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ORIGINAL ARTICLE

Klotho in placenta related pregnancy complications: A systematic review and meta-analysis

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ABSTRACT

Background: Klotho is an anti aging protein implicated in oxidative stress regulation, endothelial protection, placental senescence, and fetal growth. Its role in placenta related pregnancy complications remains unclear. To systematically review the evidence on Klotho in placenta related pregnancy complications and to quantitatively synthesize eligible studies. **Methods:** This systematic review and meta-analysis was reported in accordance with the PRISMA 2020 statement. Of 54 records identified, 9 studies were included in the systematic review and 7 in the meta-analysis. Study quality was assessed using a structured tool for non randomized studies. Random-effects models with Hartung-Knapp adjustment were used to calculate pooled standardized mean differences as Hedges' *g* with 95% confidence intervals (CIs).**Results:** Most included studies suggested reduced Klotho expression in placenta related pathological pregnancies. Meta-analysis showed significantly lower serum Klotho levels in pregnancies complicated by intrauterine growth restriction, fetal growth restriction, and small for gestational age (Hedges'*g* = -1.07, 95% CI: -1.34 to -0.80) and in pregnancies with adverse fetal outcomes (Hedges'*g* = -1.13, 95% CI: -1.29 to -0.97). Placental Klotho levels were also significantly reduced in pregnancy complications (Hedges'*g* = -1.35, 95% CI: -1.80 to -0.90). By contrast, pooled associations for serum Klotho in preeclampsia and overall pregnancy complications were not statistically significant due to substantial heterogeneity.

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Conclusions: Reduced Klotho is consistently associated with placental dysfunction, particularly in pregnancies with fetal growth impairment and adverse fetal outcomes. Placental Klotho appears to provide a more stable signal than circulating Klotho.

Keywords: Klotho, placenta, preeclampsia, fetal growth restriction, pregnancy complications, meta-analysis

INTRODUCTION

Placenta related pregnancy complications, particularly preeclampsia, intrauterine growth restriction (IUGR), fetal growth restriction (FGR), and small for gestational age (SGA), remain major causes of maternal, fetal, and neonatal morbidity.[1–3] Although these conditions differ clinically, they share a common pathophysiological basis centered on placental dysfunction, including abnormal trophoblast development, impaired uteroplacental perfusion, oxidative stress, endothelial imbalance, and inadequate fetal nutrient supply.[4] Increasing evidence therefore supports the view that these disorders represent overlapping manifestations of placental insufficiency rather than entirely separate disease entities.[5]

Klotho, originally identified as an anti aging protein, has recently attracted attention in pregnancy research because of its potential roles in oxidative stress regulation, endothelial protection, cellular senescence, and fetal growth signaling.[6,7] The alpha Klotho isoform exists in both membrane bound and soluble forms, and both may be relevant to placental biology.[6,8] Recent studies and reviews have suggested that reduced Klotho expression may contribute to placental aging, vascular maladaptation, and abnormal fetal development, thereby linking Klotho to key mechanisms underlying PE and fetal growth disorders.[9,10]

Several human studies have reported decreased Klotho levels in maternal blood, cord blood, or placental tissue in pregnancies complicated by PE, IUGR, SGA, or related adverse fetal outcomes.[11,12] In particular, lower Klotho has been associated with placental aging lesions, maternal vascular malperfusion, and impaired fetal growth. However, the existing literature remains inconsistent. Some studies have reported lower circulating or placental Klotho in complicated pregnancies, whereas others have shown preserved or even increased maternal circulating Klotho, especially in PE.[13,14] These discrepancies may reflect differences in disease phenotype, gestational timing, specimen source, and assay method.

In addition, circulating and placental Klotho may reflect different aspects of disease biology. Circulating Klotho is likely influenced by maternal systemic factors, whereas placental Klotho may more directly represent local trophoblast expression and placental stress responses.[9,13] A systematic evaluation that distinguishes between these compartments is therefore needed.

Accordingly, the present study was conducted to systematically review and quantitatively synthesize the available human evidence on Klotho in placenta related pregnancy complications. By integrating data across PE, IUGR, SGA, FGR, and adverse fetal outcomes, and by separately examining serum and placental Klotho, this study aimed to clarify whether reduced Klotho is a consistent feature of placental disease and to identify areas in which the current evidence remains heterogeneous.

METHODS

Study design

This study was designed as a systematic review and meta-analysis to investigate the association between Klotho and placenta-related pregnancy complications, with particular emphasis on circulating and placental Klotho levels in PE, IUGR, SGA, FGR, stillbirth, and adverse fetal outcomes. The review and reporting process was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement. This study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University (Approval No. 2024-478). This meta-analysis was prospectively registered in PROSPERO under the registration number CRD420261338595.

Literature search and study selection

A comprehensive search of electronic databases was performed to identify relevant studies examining Klotho in human pregnancies complicated by placenta-related disorders. The databases searched were PubMed, Embase, Cochrane CENTRAL, and PsycINFO, and the final search was conducted on February 28, 2026. For transparency and reproducibility, the full search strategy, including search terms and Boolean operators, is provided in the Supplementary file 1. Following the removal of duplicate records, the remaining studies were screened on the basis of title and abstract, and potentially eligible articles were subsequently reviewed in full text.

The study selection process is summarized in the

PRISMA flow diagram. In total, 54 records were initially identified, of which 9 duplicates were removed. The remaining 45 records underwent title and abstract screening, after which 21 articles were considered eligible for full-text assessment. Following full-text review, 12 articles were excluded, and 9 studies were ultimately included in the systematic review.[6,7,9–11,13–16] Among these, 7 studies provided sufficient quantitative data for inclusion in the meta-analysis.[9–11,13–16]

Eligibility criteria

Studies were considered eligible if they met all of the following criteria: they were original studies involving human participants; they investigated placenta-related pregnancy complications; they reported Klotho-related data in maternal, fetal, or placental specimens; and they provided sufficient information for qualitative synthesis, with extractable quantitative data available for meta-analysis where applicable.

