>		2.413	1.921	2.287	2.380	-0.157
<i>P</i>		0.0162	0.055	0.022	0.018	0.875

3.3. Perinatal outcomes

The intra-amniotic infection rate in Group A was 50.33%, which was significantly higher than that in Group B (21.00%, $P < 0.01$). Neonatal respiratory distress syndrome (3.33% vs 0%, $P < 0.01$) and meconium aspiration syndrome (0.67% vs 3.5%, $P < 0.05$) were also significantly increased. There were no significant differences in other outcomes such as neonatal asphyxia, pneumonia, hyperbilirubinemia, and death (**Table 3**).

Table 3. Comparison of the incidence of perinatal-related diseases between the premature rupture of membranes group and the normal control group

Group	Fetal distress	Intra-amniotic infection	Neonatal asphyxia	Meconium aspiration syndrome	Neonatal Respiratory distress syndrome	Neonatal Pneumonia
Group A	34 (11.33%)	151 (50.33%)	6 (2.00%)	2 (0.67%)	10 (3.33%)	23 (7.67%)
Group B	13 (6.50%)	42 (21.00%)	2 (1.00%)	7 (3.50%)	0 (0.00%)	11 (5.50%)
χ^2	3.291	43.566	0.259	3.965	-	0.889
<i>P</i>	0.070	4.0988E-11	0.611	0.046	0.007	0.346

Group	Neonatal hyperbilirubinemia	Hypoxic-ischemic encephalopathy	Neonatal mortality	Neonatal erythema	Neonatal pustulosis
Group A	134 (44.67%)	12 (4.00%)	3 (1.00%)	14 (4.67%)	5 (1.67%)
Group B	79 (39.50%)	6 (3.00%)	0 (0.00%)	11 (5.50%)	2 (1.00%)
χ^2	1.310	0.346	-	0.175	0.054
<i>P</i>	0.252	0.557	0.279	0.675	0.816

3.4. Neonatal infection indicators

The white blood cell count (22.46 ± 8.84), neutrophil count (15.80 ± 7.69), and neutrophil percentage (66.85 ± 12.94) in Group A were significantly higher than those in Group B (white blood cell count: 18.17 ± 8.39 ; neutrophil count: 12.05 ± 7.32 ; neutrophil percentage: 62.78 ± 13.66 , $P < 0.01$). There was no significant difference in PCT between the two groups, which may be related to the degree of infection and the timing of detection (Table 4).

Table 4. Comparison of neonatal laboratory infection indicators between the premature rupture of membranes group and the normal control group

Group	<i>n</i>	WBC count ($\times 10^9/L$)	Neutrophil count ($\times 10^9/L$)	Neutrophil percentage (%)	Procalcitonin (ng/mL)
Group A	300	22.460 ± 8.836	15.795 ± 7.692	66.853 ± 12.936	3.430 ± 5.857
Group B	200	18.169 ± 8.387	12.053 ± 7.317	62.776 ± 13.655	2.240 ± 5.187
<i>t</i>		4.838	275.739	251.084	177.296
<i>P</i>		2.16E-06	2.46E-06	0.004	0.114

4. Discussion

Preterm rupture of membranes (PROM) in late pregnancy is a common obstetric complication. The rupture breaks the barrier between the fetus and the external environment, providing a pathway for pathogens to ascend and infect, thus increasing the risk of infection for both mother and child^[2]. The results of this study showed that although the degree of amniotic fluid pollution in the PROM group was lower than that in the control group, the incidence of bloody amniotic fluid was significantly increased, suggesting that placental dysfunction or inflammatory reactions may occur earlier. Placental pathology examination revealed that the inflammatory cell infiltration rate in the PROM group was 48.33%, significantly higher than the 14.5% in the control group, indicating that subclinical intrauterine infection is widespread in this population, and clinicians should be highly vigilant.

Regarding infectious laboratory indicators, this study confirmed that the white blood cell count, neutrophil percentage, and procalcitonin levels in the PROM group were higher than those in normal deliveries, suggesting that these indicators can serve as important references for early identification of maternal infection risk ^[3]. Although PCT is more specific for early bacterial infections, its changes are limited in mild or early infections and need to be judged in combination with other indicators. C-reactive protein is commonly used for clinical monitoring, but it is greatly affected by stress such as childbirth and surgery, indicating that its independent predictive value is limited. Combining literature, a multi-item joint evaluation of WBC, neutrophil percentage, and PCT has more clinical practicality ^[4,5].

In terms of perinatal outcomes, the incidence of fetal intra-amniotic infection, neonatal respiratory distress syndrome, and meconium aspiration syndrome in the PROM group was significantly higher than that in the control group, indicating that intrauterine inflammation has a significant impact on fetal lung development and neonatal respiratory function. This study also found that the serum white blood cell count and neutrophil ratio were elevated in newborns in the PROM group, suggesting that the fetus had already initiated an inflammatory response in utero. It is worth noting that although some newborns did not show obvious clinical symptoms, their laboratory indicators already showed an infection trend, indicating that laboratory screening has early warning significance.

In summary, preterm rupture of membranes in late pregnancy significantly increases the risk of infection for both mother and child. Clinicians should strengthen dynamic monitoring of amniotic fluid characteristics and residual volume, evaluate infection risk based on multiple laboratory indicators such as WBC, neutrophil percentage, and PCT, rationally use prophylactic antibiotics, and individually balance the relationship between infection and premature birth ^[6]. By optimizing management strategies, it is expected to effectively improve mother and child outcomes.

