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RESEARCH ARTICLE

α-Klotho in Pregnancy Toxemia in Goats: A New Biomarker Candidate?

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Abstract

This study aimed to evaluate the potential use of serum α-Klotho as a biomarker in pregnancy toxemia. The study included 45 Damascus goats that had given birth at least once and were between 120 and 150 days of gestation, brought to the Animal Hospital of Harran University Faculty of Veterinary Medicine. The goats were divided into three groups based on β-hydroxybutyrate (BHBA) concentration in jugular vein blood samples. The control group (Control, n=15) consisted of goats with BHBA concentrations <0.8 mmol/L, the subclinical pregnancy toxemia group (Group 1, n=15) included goats with BHBA concentrations between 0.8-1.6 mmol/L, and the clinical pregnancy toxemia group (Group 2, n=15) comprised goats with BHBA concentrations between 1.6-5 mmol/L. Serum α-Klotho and non-esterified fatty acid (NEFA) concentrations in the study groups were measured using commercial kits. Significant differences were observed in α-Klotho, BHBA, and NEFA concentrations among the groups ($p<0.001$). α-Klotho concentrations were lowest in the Group 2 and highest in the control group ($p<0.001$). In contrast, BHBA and NEFA concentrations were lowest in the control group and highest in the Group 2 ($p<0.001$). Strong correlations were identified among α-Klotho, BHBA, and NEFA concentrations ($p<0.01$). A significant negative correlation was observed between α-Klotho and BHBA ($r=-0.887$, $p<0.01$) and α-Klotho and NEFA ($r=-0.951$, $p<0.01$). The significant changes in α-Klotho concentration observed in both the clinical and subclinical forms of pregnancy toxemia suggest that it may be a potential biomarker candidate for the diagnosis of the disease.

Keywords: BHBA, damascus goat, NEFA, pregnancy toxemia, α-Klotho.

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Introduction

Pregnancy requires an increase in maternal blood volume, elevated cardiac output, and the redistribution of blood flow to the uterus to meet the growing fetus's demands. The most prominent effect of normal maternal vascular adaptation during pregnancy is marked vasodilation in the uterus (Longo and Longo, 2018). However, maternal stress caused by nutritional deficiencies, hyperthermia, or metabolic disorders such as pregnancy toxemia can adversely affect the placenta's critical metabolic, transport, and hemodynamic functions (Valdes et al 2009). Excessive accumulation of free radicals can impair placental development and function, negatively impacting both fetal health and pregnancy progression (Jauniaux et al 2000).

Pregnancy toxemia (PT) is a significant metabolic disorder that occurs in small ruminants during the late stages of pregnancy, primarily due to abnormal carbohydrate and fat metabolism. This condition is more common in underweight animals [Body Condition Score (BCS) <2 on a 5-point scale] or obese animals (BCS ≥4) and is especially prevalent in individuals carrying multiple fetuses (Andrews 1997). In small ruminants, glucose serves as the primary carbon source for placental and fetal oxidative metabolism and tissue formation (Hay 1995). During late pregnancy, approximately 30–50% of maternal glucose production is utilized by the uterus and fetal tissues, with 50–70% of this amount being consumed by the uteroplacental unit (Hay 1995, Molina et al 1991). The increased energy demands of rapidly growing fetuses during this period lead to imbalances in lipid and carbohydrate metabolism, thereby elevating the risk of pregnancy toxemia (Andrews 1997, Ercan et al 2016). In the advanced stages of pregnancy, disrupted fat and carbohydrate metabolism results in an increase in free fatty acids and ketone bodies, particularly β-hydroxybutyrate (BHBA), while glucose concentrations decrease (Lacetera et al 2001). In small ruminants in negative energy balance, adipose tissue produces increased amounts of non-esterified fatty acids (NEFAs) to provide energy (Xue et al 2020). High

NEFA concentrations lead to increased expression of long-chain acyl-CoA synthetase (ACSL), long-chain acyl-CoA dehydrogenase (ACADL), and carnitine palmitoyltransferase I (CPT1), enzymes involved in fatty acid metabolism (Li et al 2013). Non-esterified fatty acids accumulate in the liver and blood, typically increasing in pregnancy toxemia (Hocquette et al 1999).