Studies were excluded if they were not original research articles, did not report Klotho-related data, or were conducted in non-human models. Review articles were retained for the qualitative synthesis to provide contextual background, but were not included in the quantitative synthesis.

Data extraction

Data were extracted in a standardized manner from all eligible studies. The following information was collected: study identifier, first author, year of publication, country, study design, total sample size, type of placenta-related pregnancy complication, Klotho measurement type, specimen type, assay method, principal findings, and relevant study notes.

The pregnancy complications of interest included PE, IUGR, SGA, FGR, and stillbirth. Klotho-related measures included soluble α-Klotho, membrane α-Klotho, Klotho mRNA, and, where relevant to the qualitative synthesis, Klotho-related genetic polymorphisms. Biological specimens included maternal serum or plasma, placental tissue, umbilical cord blood, and fetal membranes. Assay methods included enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC), polymerase chain reaction (PCR), western blotting (WB), and single nucleotide polymorphism (SNP) assays.

Quality assessment

The methodological quality of the included studies was assessed using a structured scoring framework based on commonly applied criteria for non-randomized studies. The following domains were evaluated: clearly stated aim, inclusion of consecutive patients, prospective data collection, endpoints appropriate to the study aim, unbiased assessment of study endpoints, follow-up period appropriate to the study aim, loss to follow-up less than 5%, prospective calculation of study size, adequacy of the control group, contemporaneity of comparison groups, baseline equivalence of groups, and adequacy of statistical analyses.

Each domain was scored individually, and a total quality score was calculated for each study. For review articles, domains that were not applicable because of study design were recorded accordingly.

Outcomes of interest

Quantitative synthesis was performed for outcomes with sufficient comparable data. The primary outcomes included serum Klotho levels in PE, serum Klotho levels in IUGR/SGA/FGR, placental Klotho levels in pregnancy complications, serum Klotho levels in overall pregnancy complications, and serum Klotho levels in relation to adverse fetal outcomes.

Statistical analysis

Meta-analyses were performed for outcomes with adequate quantitative data. Because Klotho levels were measured across different study populations and analytical platforms, pooled effect sizes were calculated using the standardized mean difference and expressed as Hedges'g with 95% confidence intervals (CIs). A negative effect size indicated lower Klotho levels in the case group relative to the control group. The study selection process was presented using a PRISMA flow diagram. The characteristics of the included studies and the results of the quality assessment were summarized in tabular form. Forest plots were generated for each pooled outcome, and the results of the leave-one-out sensitivity analyses were presented in a supplementary Table 1.

Only studies involving human participants were included in this review. Review articles were incorporated into the qualitative synthesis to provide interpretive context for the existing evidence base, whereas only original studies with extractable quantitative data were included in the meta-analysis. Given the expected clinical and methodological variability among studies, all pooled analyses were conducted using random-effects models. Hartung-Knapp adjustment was applied to the random-effects estimates. Statistical heterogeneity was evaluated using Cochran's Q test, tau-squared (τ^2), and the I-squared (I^2) statistic. Larger I^2 values were interpreted as indicating greater between-study inconsistency.

Sensitivity analysis

To assess the robustness of the pooled estimates, leave-one-out sensitivity analyses were performed for each meta-analysis. In these analyses, individual studies were sequentially omitted, and the pooled effect size, 95% CI,

P-value, and I^2 were recalculated after each omission. This procedure was used to determine whether any single study exerted a disproportionate influence on the overall results or on the magnitude of heterogeneity.

RESULTS

Study selection

The study selection process is shown in Figure 1. A total of 54 records were initially identified through database searching. After removal of 9 duplicate records, 45 records remained for title and abstract screening. Of these, 24 records were excluded based on title and abstract. The full texts of the remaining 21 articles were assessed for eligibility. Twelve articles were subsequently excluded for the following reasons: not original research (n = 4), no Klotho-related data reported (n = 3), and non-human study design (n = 5). Ultimately, 9 studies were included in the systematic review, of which 7 provided sufficient quantitative data for meta-analysis.

Characteristics of included studies

The characteristics of the included studies are summarized in Table 1. A total of nine studies published between 2012 and 2025 were included in the systematic review, comprising seven original studies and two review articles. The studies were conducted in different geographic regions, including the United States, China, Israel, Italy, and Europe. Among the original studies, the main study designs were case-control and cross-sectional studies.

The included studies investigated several placenta-related pregnancy complications, primarily PE, IUGR, SGA, FGR, and stillbirth. PE and IUGR were the most frequently studied conditions, either alone or in combination, while SGA and stillbirth were less commonly reported.

Klotho was assessed in multiple biological specimens, including maternal serum or plasma, placental tissue, umbilical cord blood, and fetal membranes. Placenta and maternal circulation were the most commonly examined sample types. Most studies evaluated α-Klotho, including soluble and membrane-bound forms, whereas some also assessed Klotho mRNA expression or genetic polymorphisms. The principal assay methods included ELISA, immunohistochemistry, polymerase chain reaction, western blotting, and SNP assay.

Overall, most original studies suggested that Klotho expression was reduced in placenta-related pathological pregnancies, particularly in PE and IUGR. Lower circulating, cord blood, or placental Klotho levels were associated with fetal growth restriction, placental aging, maternal vascular malperfusion, or altered endocrine signaling. However, some inconsistency was observed. In particular, one study reported increased maternal plasma α-Klotho levels in PE, while placental Klotho expression did not differ significantly from controls. In addition, the study on stillbirth mainly focused on Klotho-related polymorphisms rather than quantitative Klotho expression.