5. Conclusion

Preterm rupture of membranes in late pregnancy significantly increases the risk of infection for both mother and child. Attention should be paid to the combined detection of amniotic fluid monitoring and inflammatory indicators, rational use of antibiotics, and the development of individualized management strategies to optimize mother and child outcomes.

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Disclosure statement

The authors declare no conflict of interest.

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Clinical Effect of HPV-DNA Typing Detection Combined with TCT in Cervical Cancer Screening

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Abstract: *Objective:* To explore the importance of combined diagnosis (TCT+HPV-DNA) in cervical cancer screening for diagnosis and treatment. *Methods:* From March 2024 to December 2024, 35 cases were diagnosed as positive by the gold standard (colposcopy biopsy), and then screened by TCT and HPV-DNA typing respectively, and the different results were analyzed. *Results:* Compared with TCT+HPV-DNA typing, the coincidence rate, specificity and sensitivity of TCT and HPV-DNA typing were significantly lower ($P<0.05$). *Conclusion:* Combined diagnosis (i.e. TCT+HPV-DNA typing test) in cervical cancer screening can ensure the accuracy of the results and prompt patients to obtain targeted treatment plans at an early stage.

Keywords: Cervical cancer; Screening; TCT; Detection of HPV-DNA typing

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1. Introduction

Currently, women worldwide are suffering from the impact of cervical cancer. According to the World Health Organization, a large number of women are diagnosed with cervical cancer every year, and the disease is beginning to affect younger women globally. In China, cervical cancer, as a high-incidence malignant tumor, has a significant impact on women's health and quality of life. However, it is worth noting that cervical cancer is one of the few malignant tumors in recent years with a determined etiology that can be actively prevented and controlled through early screening. Therefore, to ensure effective control of cervical cancer mortality and incidence, it is of great significance to implement effective and scientific screening methods early. In recent years, TCT examination and HPV-DNA typing detection have become common methods for cervical cancer screening. TCT screening mainly focuses on cellular morphology, carefully observing cervical cell conditions with slide preparation techniques, detecting abnormal changes in a timely manner, and subsequently identifying early cervical cancer and precancerous lesions. However, it should be noted that there are some issues with the TCT screening method, including the lack of typical bacterial morphology or insufficient cell collection data, which may increase the risk of subsequent diagnostic errors. HPV-DNA typing detection focuses on the viral level, detecting the gene sequence of human papillomavirus, promptly determining the type of infection, and understanding the patient's risk of subacute infection or HPV infection ^[1]. However, this method can only

clarify the status of viral infection but cannot reflect the morphological changes of cervical cells affected by the virus, making it difficult to accurately determine the degree of lesions. In view of this, this study selected 62 patients with suspected cervical cancer to carry out research work, followed by different screening methods such as TCT, TCT + HPV-DNA typing detection, etc. The aim is to determine the best diagnostic method to ensure that patients' conditions are controlled at an early stage.

2. Materials and methods

2.1. General information

In this study, 62 patients suspected of having cervical cancer were randomly selected using a random number table for research from March 2024 to December 2024. The mean age was 38.42 ± 5.57 years old (range 29–68 years old), and the mean weight was 58.79 ± 4.47 kg (range 38–76 kg).

Inclusion criteria: (1) Normal organ function; (2) No contraindications to the diagnostic methods used in this study; (3) Signed informed consent; (4) History of sexual activity. Exclusion criteria: (1) Recent pelvic radiation or treatment for cervical disease; (2) Reproductive system infection or communication difficulties.

2.2. Methods

Patients were instructed to abstain from sexual activity and avoid using vaginal medications for 3 days before the examination. HPV-DNA genotyping: Patients were positioned in the lithotomy position to fully expose the cervix. The surface secretions were wiped clean with a sterile swab. A sampler was inserted into the external orifice and rotated clockwise for 5 turns, held for 10 seconds, then broken off, and the sample was preserved for timely examination. After centrifugation, 15 high-risk HPV types were detected using PCR-reverse dot blot hybridization. The samples were first subjected to PCR amplification, and the PCR products were then hybridized with specific probes on a membrane strip to analyze the final results. TCT: The patient's position was the same as for HPV-DNA genotyping. The cervix was exposed, and secretions were wiped away. A sampling brush was inserted to a depth of 1 cm and rotated clockwise for 5 turns. The sampler was placed in Thinprep cell preservation solution. After 10 oscillations, the sample was processed for the separation of inflammatory cells, blood secretions, mucus, and epithelial cells. A thin cell smear was prepared using a liquid-based thin-layer method and stained for microscopic examination. Colposcopy: The patient's position was the same as for HPV-DNA genotyping. The surface secretions of the cervix were wiped clean with a sterile swab. The color and shape of the cervix were observed under a low-power microscope, and blood vessels were examined through a filter. The cervix was treated with glacial acetic acid solution to observe the morphology and color of the blood vessels. Abnormal areas were sampled for examination.

2.3. Observation indicators

2.3.1. Analysis of diagnostic results using different diagnostic methods

Calculate the concordance rate, specificity, and sensitivity of patients undergoing different diagnostic methods. The concordance rate = number of correctly diagnosed cases / total number of cases; sensitivity (true positive rate) = number of true positives / (number of true positives + number of false negatives) $\times 100\%$; specificity = number of true negatives / (number of true negatives + number of false positives) $\times 100\%$. Here, true negatives mainly refer to cases where the disease is absent and the diagnosis is negative; true positives mainly refer to cases where the disease is actually present and the diagnosis is positive.