The intrauterine period is a critical process that forms the foundation of lifelong health and well-being. During this phase, the complex interaction between genetic and environmental factors can determine an individual's susceptibility to various diseases later in life. Initially identified in the kidneys, the Klotho protein has gained attention as an important regulator of healthy metabolism and longevity (Prud'homme et al 2022). Research initially focused on its anti-aging effects (Kuro et al 1997), but recent studies have revealed that Klotho also plays significant roles in intrauterine life and organogenesis (Kanbay et al 2024). The soluble isoforms of α-Klotho (αKL) can be detected in blood, cerebrospinal fluid, and urine (Dalton et al 2017). Various studies have highlighted αKL's role in fundamental biological processes such as nitric oxide production, angiogenesis, and antioxidant enzyme synthesis (Xu et al 2015). These functions suggest that αKL may be an important factor in protecting the placenta and newborn during pregnancy.

α-Klotho plays a role in the maintenance of vitamin D and calcium homeostasis, the modulation of cellular senescence (Liu et al 2007), and the production of enzymes that buffer reactive oxygen species (Lin and Sun, 2015). Additionally, alterations in circulating concentrations of α-Klotho have been demonstrated in obese and diabetic individuals (Wan et al 2017).

This study aims to evaluate the potential use of serum αKL as a biomarker in pregnancy toxemia and determine the changes in its concentrations between healthy pregnancy and pregnancy toxemia. Our hypothesis is that since the α-Klotho protein plays an important role in healthy pregnancies and intrauterine development, the elevated concentrations of BHBA and NEFA caused by a complex pregnancy pathology such as pregnancy toxemia which affects both maternal and fetal health may lead to significant changes in α-Klotho concentrations; and that these changes could be used for diagnostic purposes.

Material and Methods

This study was conducted in accordance with the approval of the Harran University Local Ethics Committee for Animal Experiments (HRÜ-HADYEK) under the permit number 2025/001/07.

Selection of Animals and Experimental Protocol

The study material consisted of 45 Damascus goats, each having given birth at least once and between the 120th and 150th days of gestation, brought to the Animal Hospital of Harran University Faculty of Veterinary Medicine. Our study was conducted over a three-month period in January, February and March 2025. Although the study was conducted during the last month of pregnancy, care was taken to ensure that the goats selected for each group were at similar gestational days. This approach aimed to minimize potential variations in the measured parameters that could arise due to differences in gestational age. The goats were divided into three experimental groups based on the BHBA concentration in blood samples taken from the jugular vein (Andrews 1997). The control group (Control, n=15) consisted of goats with BHBA concentrations <0.8 mmol/L, the subclinical pregnancy toxemia group (Group 1, n=15) consisted of goats with BHBA concentrations between 0.8-1.6 mmol/L, and the clinical pregnancy toxemia group (Group 2, n=15) consisted of goats with BHBA concentrations between 1.6-5 mmol/L. The diagnosis was confirmed by measuring the BHBA concentrations in blood samples taken from the jugular vein using rapid test kits (Free Style Optium Neo H-Abbott®) (sensitivity: 93.6%–75.8%, specificity: 37.8%–70.3%). Goats with BHBA concentrations above 5 mmol/L and those in a coma were excluded from the study. Following blood sample collection, the necessary treatments (%30 dextrose, propylene glycol, B-complex vitamins, and calcium) were administered to the subclinical and clinical pregnancy toxemia groups. All goats recovered fully after the treatment (Figure 1).

Collection of Blood Samples and Laboratory Analysis

After the BHBA measurement in all groups, blood samples were collected from the jugular vein. The collected blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum samples were stored at -20°C until the day of analysis. To determine the serum α-Klotho concentrations in the study groups, a commercial ELISA kit (Goat alpha-Klotho (α-KL) ELISA Kit, MBS2614439, MyBioSource, Inc., USA) (sensitivity: 0.05 ng/mL) was used. For the measurement of NEFA concentrations, a commercial ELISA kit (Goat Non-ester Fatty Acid (NEFA) ELISA Kit, MBS755166, MyBioSource, Inc., USA), (sensitivity: 0.1 µmol/mL) was used. Measurements were performed according to manufacturer's instructions.