Quality assessment

The methodological quality of the included original studies is summarized in Table 2. Overall, study quality was moderate to high, with total scores ranging from 19 to 24. Franklin (2019) and Ferrari (2012) achieved the highest score of 24, while Loichinger (2016) and Zeng (2025) scored 23, indicating generally strong methodological quality. Fan (2016) and Miranda (2014) each scored 20, mainly because of limitations in prospective data collection. Sabren (2024) had the lowest score of 19, with lower ratings in prospective data collection, unbiased assessment, sample size calculation, control group adequacy, and baseline equivalence.

All studies scored well for clearly stated aims, appropriate endpoints, contemporary groups, and statistical analysis. By contrast, follow up was limited across studies, with all receiving a score of 1 in this domain. Overall, the included studies were considered methodologically acceptable for qualitative and quantitative synthesis.

Meta-analysis of serum klotho levels in preeclampsia

Five studies were included in the meta-analysis of serum Klotho levels in PE: Zeng (2025), Fan (2016), Miranda (2014), Sabren (2024), and Loichinger (2016) (Figure 2). The pooled random-effects analysis showed that serum Klotho levels tended to be lower in women with PE than in controls, but the overall difference was not statistically significant (Hedges'g = -0.61, 95% CI: -1.48 to 0.27).

Substantial between-study heterogeneity was observed (I^2 = 96.9%, τ^2 = 0.48, P < 0.01). Four studies reported lower serum Klotho levels in PE, including Zeng (2025) (Hedges' g = -1.28, 95% CI:-1.63 to -0.93), Fan (2016) (Hedges'g = -1.25, 95% CI: -1.53 to -0.97), Miranda (2014) (Hedges'g = -0.55, 95% CI: -0.71 to -0.38), and Sabren (2024) (Hedges'g = -0.42, 95% CI: -0.73 to -0.12). In contrast, Loichinger (2016) showed higher serum Klotho levels in PE than in controls (Hedges'g = 0.43, 95% CI: 0.23 to 0.63), opposite to the direction reported in the other studies.

META-ANALYSIS OF SERUM KLOTHO LEVELS IN IUGR, SGA, AND FGR

Five studies were included in the analysis of serum

Table 1: Characteristics of studies included in the systematic review

Study ID	First author	Year	Country	Study design	Sample size	Complication	Klotho measurement	Specimen type	Key findings
1	Sabren	2024	Israel	Case-control	49	PE, IUGR	Membrane α-Klotho	Placenta, Serum	Lower serum α-Klotho and placental mKL in PE/IUGR.
2	Fan	2016	China	Cross-sectional	42	PE, IUGR	α-Klotho (mRNA, protein)	Placenta, Serum, Umbilical Cord	Lower placental and serum α-Klotho in PE; linked to birth weight and renal function.
3	Franklin	2019	USA	Case-control	54	IUGR	Soluble α-Klotho, mRNA Klotho	Serum, Umbilical Cord, Placenta	Lower cord α-Klotho in IUGR; linked to placental aging and angiopoietin-2.
4	Loichinger	2016	USA	Case-control	61	Preeclampsia (PE)	Soluble α-Klotho, Membrane α-Klotho	Plasma, Placenta, Fetal membranes	Higher maternal α-Klotho in PE; placental Klotho unchanged.
5	Miranda	2014	USA	Cross-sectional	327	PE, SGA	Soluble α-Klotho	Plasma, Cord Blood, Placenta	Lower maternal α-Klotho in PE/SGA; associated with fetal growth restriction.
6	Ferrari	2012	Italy	Case-control	96	Stillbirth	Klotho (mRNA, SNP)	Placenta, Serum	No overall Klotho SNP effect; eNOS rs1800783 linked to AGA stillbirth.
7	Zeng	2025	China	Case-control	52	IUGR	Soluble α-Klotho, mRNA Klotho	Serum, Umbilical Cord, Placenta	Lower Klotho in maternal, cord, and placental samples in IUGR; altered GH/IGF-1.
8	Kanbay	2024	Europe	Review	N/A	PE, IUGR, SGA	α-Klotho (soluble, membrane)	Serum, Placenta, Umbilical Cord	Review suggests lower α-Klotho in PE/SGA and relevance to fetal development.
9	Cecati	2025	Italy	Review	N/A	PE, SGA	α-Klotho (mKlotho and sKlotho)	Serum, Placenta	Review links reduced Klotho to PE, placental aging, and abnormal fetal growth.

PE, preeclampsia; IUGR, intrauterine growth restriction; SGA, small for gestational age; FGR, fetal growth restriction; ELISA, enzyme linked immunosorbent assay; WB, western blot; IHC, immunohistochemistry; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; mRNA, messenger ribonucleic acid.

Table 2: Methodological quality assessment of the included studies

Study	Clear aims	Consecutive patients	Prospective data	Appropriate endpoints	Unbiased assessment	Follow up	Loss follow<5%	Sample size	Control group	Contemporary groups	Baseline equivalence	Statistical analysis	Total score
Sabren (2024)	2	2	1	2	1	1	2	1	1	2	1	2	18
Fan (2016)	2	1	0	2	2	1	2	1	2	2	2	2	19
Franklin (2019)	2	2	2	2	2	1	2	2	2	2	2	2	23
Loichinger (2016)	2	2	1	2	2	1	2	2	2	2	2	2	22
Miranda (2014)	2	2	0	2	2	1	2	2	2	2	2	2	21
Ferrari (2012)	2	2	2	2	2	1	2	2	2	2	2	2	23
Zeng (2025)	2	2	2	2	2	1	2	2	1	2	2	2	22

Klotho levels in pregnancies complicated by IUGR, SGA, or FGR: Miranda (2014), Franklin (2019), Zeng (2025), Fan (2016), and Loichinger (2016) (Figure 3). The pooled random-effects model demonstrated significantly lower serum Klotho levels in affected pregnancies than in controls (Hedges'g = -1.07, 95% CI: -1.34 to -0.80).