2.3.2. Analysis of differences in diagnostic efficacy among different diagnostic methods

Compare and statistically analyze the differences in concordance rate, specificity, and sensitivity among TCT testing, HPV-DNA genotyping, and TCT + HPV-DNA genotyping test results.

2.4. Statistical methods

Statistical analysis was performed using SPSS 24 software. Count data were expressed as percentages (%) and compared using the chi-square test. A P -value < 0.05 was considered statistically significant.

3. Results

3.1. Analysis of diagnostic results using different diagnostic methods

The diagnostic accuracy of TCT was 80.65% (50/62), specificity was 47.83% (11/23), and sensitivity was 38.46% (15/39). The diagnostic accuracy of HPV-DNA genotyping was 83.87% (52/62), specificity was 58.33% (14/24), and sensitivity was 44.74% (17/38). The diagnostic accuracy of combined TCT and HPV-DNA genotyping was 98.39% (61/62), specificity was 97.06% (33/34), and sensitivity was 92.86% (26/28). See **Table 1**.

Table 1. Analysis of diagnostic results of different diagnostic methods (%)

Gold standard	TCT		HPV-DNA Typing Test		TCT + HPV-DNA Typing Test		Total
	Positive	Negative	Positive	Negative	Positive	Negative	
Positive	11	24	14	21	33	2	35
Negative	12	15	10	17	1	26	27
Total	23	39	24	38	34	28	62

3.2. Analysis of diagnostic efficacy differences among different diagnostic methods

Compared with TCT+HPV-DNA typing detection, the coincidence rate, specificity, and sensitivity of TCT and HPV-DNA typing detection were significantly lower ($P < 0.05$). See **Table 2**.

Table 2. Analysis of diagnostic efficacy differences among different diagnostic methods (%)

Group	Number of Cases	Concordance Rate	Specificity	Sensitivity
TCT	62	80.65%*	47.83%*	38.46%*
HPV-DNA genotyping test	62	83.87%*	58.33%*	44.74%*
TCT + HPV-DNA genotyping test	62	98.39%	97.06%	92.86%

Note: Compared with TCT+HPV-DNA typing detection, * $p < 0.05$.

4. Discussion

4.1. Clinical manifestations, pathogenesis, and screening significance of cervical cancer

Cervical cancer, as the most common female reproductive system tumor after breast cancer, lacks typical manifestations in its early stages. Only a few patients may experience changes in the texture or increase in vaginal discharge, which is often overlooked. However, as the disease progresses, typical symptoms such as contact vaginal bleeding may appear, for example, spot bleeding after gynecological examination or bleeding signs after

sexual activity. Irregular vaginal bleeding may occur during non-menstrual periods. In the late stages, when surrounding tissues are invaded by cancer tissue, symptoms such as lower extremity swelling and pain, urgency and frequency of urination, and constipation may manifest. In severe cases, uremia and ureteral obstruction may develop, which not only reduces the patient's quality of life but also shortens their life expectancy. Persistent infection with high-risk human papillomavirus (HPV) is closely linked to the pathogenesis of cervical cancer. The E6 and E7 oncogenes of HPV integrate into the host cell genome, leading to complete inhibition of the tumor suppressor genes p53 and Rb, ultimately disrupting cell cycle regulation ^[3]. Abnormal differentiation and proliferation of cervical epithelial cells gradually evolve into precancerous lesions, and improper or untimely intervention can lead to cervical cancer. Additionally, factors such as multiple sexual partners, reduced immune function, and smoking can accelerate the progression of the disease. Therefore, the combined application of TCT and HPV-DNA typing detection in cervical cancer screening has important clinical value ^[2]. These two methods can complement each other, allowing for the understanding of the viral infection status and observation of changes in cell morphology, thereby enabling early prevention in the precancerous stage of cervical cancer.

4.2. Limitations of single screening techniques for cervical cancer and the clinical value of combined testing

Scholars have indicated that TCT (ThinPrep Cytologic Test) and HPV-DNA typing have become commonly used diagnostic techniques for cervical cancer. TCT focuses on the morphological analysis of cervical cells, but it may be ineffective in recognizing atypical cells, poor slide quality, or inadequate cell collection, leading to increased misdiagnosis. HPV-DNA typing can accurately identify the type of virus infection, but it cannot determine the morphological changes caused by virus-infected cervical cells, resulting in a high risk of overdiagnosis. If used singly, both methods have limitations, making it difficult for most patients with early-stage cervical cancer or precancerous lesions to detect timely manner and miss the best treatment opportunity. Therefore, the combined application of TCT and HPV-DNA typing in cervical cancer screening has important clinical value. These two methods can form a complementary relationship, allowing for the observation of cell morphological changes besides understanding the state of viral infection, enabling accurate assessment of the risk of cervical lesions and improving the detection rate of precancerous lesions and cervical cancer to a certain extent. This allows patients to receive effective treatment at the optimal time, reducing personal mortality and morbidity rates and ensuring women's health.