Statistical Analysis

SPSS v26 software statistical analysis. was used both visual approaches (histograms and probability maps) and analytical tests (Kolmogorov-Smirnov and Shapiro-Wilk tests) were used to check if the variables were normal. For variables that follow a normal distribution, descriptive

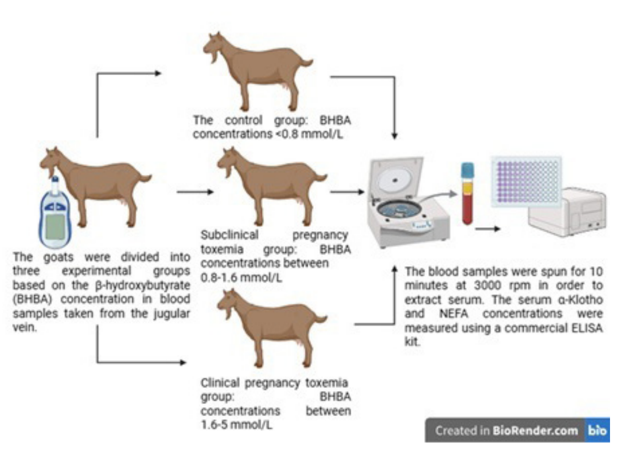

Figure 1. Study design

statistics are shown as mean ± standard deviation. Since the measured parameters were determined to follow a normal distribution, one-way ANOVA was applied for group comparisons. When a substantial difference was identified between the groups, pairwise comparisons were performed utilizing the Tukey post-hoc test. Correlation analysis between the data was performed using Pearson correlation, and statistical significance was set at the 5% level.

Results

Serum α-Klotho, BHBA, and NEFA concentrations according to the presence of pregnancy toxemia are presented in Table 1. Significant differences in α-Klotho, BHBA, and NEFA concentrations were found between the groups ($p<0.001$). BHBA and NEFA concentrations were the lowest in the control group and the highest in the clinical PT group ($p<0.001$), while the α-Klotho concentrations was the lowest in the clinical PT group and the highest in the control group ($p<0.001$). The correlation findings between α-Klotho, BHBA, and NEFA concentrations based on the clinical form of pregnancy toxemia in goats are presented in Table 2. Strong correlations were found among α-Klotho, BHBA, and NEFA concentrations ($p<0.01$). A significant negative correlation was observed between α-Klotho and BHBA, as well as α-Klotho and NEFA ($r=-0.887$, $p<0.01$; $r=-0.951$, $p<0.01$, respectively). Additionally, a significant positive correlation was found between BHBA and NEFA ($r=0.835$, $p<0.01$).

Discussion

Pregnancy is a predominant physiological condition in which metabolic changes occur due to the increasing nutritional demands of the developing fetus. In addition to these changes, placentogenesis is closely associated with various pathophysiological changes in the feto-maternal compartment (Ekici et al 2021). Kasimanickam (2016) reported in their studies that in sheep with PT, genes associated with vascular remodeling were lower, while genes related to hypoxia were higher in the uteroplacental compartment. Some studies in humans have focused on the involvement of α-Klotho (αKL), a novel anti-aging factor, in normal placentation during pregnancy (Roberts and Post, 2008, Sabren et al 2024). These studies suggest that αKL plays a critical role as a factor contributing to the protection and care of the placenta and neonate in healthy pregnancies (Roberts and Post 2008, Xu and Sun, 2015). In this context, it has been suggested that changes in αKL concentrations may be associated with the development of pregnancy complications (Sabren et al 2024). Some research has investigated changes in αKL concentrations during pregnancy, but inconsistent results have been obtained. This has led to a lack of consensus regarding whether αKL concentrations are significant in the development of healthy or complicated pregnancies. However, existing studies are generally based on research conducted in humans, with only a few referring to maternal αKL concentrations during the progression of healthy pregnancies.