Moderate-to-substantial heterogeneity was observed (I^2 = 67.6%, τ^2 = 0.03, P = 0.01). Notably, all included studies showed a consistent direction of effect, indicating lower circulating Klotho levels in the case groups. The largest effect size was observed in Miranda (2014) (Hedges'g = -1.32, 95% CI: -1.56 to -1.09), followed by Franklin (2019) (Hedges'g = -1.20, 95% CI: -1.50 to -0.90), Zeng (2025) (Hedges'g = -1.12, 95% CI: -1.41 to -0.84), Fan (2016)(Hedges'g = -0.87, 95% CI: -1.19 to -0.55), and Loichinger (2016) (Hedges'g = -0.83, 95% CI: -1.03 to -0.63).

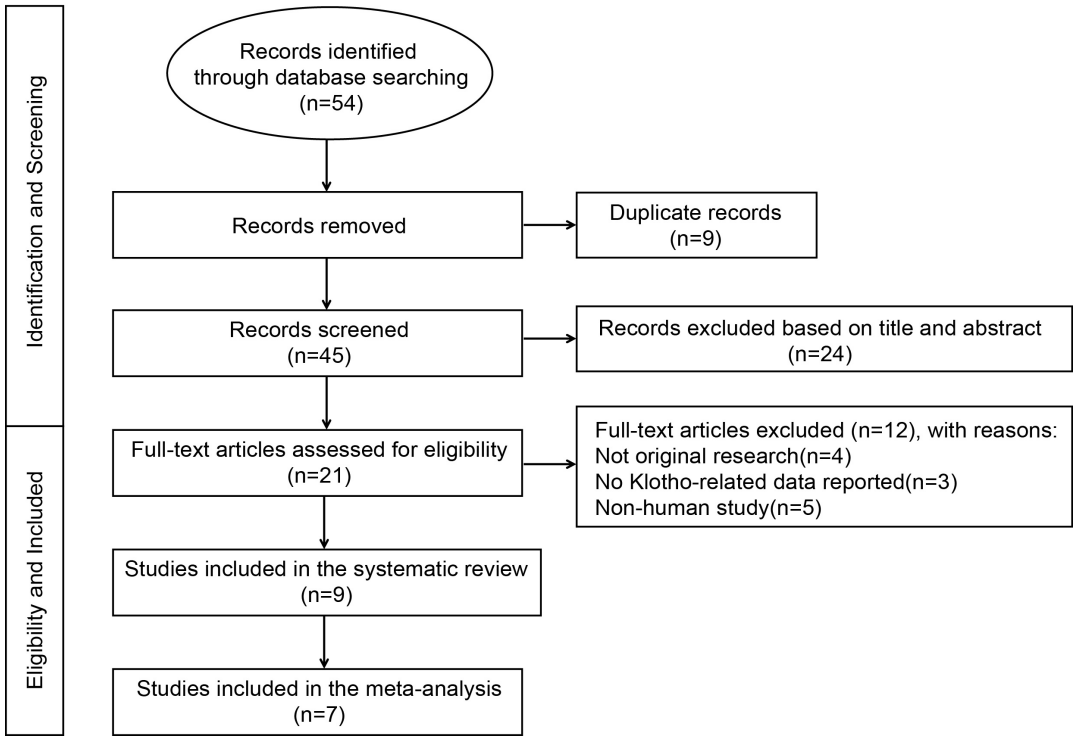

Figure 1. PRISMA flow diagram of study selection. A total of 54 records were identified through database searching. After removal of 9 duplicates, 45 records were screened, 24 were excluded after title and abstract review, and 21 full text articles were assessed for eligibility. Finally, 9 studies were included in the systematic review, of which 7 were eligible for meta-analysis.

Meta-analysis of placental Klotho levels in pregnancy complications

Three studies, namely Zeng (2025), Franklin (2019), and Fan (2016), were included in the meta-analysis of placental Klotho levels in pregnancy complications (Figure 4). The pooled random-effects model showed that placental Klotho levels were significantly lower in complicated pregnancies than in controls (Hedges'g = -1.35, 95% CI: -1.80 to -0.90).

Heterogeneity was low to moderate and not statistically significant (I^2 = 40.4%, τ^2 = 0.01, P = 0.19). All three studies consistently demonstrated reduced placental Klotho expression in the case groups. The individual effect sizes were -1.54 (95% CI: -1.82 to -1.27) for Zeng (2025), -1.32 (95% CI: -1.56 to -1.09) for Franklin (2019), and -1.17 (95% CI: -1.47 to -0.87) for Fan (2016).

Meta-analysis of serum Klotho levels in overall pregnancy complications

Six studies were included in the pooled analysis of serum Klotho levels across overall pregnancy complications: Zeng (2025), Franklin (2019), Miranda (2014), Fan (2016), Sabren (2024), and Loichinger (2016)(Figure 5). The pooled random-effects estimate suggested lower serum Klotho levels in complicated pregnancies than in controls, but the association did not reach statistical significance (Hedges'g = -0.72, 95% CI: -1.51 to 0.07).

Marked heterogeneity was present (I^2 = 98.1%, τ^2 = 0.56, P < 0.01). Most studies reported reduced serum Klotho levels in cases, including Zeng (2025) (Hedges'g = -1.30, 95% CI: -1.51 to -1.10), Franklin (2019) (Hedges'g = -1.15, 95% CI: -1.43 to -0.87), Miranda (2014) (Hedges'g = -1.10, 95% CI: -1.38 to -0.83), Fan (2016) (Hedges'g = -1.06, 95% CI: -1.35 to -0.78), and Sabren (2024) (Hedges'g = -0.39, 95% CI: -0.61 to -0.16). However, Loichinger (2016) reported an opposite effect direction, showing higher serum Klotho levels in the complication group than in controls (Hedges'g = 0.67, 95% CI: 0.48 to 0.86).