4.3. Clinical benefits and value of combined TCT and HPV-DNA genotyping in cervical cancer screening

With the use of cytological screening techniques, the screening rate for cervical cancer has improved to some extent. A series of operations, including slide preparation and TCT sampling, requires systematic processing of specimens according to standard requirements to avoid external factors affecting specimen quality. This helps to view the distribution of bacteria evenly and clearly, increasing bacterial identification and preventing cell contamination or loss, enabling the timely detection of abnormal cervical epithelial cells ^[4]. However, studies have shown that among adult women who have regular sexual activity, the positive rate of TCT is relatively low, and misdiagnosis is prone to occur, leading to limitations in clinical use ^[5]. The occurrence and development of cervical cancer and precancerous lesions are closely related to HPV infection, and clinical practice has incorporated HPV-DNA typing as a core screening method. The results of this study indicate that compared with TCT+HPV-DNA

typing, the coincidence rate, specificity, and sensitivity of TCT and HPV-DNA typing are significantly lower^[6]. This suggests that TCT+HPV-DNA typing plays a crucial role in early cervical cancer screening, providing valuable information support for clinical disease differential diagnosis. Whether it is the HPV-DNA typing method or the TCT detection method, their sensitivity, specificity, and coincidence rate are not as good as the combined use of the two methods when used singly. The TCT examination method observes cell chromatin, size, and structure under a microscope to determine whether the patient has lesions^[7]. However, during the actual operation, factors such as the pathologist's subjective interpretation ability, cell preservation conditions, and the standardization of cell collection can directly affect the final examination results. In other words, differences in physicians' interpretation criteria can increase missed diagnoses and misdiagnoses, especially when faced with atypical hyperplastic cells. If the cell collection volume is insufficient and it is difficult to obtain diseased cells, the final detection coincidence rate and sensitivity will decrease accordingly. HPV-DNA typing can accurately detect viral infections, but it cannot effectively determine the presence of subtypes or viruses, and the degree of cervical cell lesions caused by viral infections is also difficult to visually demonstrate^[8].

In clinical practice, some HPV-positive patients remain in the process of transient viral infection without typical cervical lesions. If the diagnosis is made based on HPV test results alone, it may lead to overdiagnosis and significantly reduce specificity. Moreover, some patients experience excessively low viral loads, making HPV test results appear false-negative and ultimately affecting sensitivity. Due to the various issues that arise when these two examination methods are used singly, they cannot meet the high requirements of current precise cervical cancer screening. Based on this, to ensure that the above problems are effectively avoided, the implementation of combined detection methods is extremely important. This can effectively integrate information on cell morphological changes and viral infections, enabling clinicians to obtain more accurate and comprehensive evidence during disease diagnosis. It is helpful to improve the effectiveness of cervical cancer screening and precancerous lesion screening, providing many conveniences. In exploring the clinical value of combining HPV-DNA typing and TCT in cervical cancer screening, Xiong found that using colposcopy pathology results as the gold standard, the positive rate of the gold standard diagnosis was 51.43%, and the negative rate was 48.57% among the 70 selected patients undergoing cervical cancer screening^[9]. The combined diagnosis of HPV-DNA typing + TCT achieved a specificity of 94.12%, an accuracy of 95.71%, a positive predictive value of 94.59%, and a negative predictive value of 96.97%. The sensitivity was as high as 97.22%. Compared with the single TCT examination method, which had a negative and positive predictive value of 73.68% and 75.00%, and an accuracy, specificity, and sensitivity of 74.29%, 70.59%, and 77.78%, respectively, and the single HPV-DNA typing method, which had a negative and positive predictive value of 75.00% and 73.53%, and an accuracy, specificity, and sensitivity of 74.29%, 73.53%, and 75.00%, respectively, the results of the HPV-DNA typing + TCT detection method were consistent with those of colposcopy pathology, effectively improving the detection rate of cervical cancer. These findings align with the results of this study, confirming the advantages of combining HPV-DNA typing and TCT in cervical cancer screening.

5. Conclusion

In summary, there is a close relationship between HPV infection and the occurrence of cervical cancer, and the core cause of cervical cancer lesions is persistent infection with high-risk HPV. Therefore, it is crucial to actively carry out HPV-DNA typing diagnosis in cervical cancer patients. However, although single HPV-DNA

typing can detect viral infection, it can only provide risk indications and cannot directly reflect the degree of lesions. Therefore, to compensate for the limitations of single diagnostic methods, clinicians should consider using two methods together, namely HPV-DNA typing + TCT. This approach can not only improve diagnostic specificity and sensitivity but also ensure the accuracy of the final diagnosis, making it worthy of clinical adoption. However, it is important to note that this study has limitations such as time constraints and sample size restrictions. To verify the accuracy of the results, future clinical studies should extend the study duration and expand the sample size to further confirm the reliability of the findings.

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Disclosure statement

The authors declare no conflict of interest.

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Expression of CLDN18.2, CDX2, SATB2, and PAX8 in Primary and Gastrointestinal-Derived Mucinous Ovarian Carcinoma

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Abstract: *Objective:* Primary and metastatic mucinous ovarian carcinomas share similar morphologies but have significant differences in prognosis. This study aims to explore the immunophenotypic characteristics of primary and gastrointestinal-derived mucinous ovarian carcinomas. *Methods:* A total of 230 cases of primary and gastrointestinal-derived mucinous ovarian tumors surgically removed at Junning County People's Hospital and the Affiliated Hospital of Qingdao University from 2014 to 2024 were randomly selected. These included 67 cases of primary mucinous ovarian carcinoma, 56 cases of primary borderline mucinous ovarian tumor, 61 cases of colorectal-derived mucinous ovarian carcinoma (including 26 cases from the appendix and 35 cases from the colorectum), 26 cases of gastric-derived mucinous ovarian carcinoma, and 20 cases of mucinous cystadenoma of the ovary. All specimens were reviewed and confirmed by two experienced pathologists according to the 2020 WHO classification criteria. Immunohistochemistry was used to detect the expression differences of CLDN18.2 in primary mucinous ovarian tumors. Furthermore, the expressions of CLDN18.2, CDX2, SATB2, and PAX8 were jointly detected in primary and gastrointestinal metastatic mucinous ovarian tumors to explore the immunoexpression characteristics of multiple immune markers in primary ovarian and upper and lower gastrointestinal-derived ovarian metastatic mucinous carcinomas. *Results:* 1. CLDN18.2 showed varying degrees of expression in mucinous cystadenoma of the ovary, borderline mucinous ovarian tumor, and primary mucinous ovarian carcinoma, but was not expressed in normal ovarian and fallopian tube tissues. 2. In primary mucinous ovarian carcinoma, CLDN18.2 and PAX8 showed high expression, while CDX2 and SATB2 showed lower expression. In gastric-derived mucinous ovarian carcinoma, CLDN18.2 and CDX2 showed high expression, while SATB2 and PAX8 were almost not expressed. In colorectal-derived mucinous ovarian carcinoma, CDX2 and SATB2 showed high expression, while CLDN18.2 and PAX8 showed low expression. *Conclusion:* CLDN18.2 shows high expression in both primary and gastric-derived mucinous ovarian carcinomas and can be used as an auxiliary method for differentiating primary and gastrointestinal-derived mucinous ovarian carcinomas along with CDX2, SATB2, and PAX8.

Keywords: Mucinous ovarian carcinoma; Gastrointestinal-derived mucinous ovarian carcinoma; CLDN18.2; Immunohistochemistry; Differential diagnosis

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1. Introduction

Ovarian cancer poses a severe threat to women's health. Although it ranks third in the incidence of female reproductive system tumors, it has the highest fatality rate^[1]. Most women in developed countries die from ovarian cancer^[2]. Epithelial ovarian carcinoma (EOC) is the main pathological type of ovarian cancer^[3], accounting for approximately 90% of cases^[4]. Mucinous ovarian carcinoma (MOC) is one of the subtypes of EOC, with unique clinical, histological, and molecular characteristics^[5]. However, it is difficult to distinguish the primary source of the tumor^[6]. Compared to primary mucinous ovarian carcinoma (PMOC), metastatic mucinous ovarian carcinoma (MMOC) is more common, mainly originating from the gastrointestinal tract and pancreatobiliary system^[7]. There are differences in the prognosis of PMOC and MMOC^[8], so correctly identifying the source of ovarian tumors is crucial for clinical management.

CLDNs are a family of tight junction proteins composed of at least 27 protein members^[9]. Evidence suggests that CLDNs proteins are key structural and functional components of tight junction proteins, playing a critical role in tumorigenesis and inflammation^[10]. CLDN18, a member of the CLDNs family, has two subtypes: CLDN18.1 and CLDN18.2. In normal tissues, CLDN18.1 is expressed in the lungs^[11], while CLDN18.2 is only expressed in differentiated gastric mucosal epithelial cells^[12]. CLDN18 is highly expressed in gastric cancer, esophageal cancer, pancreatic cancer, ovarian cancer, cholangiocarcinoma, and lung adenocarcinoma^[13]. Studies by Halimi *et al.*^[14,15] have shown positive expression of CLDN18 in gastric-type mucinous cystadenoma and gastric-type mucinous borderline tumors of mucinous ovarian tumors.

CDX2 encodes a transcription factor that plays an important role in regulating the development and differentiation of intestinal epithelial cells^[16]. Research indicates that CDX2 is expressed in both normal and neoplastic intestinal epithelial tissues, serving as a marker for intestinal-derived tumors^[16,17].

SATB2 is a human DNA-binding protein involved in transcriptional regulation and chromatin remodeling, with high expression specifically localized in the epithelial cells of the lower digestive tract^[18]. Recently, the role of STAB2 in the differential diagnosis of colorectal cancer has gradually been recognized. Aldaoud's^[19] study shows that SATB2 is a sensitive and highly specific marker in colorectal cancer. Besides CDX2, CK7, and CK20, PAX8 has also been mentioned for distinguishing the primary source of mucinous ovarian cancer^[19]. The paired box gene (PAX) encodes a family of nine transcription factors involved in organogenesis during human development^[20]. Bowen *et al.*'s^[21] studies have confirmed that PAX8 is an important marker of genital tract origin.

Currently, no research has been found on the immunohistochemical expression characteristics of combined applications of CLDN18.2, CDX2, SATB2, and PAX8 in primary ovarian and gastrointestinal-derived mucinous carcinomas. Therefore, this study intends to investigate the immunophenotypic characteristics of different sources of ovarian mucinous carcinomas by jointly detecting the expression of several immune markers in primary ovarian and gastrointestinal-derived ovarian mucinous carcinomas.

2. Materials and methods

2.1. General information

A total of 230 cases of primary and gastrointestinal-derived mucinous ovarian tumors were randomly selected from the surgical resection cases at Junnan County People's Hospital and the Affiliated Hospital of Qingdao University from 2014 to 2024. These included 67 cases of primary mucinous ovarian cancer, 56 cases of primary borderline mucinous ovarian tumors, 61 cases of mucinous ovarian cancer derived from the large intestine (including 26 cases from the appendix and 35 cases from the colon and rectum), 26 cases of ovarian mucinous carcinoma derived

from the stomach, and 20 cases of ovarian mucinous cystadenoma. Additionally, normal ovarian and fallopian tube tissues from 10 patients who underwent total hysterectomy due to uterine fibroids were selected as controls. All specimens were reviewed and confirmed by two experienced pathologists based on the 2020 WHO classification criteria. None of the selected patients received radiotherapy or chemotherapy before surgery. All study subjects signed informed consent, and the study protocol was approved by the Medical Research Ethics Committee of Junnan County People’s Hospital.