Meta-analysis of serum Klotho levels in relation to fetal outcomes

Four studies were included in the meta-analysis of serum Klotho levels in relation to adverse fetal outcomes: Franklin (2019), Miranda (2014), Zeng (2025), and Fan (2016)(Figure 6). The pooled random-effects model showed that serum Klotho levels were significantly lower in pregnancies with adverse fetal outcomes than in controls (Hedges'g = -1.13, 95% CI: -1.29 to -0.97).

No heterogeneity was detected across studies (I^2 = 0.0%, τ^2 = 0, P = 0.64), indicating excellent consistency. All four studies demonstrated concordant results, with

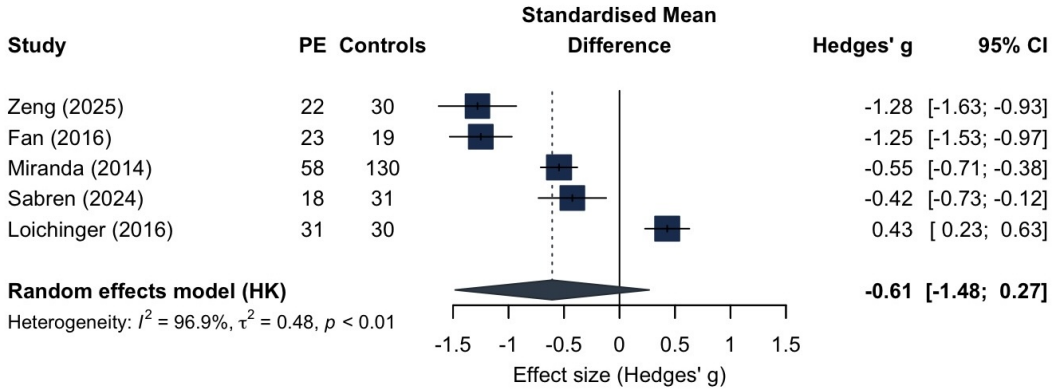

Figure 2. Forest plot of serum Klotho levels in preeclampsia. The pooled analysis showed a non significant trend toward lower serum Klotho levels in preeclampsia compared with controls (Hedges'*g* =-0.61, 95% CI: -1.48 to 0.27), with substantial heterogeneity (*I2* = 96.9%). Most studies showed decreased serum Klotho in preeclampsia, although one study reported an opposite effect direction. PE, preeclampsia.

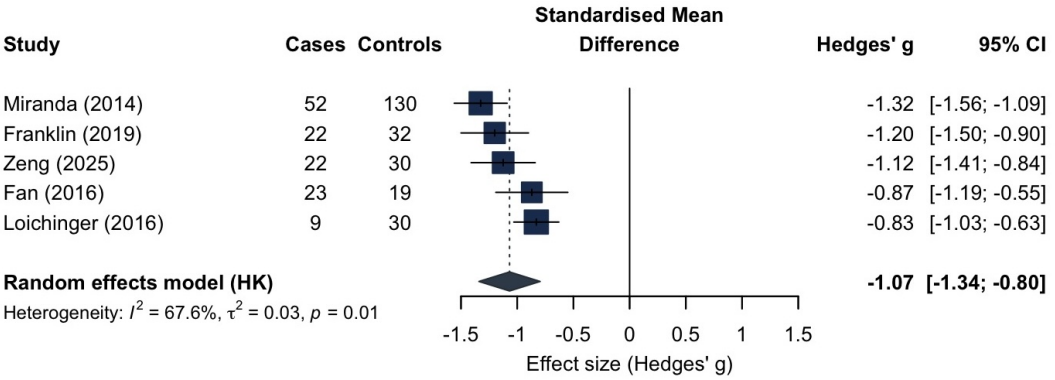

Figure 3. Forest plot of serum Klotho levels in pregnancies complicated by IUGR, SGA, and FGR. The pooled analysis showed significantly lower serum Klotho levels in pregnancies complicated by IUGR, SGA, and FGR than in controls (Hedges'*g* =-1.07, 95% CI: -1.34 to -0.80), with moderate heterogeneity (*I2* = 67.6%). All included studies showed a consistent direction of effect. IUGR, intrauterine growth restriction; SGA, small for gestational age; FGR, fetal growth restriction.

lower serum Klotho levels in the adverse outcome groups. The individual effect sizes were -1.23 (95% CI: -1.54 to -0.92) for Franklin (2019), -1.17 (95% CI: -1.44 to -0.90) for Miranda (2014), -1.14 (95% CI: -1.35 to -0.92) for Zeng (2025), and -0.96 (95% CI: -1.26 to -0.67) for Fan (2016).

Sensitivity analysis

Leave-one-out sensitivity analyses were performed for all pooled outcomes, and the results are summarized in Supplementary Table 1.

For serum Klotho and PE, the pooled effect remained negative after sequential omission of each study, ranging from -0.45 to -0.87. However, statistical significance was achieved only when Loichinger (2016) was excluded (Hedges'*g* = -0.87, 95% CI:-1.58 to -0.15, *P* = 0.031), while heterogeneity remained high (I^2 = 90.4%). Exclusion of any other study did not materially change the non-significant overall result, with I^2 values remaining above 96%. These findings indicate that the PE analysis was sensitive to the inclusion of Loichinger (2016).

For serum Klotho and IUGR/SGA/FGR, the pooled association remained consistently significant regardless of which study was omitted, with pooled effect sizes ranging from -0.99 to -1.15 and *P*-values < 0.001. Although the degree of heterogeneity varied from 42.0% to 75.1%, the direction and statistical significance of the association remained unchanged, indicating robust findings.

For placental Klotho and pregnancy complications, omission of Fan (2016) or Zeng (2025) still yielded statistically significant pooled effects (Fan omitted: Hedges'*g* = -1.42, 95% CI: -2.81 to -0.03, *P* = 0.049; Zeng omitted: Hedges'*g* = -1.27, 95% CI: -2.22 to -0.31, *P* = 0.038). By contrast, omission of Franklin (2019) resulted in a non-significant pooled association (Hedges'*g* = -1.36, 95% CI -74.00 to 1.02, *P* = 0.087), suggesting that the statistical significance of this analysis was influ-

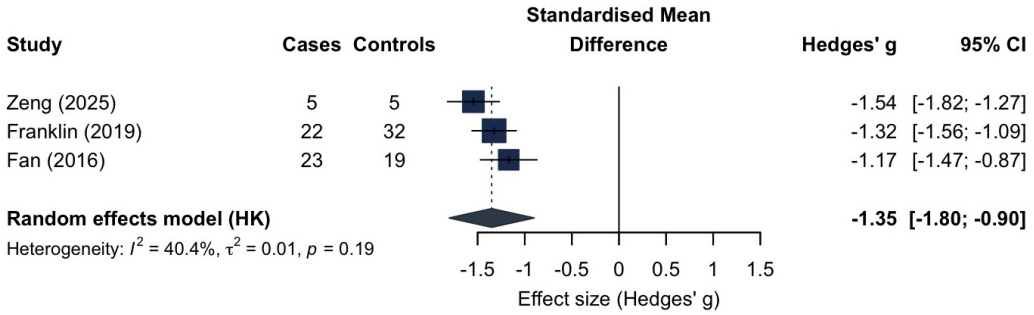

Figure 4. Forest plot of placental Klotho levels in pregnancy complications. Placental Klotho levels were significantly lower in pregnancy complications than in controls (Hedges'*g* = -1.35, 95% CI :-1.80 to -0.90), with low to moderate heterogeneity (*I2* = 40.4%). All included studies showed reduced placental Klotho expression in the affected group.

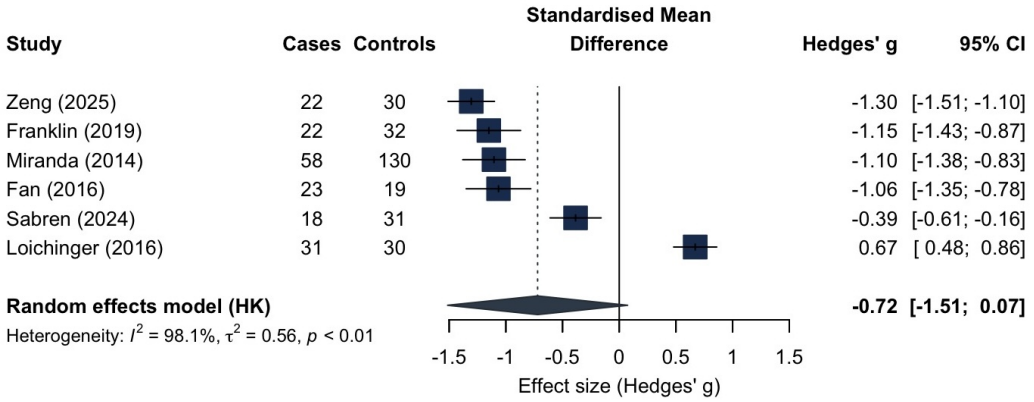

Figure 5. Forest plot of serum Klotho levels in overall pregnancy complications. The pooled analysis showed a non significant trend toward reduced serum Klotho levels in overall pregnancy complications compared with controls (Hedges'*g* = -0.72, 95% CI: -1.51 to 0.07), with marked heterogeneity (*I2* = 98.1%). Most studies suggested lower serum Klotho levels, although one study reported higher levels in the complication group.

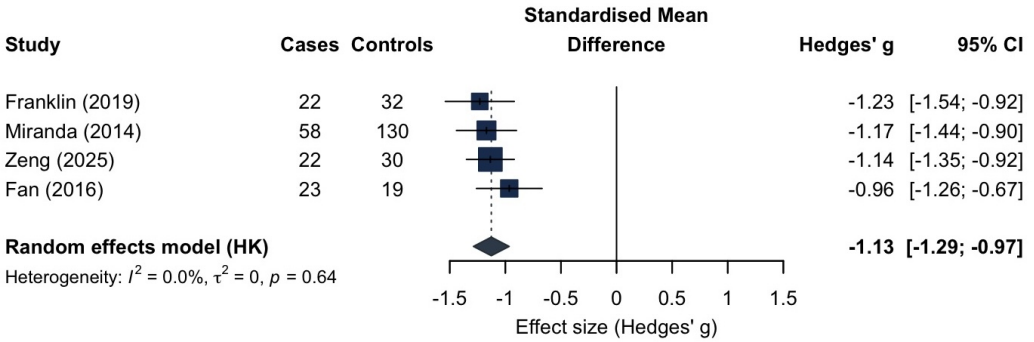

Figure 6. Forest plot of serum Klotho levels in relation to adverse fetal outcomes. Serum Klotho levels were significantly lower in pregnancies with adverse fetal outcomes than in controls (Hedges'*g* =-1.13, 95% CI: -1.29 to -0.97), with no heterogeneity across studies (*I2* = 0.0%). The association was consistent in all included studies.

enced to some extent by the limited number of included studies.