2.2. Reagents and methods

Surgical specimens were routinely fixed and embedded in paraffin. The paraffin-embedded tissues were continuously sectioned at a thickness of 4μm and subjected to immunohistochemical staining for CLDN18.2 (abcam, ab203563; 1:500), CDX2 (ABclonal Technology Co, a20222; 1:50), SATB2 (abcam, ab92446; 1:50), and PAX8 (abcam, ab53490; 1:100). Result interpretation: The slides were reviewed by experienced pathologists in a double-blind manner. Positive expression of CLDN18.2 was defined as staining of the outer basement membrane, while CDX2, SATB2, and PAX8 were all expressed in the cell nucleus. A semi-quantitative scoring system was used to evaluate staining intensity and the percentage of stained cells. Staining intensity was scored as follows: 0 for no color; 1 for light yellow, 2 for brown yellow, and 3 for tan. The percentage of stained cells was scored as follows: < 5% positive cells were scored as 0; 5–25% as 1; 26–50% as 2; 51–75% as 3; and 76–100% as 4. The sum of the two scores represented the staining score, with a total score > 2 considered positive and ≤ 2 considered negative.

2.3 Statistical methods

SPSS 26.0 software was used for statistical analysis. The chi-square test (χ^2 test) was selected as the statistical method, and a *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Expression of CLDN18.2 in mucinous ovarian tumors

The results showed that CLDN18.2 immunohistochemical staining was mainly localized to the outer edge of the cell basement membrane, and positive CLDN18.2 staining appeared as brown yellow or tan. CLDN18.2 was not expressed in normal ovaries but showed varying degrees of expression in mucinous cystadenomas, borderline mucinous ovarian tumors, and primary mucinous ovarian cancers (**Table 1, Figure 1**).

Table 1. Expression of CLDN18.2 in mucinous ovarian tumors

Category	Staining intensity				Staining percentage					CLDN18.2	
	0	1	2	3	0	1	2	3	4	Positive	Negative
Primary mucinous Ovarian cancer	3	4	25	35	3	4	15	15	30	56 (83.6%)	11 (16.4%)
Borderline mucinous Tumor	5	7	28	16	6	17	28	5	0	26 (46.4%)	30 (53.6%)
Mucinous Cystadenoma	3	12	5	0	3	10	4	3	0	3 (15%)	17 (85%)

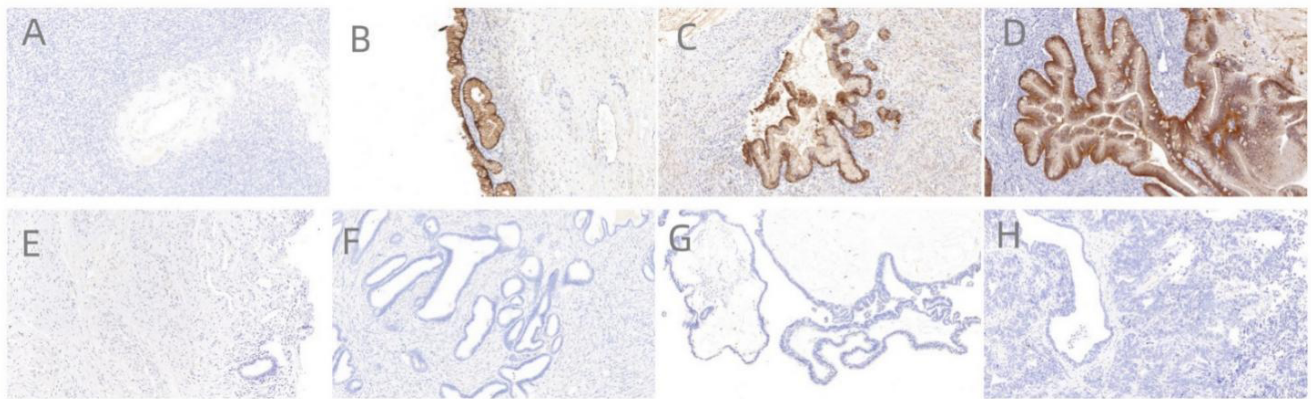


Figure 1. The expression of CLDN18.2 in normal ovary (A), mucinous cystadenoma (B), borderline mucinous tumor (C), primary mucinous ovarian cancer (D), normal fallopian tube (E), serous cystadenoma (F), borderline serous tumor (G), and high-grade serous ovarian cancer (H) at 200x magnification.

3.2. Immunohistochemical characteristics of CLDN18.2, CDX2, SATB2, and PAX8 in primary mucinous ovarian cancer and gastrointestinal-derived mucinous ovarian cancer