For serum Klotho and overall pregnancy complications, the pooled effect remained negative after exclusion of each study, ranging from -0.60 to -1.00. The association became statistically significant only when Loichinger (2016) was omitted (Hedges'*g* = -1.00, 95% CI: -1.45 to -0.55, *P* = 0.003), and heterogeneity decreased from 98.1% to 89.8%, although it remained substantial. Exclusion of the other studies did not materially alter the non-significant overall result. These findings again identify Loichinger (2016) as a major source of heterogeneity.

For serum Klotho and fetal outcomes, the pooled effect

remained highly stable in all leave-one-out analyses, with Hedges'*g* values ranging from -1.10 to -1.17. All pooled estimates remained statistically significant ($P = 0.001$ to 0.005), and heterogeneity remained absent in all iterations ($I^2 = 0$), supporting the robustness of this finding.

Overall synthesis of findings

Taken together, the quantitative synthesis showed that reduced Klotho levels were most consistently observed in pregnancies complicated by fetal growth impairment and adverse fetal outcomes, both in serum and placental samples. The evidence for PE and for overall pregnancy complications showed the same general direction in most studies, but these pooled analyses were weakened by substantial heterogeneity, largely driven by one study with an opposite effect direction. Overall, the findings support an association between decreased Klotho expression and placenta-related pregnancy complications, particularly those involving abnormal placental function and impaired fetal growth.

DISCUSSION

The present systematic review and meta-analysis synthesized the available evidence on Klotho in placenta-related pregnancy complications and showed three main findings. First, reduced Klotho was most consistently observed in pregnancies complicated by fetal growth impairment, including IUGR, SGA, and FGR, as well as in pregnancies with adverse fetal outcomes. Second, placental Klotho expression was significantly decreased in complicated pregnancies, with relatively low between-study heterogeneity. Third, although most studies also suggested lower circulating Klotho levels in PE and in overall placenta-related pregnancy complications, these associations did not reach statistical significance in the pooled analyses because of marked heterogeneity, largely driven by one study showing an opposite direction of effect. Taken together, these findings support the concept that Klotho is involved in placental dysfunction, particularly in pathways linked to placental aging, oxidative stress, vascular maladaptation, and impaired fetal growth.[6,7]

Our findings are broadly consistent with recent reviews that have positioned Klotho as a candidate regulator of healthy placentation and fetal development. A 2024 narrative review by Kanbay *et al.*[6] emphasized that Klotho may function not only as an anti-aging factor but also as a placental regulator linked to oxidative stress control, endothelial homeostasis, and fetal growth signaling. That review also suggested that reduced placental Klotho may contribute to PE and IUGR through accelerated placental senescence and dysregulated insulin-like growth factor (IGF) signaling. Similarly, a 2025 review by Cecati *et al.*[7]. framed PE as a model of accelerated aging and discussed Klotho as a molecular link between oxidative stress, placental aging, and later maternal cardiovascular risk. The overall pattern observed in our meta-analysis, especially the strong signal for fetal growth disorders and placental Klotho reduction, aligns well with these newer conceptual models.[10,17]

One of the most important findings of the present study is the relatively consistent reduction in serum Klotho in pregnancies affected by IUGR, SGA, and FGR. In contrast to the PE analysis, the direction of effect was uniform across all included studies, and the pooled effect remained robust in leave-one-out sensitivity analyses. This consistency is in line with Franklin *et al.*[12], who showed that cord blood alpha-Klotho was decreased in small-for-gestational-age infants and was associated with maternal vascular malperfusion sublesions and angiopoietin-2, suggesting a link between Klotho deficiency and vascular-mediated placental aging. More recently, Zeng *et al.*[14] reported significantly lower Klotho levels in maternal blood, cord blood, and placental tissue in IUGR pregnancies, together with altered growth hormone (GH) and IGF-1 profiles, supporting the view that Klotho may be integrated into endocrine and metabolic pathways relevant to fetal growth. These observations are also compatible with the broader literature on placental insufficiency, in which disturbed angiogenesis, oxidative injury, and villous maturation abnormalities converge to impair nutrient transfer and fetal growth.[6,9]

The pooled reduction in placental Klotho across pregnancy complications is another notable result. Compared with the serum analyses, the placental analysis showed a more stable and homogeneous signal, which may indicate that tissue-level Klotho changes more directly reflect local placental pathology than circulating measurements do.[10] This interpretation is supported by several lines of recent evidence. Kanbay *et al.*[16] summarized data indicating that lower placental Klotho expression is linked to placental aging and impaired intrauterine development. In addition, a 2024 study on placentas exposed to maternal vascular malperfusion found reduced alpha-Klotho gene and protein expression, reinforcing the association between Klotho deficiency and placental aging-related lesions. The consistency of our placental meta-analysis therefore strengthens the hypothesis that Klotho is not simply a secondary serum marker, but may be directly involved in trophoblast and villous homeostasis.[14,17]

Biologically, the present findings are plausible. Klotho has recognized anti-oxidative, anti-senescent, and vasculoprotective properties. Experimental and translational studies suggest that soluble Klotho can inhibit insulin/IGF-1/PI3K/Akt signaling, enhance antioxidant

defense through FoxO-related pathways, and attenuate endothelial dysfunction.[18,19] In the placental context, lower Klotho expression may favor oxidative stress, trophoblast injury, and accelerated senescence, thereby contributing to maternal vascular malperfusion, villous abnormalities, and restricted fetal growth.[20] Recent reviews of placental aging have specifically highlighted Klotho as one of the anti-aging proteins most plausibly linked to placental epigenetic aging and downstream offspring consequences.[7] Our findings, especially the stronger associations with fetal growth impairment than with PE alone, suggest that Klotho may be particularly relevant to the placental insufficiency axis rather than to the full heterogeneous clinical syndrome of PE.