3.2.1. Comparison between primary mucinous ovarian cancer (PMOC) and gastrointestinal-derived mucinous ovarian cancer

The positive expression rate of CLDN18.2 in PMOC was 92.5% (62/67), which was significantly higher than that in colon-derived mucinous ovarian cancer (37.7%). The difference between the two was statistically significant ($P < 0.05$), but there was no significant difference compared to stomach-derived mucinous ovarian cancer (96.2%) ($P > 0.05$). CDX2 had a positive expression rate of 29.9% (20/47) in PMOC, which was significantly lower than that in stomach-derived mucinous ovarian cancer (61.5%) and colon-derived mucinous ovarian cancer (90.2%). The differences were statistically significant ($P < 0.05$). The positive expression of SATB2 in PMOC and stomach-derived mucinous ovarian cancer accounted for only 4.5% (3/67) and 4.8% (1/26), respectively, while the positive expression in colon-derived mucinous ovarian cancer was as high as 91.8% (56/61). The difference between PMOC and colon-derived mucinous ovarian cancer was statistically significant ($P < 0.05$), but there was no significant difference compared to stomach-derived mucinous ovarian cancer ($P > 0.05$). The expression rate of PAX8 in PMOC was 43.3% (29/67), while only 1 case (1.6%) of colon-derived mucinous ovarian cancer showed positive expression, and no positive expression was observed in stomach-derived mucinous ovarian cancer. Compared with gastrointestinal-derived mucinous ovarian cancer, the positive expression rate of PAX8 in PMOC was higher, and the difference was statistically significant ($P < 0.05$).

3.2.2. Comparison between colon-derived mucinous ovarian cancer and stomach-derived mucinous ovarian cancer

CLDN18.2 was positively expressed in 37.7% (23/61) of colon-derived mucinous ovarian cancers, while the positive expression rate of CLDN18.2 in stomach-derived mucinous ovarian cancer was 96.2% (25/26). The positive expression rate of CLDN18.2 in stomach-derived mucinous ovarian cancer was significantly higher than that in colon-derived mucinous ovarian cancer, and the difference was statistically significant ($P < 0.05$). CDX2 was positively expressed in 90.2% (55/61) of colon-derived mucinous ovarian cancers, while the positive expression rate in stomach-derived mucinous ovarian cancer was 61.5% (16/26). The positive expression rate

of CDX2 in colon-derived mucinous ovarian cancer was higher than that in stomach-derived mucinous ovarian cancer, and the difference was statistically significant ($P < 0.05$). SATB2 had a positive expression rate of 91.8% (56/61) in colon-derived mucinous ovarian cancer, but only 1 case (4.8%) showed positive expression in stomach-derived mucinous ovarian cancer. The positive expression rate of SATB2 in colon-derived mucinous ovarian cancer was significantly higher than that in stomach-derived mucinous ovarian cancer, and the difference was statistically significant ($P < 0.05$). PAX8 was positively expressed in only 1 case (1.6%) of colon-derived mucinous ovarian cancer and was not expressed in stomach-derived mucinous ovarian cancer. There was no significant difference between the two ($P > 0.05$).

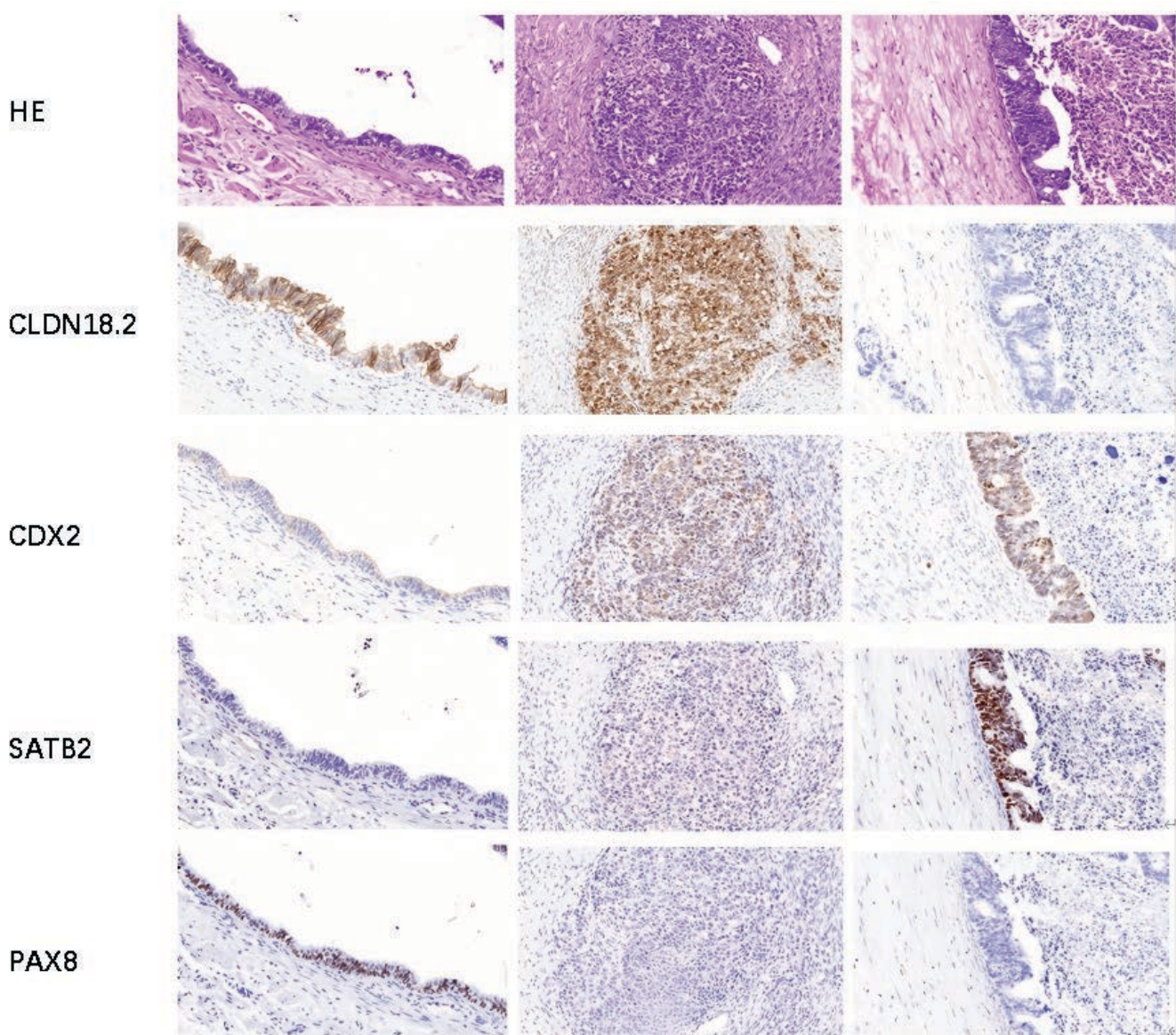


Figure 2. Expression of CLDN18, CDX2, SATB2, and PAX8 in PMOC, metastatic mucinous ovarian cancer from gastrointestinal origin.

Table 2. Expression of CLDN18.2, CDX2, SATB2, and PAX8 in PMOC and mucinous ovarian cancer of gastrointestinal origin

		PMOC (<i>n</i> = 67) (%)	Metastatic colorectal (<i>n</i> = 61) (%)	Gastric metastatic (<i>n</i> = 26) (%)	<i>P</i> -value 1	<i>P</i> -value 2	<i>P</i> -value 3
CLDN18.2	+	62 (92.5%)	23(37.7%)	25(96.29%)	< 0.001	0.867	< 0.001
	-	5 (7.5%)	38(62.3%)	1(4.8%)			
CDX2	+	20 (29.9%)	55 (90.2%)	16(61.5%)	< 0.001	0.005	0.004
	-	47 (70.1%)	6 (9.8%)	10 (28.5%)			
SATB2	+	3 (4.5%)	56 (91.8%)	1 (4.8%)	< 0.001	1.000	< 0.001
	-	64(95.5%)	5(8.2%)	25(96.2%)			
PAX8	+	29 (43.3%)	1 (1.6%)	0 (0.0%)	< 0.001	< 0.001	1.000
	-	38 (56.7%)	60(98.4%)	26(100%)			

P-value 1: Comparison between PMOC and mucinous ovarian cancer of colonic origin; *P*-value 2: Comparison between PMOC and mucinous ovarian cancer of gastric origin; *P*-value 3: Comparison between colonic and gastric origin groups.

4. Discussion

Mucinous ovarian tumors are classified as benign, borderline, and malignant, but these three forms can coexist within the same pathological tissue [8,22]. Many scholars believe that mucinous ovarian cancer (MOC) progresses from benign to borderline and then to malignant pathology [23]. Distinguishing between metastatic and primary ovarian malignancies is crucial. CLDN18 is one of 27 proteins that constitute the claudin family, essential for forming tight junctions and maintaining the polarity of epithelial and endothelial cells [24]. Experimental evidence suggests that abnormal expression of CLDN18 and CLDN18.2 in gastric cancer is significant for diagnosis, treatment, and prognosis [12,25–28]. This study demonstrates that CLDN18.2 is positively expressed in both mucinous cystadenoma and borderline mucinous ovarian tumors, often focal but sometimes diffuse. In PMOC, the positive expression rate of CLDN18.2 protein is significantly increased, with 51 out of 60 positive cases showing diffuse expression. Based on CLDN18.2 expression levels in mucinous ovarian tumors, it has certain diagnostic value for PMOC.

Eighty percent of MOC cases originate from metastases, primarily from the gastrointestinal tract [26]. Distinguishing the primary source of MOC can be challenging due to the histological similarity between some primary and metastatic mucinous carcinomas. Although numerous studies have confirmed that clinical features like tumor size and laterality can distinguish between PMOC and MMOC [6,29–31], no single feature can unequivocally differentiate them [6]. Immunohistochemistry is the most commonly used method to distinguish between primary and metastatic tumors. Experiments have confirmed the differential diagnostic value of CK7, CK20, CDX2, SATB2, PAX8, and others in ovarian cancer [17–21,32]. However, relying on a single antibody is insufficient for determining the tumor's origin, and several antibodies are typically required for differential diagnosis. In our study, we first used CLDN18.2, CDX2, SATB2, and PAX8 to differentiate the primary source of mucinous ovarian cancer. Our results show that CLDN18.2 expression is higher in PMOC and gastric-derived mucinous ovarian cancer than in colonic-derived mucinous ovarian cancer. CDX2 expression is higher in colonic and gastric-derived mucinous ovarian cancers than in PMOC. SATB2 shows high expression in colonic-derived mucinous ovarian cancer but minimal expression in PMOC and gastric-derived mucinous ovarian cancer. PAX8 is positively expressed in about half of PMOC cases but rarely in gastrointestinal-derived mucinous ovarian cancer. Based on these results, the

common immunohistochemical phenotype in PMOC is CLDN18.2(+), CDX2(-), SATB2(-), PAX8(+), in colonic-derived mucinous ovarian cancer is CLDN18.2(-), CDX2(+), SATB2(+), PAX8(-), and in gastric-derived mucinous ovarian cancer is CLDN18.2(+), CDX2(+), SATB2(-), PAX8(-).

5. Conclusion

In summary, our findings provide an experimental basis for the differential diagnosis of CLDN18.2 in mucinous ovarian cancer. However, further research is needed to understand the mechanism of CLDN18.2's role in the development of mucinous ovarian cancer and whether it can be a new therapeutic target.

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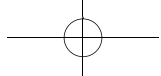
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Hospital



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