The findings for PE require more cautious interpretation. Although most included studies reported reduced circulating Klotho in PE, the pooled effect was not statistically significant and heterogeneity was extremely high. Sensitivity analysis showed that the exclusion of Loichinger *et al.* rendered the pooled association significant, identifying that study as a major source of heterogeneity. This discrepancy is not trivial and likely reflects genuine biological and methodological differences rather than simple statistical noise. Loichinger *et al.*[13] examined women in the last trimester and found increased maternal plasma alpha-Klotho in PE, with higher levels associated with reduced accelerated villous maturation. The authors interpreted this as a potentially compensatory systemic response. By contrast, other studies, including Miranda *et al.* [9] and Fan *et al.*[11], reported lower maternal or placental Klotho in PE and linked these reductions to poorer fetal growth or placental dysfunction. Sabren *et al.*[16] further complicated the picture by showing that blood alpha-Klotho concentrations could be relatively preserved during pregnancy while placental membrane Klotho and Klotho activity were reduced in complicated pregnancies. Collectively, these findings suggest that circulating and placental Klotho may not move in parallel in PE, and that gestational timing, disease severity, concomitant fetal growth restriction, specimen type, and the specific Klotho isoform measured may substantially affect the observed direction and magnitude of association.

This distinction between systemic and placental Klotho may be central to understanding the literature. Circulating Klotho during pregnancy may be influenced not only by placental production but also by maternal kidney function, endothelial status, cleavage of membrane Klotho by ADAM10/17, and volume-related physiological changes.[13] Placental Klotho, in contrast, may more directly reflect local trophoblast expression and placental stress biology. The stronger and less heterogeneous placental association observed in our analysis supports this interpretation.[10,16] It also offers one explanation for why fetal growth-related outcomes, which are closely tied to placental function, showed a clearer signal than PE as a broad syndrome. PE is clinically and biologically heterogeneous, encompassing early- and late-onset forms, variable placental contribution, and different degrees of maternal endothelial involvement.[6] Therefore, a biomarker closely linked to placental aging may perform better in identifying placental insufficiency phenotypes than PE considered as a single pooled diagnosis.

Our findings also have potential clinical implications, although they should be interpreted cautiously. The robustness of the association between lower Klotho and fetal growth-related outcomes suggests that Klotho may have value as part of a biomarker panel for placental insufficiency.[6,7] However, the current evidence is not sufficient to support standalone clinical use. Recent reviews have similarly concluded that Klotho is promising but not yet validated as a routine biomarker in pregnancy, largely because of inconsistent study designs, small samples, and variability in biological matrices and assay methods.[14] In practical terms, future work may benefit from evaluating Klotho alongside established angiogenic, inflammatory, and placental aging markers rather than as an isolated analyte.

Several limitations of the present study should be acknowledged. First, the number of original studies eligible for quantitative synthesis was small, particularly for placental analyses. Second, substantial heterogeneity was present in some pooled models, especially those involving PE and overall serum Klotho. Third, the included studies differed in study design, gestational age at sampling, complication definitions, sample source, and assay methodology. Fourth, some outcomes grouped related but not identical clinical entities, such as IUGR, SGA, and FGR, which may share overlapping but not identical pathophysiology. Fifth, two review articles were included in the qualitative synthesis to provide context, but they could not contribute quantitative data. Finally, publication bias could not be formally assessed because fewer than 10 studies were available for each outcome, precluding funnel plot analysis.These limitations mean that the pooled estimates should be interpreted as evidence of direction and magnitude trends rather than as definitive clinical thresholds.

At the same time, this study has several strengths. It integrates both circulating and placental evidence, includes sensitivity analyses for all pooled outcomes, and distinguishes between PE-related findings and growth-restriction-related findings rather than treating all placenta-related disorders as a single entity. The resulting pattern is biologically coherent: the association is strongest where placental insufficiency and fetal growth impairment are most central, and less consistent where maternal systemic adaptation and syndrome hetero-

geneity are greater.

Future research should focus on well-phenotyped prospective cohorts with standardized timing of biospecimen collection, clear separation of early- and late-onset PE, harmonized definitions of fetal growth disorders, and explicit differentiation between soluble and membrane Klotho. Parallel assessment of placental histopathology, angiogenic markers, oxidative stress markers, and senescence-related pathways would help clarify whether Klotho is a causal mediator, a compensatory response, or a downstream marker of placental injury. Mechanistic studies are also needed to define how Klotho interacts with GH/IGF-1 signaling, fibroblast growth factor 23 (FGF23)-related pathways, endothelial dysfunction, and trophoblast senescence in human pregnancy. Recent work in IUGR and placental aging provides a strong rationale for such studies, but the field remains at an early stage.

CONCLUSION

The present systematic review and meta-analysis indicates that reduced Klotho is consistently associated with placental dysfunction, particularly in pregnancies complicated by fetal growth impairment and adverse fetal outcomes. Placental Klotho appears to provide a clearer signal than circulating Klotho, while evidence in PE remains heterogeneous. Overall, these findings support the emerging view that Klotho is involved in the biology of placental aging and insufficiency and may represent a promising biomarker and mechanistic target in placenta-related pregnancy complications, although further validation is required.

DECLARATIONS

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Author contributions
Li X, Liu TJ and Li YL designed the research protocol; XL, Li X, Liu TJ and Li YL conducted the study; Li X, Liu TJ and Li YL analyzed the data; Li X, Liu TJ and Li YL drafted the manuscript; Li X, Liu TJ, Li YL, Zeng SY, Yuan P, Hong YQ and Qi HB critically revised the manuscript; Hong YQ and Qi HB provided funding resources. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Ethical approval
This study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University (Approval No. 2024-478). This meta-analysis was prospectively registered in PROSPERO under the registration number CRD420261338595.

Informed consent
No applicable.

Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Use of large language models, AI and machine learning tools
None declared.

Data availability statement
Data are available upon reasonable request to the corresponding author.

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