In the present study, significant findings were obtained suggesting that αKL could be used as a novel biomarker for the diagnosis of pregnancy toxemia, one of the most common pregnancy complications in small ruminants. In addition, it has been determined that further studies are needed in this field for α-Klotho to serve as a biological indicator for the early diagnosis and management of pregnancy toxemia. Insulin not only affects glucose homeostasis but also inhibits the degradation of triacylglycerols in adipocytes (Wang et al 2008) and restricts the development of hepatic

Table 1. α-Klotho, BHBA and NEFA levels according to pregnancy toxemia status (mean ± standard deviation, n=15)

	Control	Subclinical PT	Clinical PT	p value
α-Klotho (mg/dl)	2.81±0.06[a]	2.06±0.04[b]	1.13±0.02[c]	<0.001
BHBA (mmol/L)	0.58±0.03[a]	1.28±0.07[b]	3.94±0.21[c]	<0.001
NEFA (mmol/L)	0.21±0.01[a]	0.5±0.02[b]	0.79±0.02[c]	<0.001

[a,b,c]Different letters in the same line indicate a statistically significant difference. β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), pregnancy toxemia (PT)

Table 2. Correlation findings between α-Klotho, BHBA, and NEFA levels in goats with pregnancy toxemia				
Correlation analysis		α-Klotho	BHBA	NEFA
α-Klotho	r	1		
BHBA	r	-0.887	1	
	p	<0.001		
NEFA	r	-0.951	0.835	1
	p	<0.001	<0.001	

β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA).

ketone bodies (Laffel 1999). Insulin resistance therefore hinders glucose delivery to maternal skeletal muscle and adipose tissue, elevates lipolysis, and stimulates ketone body production. In small ruminants that cannot meet their energy demands during late pregnancy, a physiological peripheral insulin resistance (IR) occurs as an adaptive mechanism, characterized by decreased insulin production, reduced insulin sensitivity, or both (Duehlmeier et al 2013). Hypoglycemia, hyperglycemia, lipemia, and hyperketonemia are prevalent manifestations in pregnancy toxemia, with insulin resistance playing an important role to the disease's genesis and progression (Marteniuk and Herdt 1998). In human patients with obesity and diabetes, alterations in circulating α-Klotho concentrations have been demonstrated and linked to overall energy metabolism (Amitani et al 2013, Wan et al 2017). Moreover, genetic deletion of α-Klotho has been shown to suppress the expression of Uncoupling Protein 1 (UCP1) gene, reduce temperature in brown adipose tissue, and increase food intake relative to body weight (Mori et al 2000). In mice, genetic inactivation of α-Klotho also enhances insulin sensitivity (Kurosu et al 2006). In the present study, the observed decrease in α-Klotho levels during pregnancy toxemia is thought to reflect an attempt by the body to protect and repair itself against anorexia and insulin resistance resulting from pregnancy toxemia.

The present knowledge regarding the function of Klotho in physiological and pathological states has prompted the assessment of its viability as a biomarker for healthy gestation and fetal well-being (Cilingir et al 2019). Klotho has been proposed as a diagnostic tool for various pregnancy-related diseases in humans, such as intrahepatic pregnancy cholestasis (IPC), preeclampsia, and gestational diabetes (Cilingir et al 2019). However, existing clinical studies present differing results. In a study comparing 23 participants with healthy pregnancies to 19 participants with severe preeclampsia, it was found that maternal α-Klotho concentrations <830 pg/mL predicted preeclampsia with 89.5% sensitivity (95% confidence interval: 66.9-98.7) and 73.1% specificity (95% confidence interval: 52.2-84.4) (Fan et al 2016). In another study, when comparing 36 women with preeclampsia, 28 healthy women, and 10 women with chronic hypertensive pregnancies, it was found that serum Klotho concentrations above 12.48 pg/mL detected preeclampsia with 100% sensitivity and 96% specificity (Cilingir et al 2019). Miranda et al (2014) showed that maternal plasma α-Klotho concentrations were higher in women with uncomplicated pregnancies, while plasma Klotho concentrations were lower in mothers who gave birth to small for gestational age (SGA) neonates, regardless of the presence of preeclampsia, compared to those who gave birth to appropriate for gestational age neonates. The data in our study support the literature on pregnancy complications such as pregnancy toxemia and changes in αKL concentrations. However, as will be discussed later, it is important to consider other factors that may influence αKL concentrations between healthy and complicated pregnancies and this will help clarify the specificity of αKL for pregnancy toxemia.

α-Klotho inhibits the insulin/IGF-1 signaling pathway, activates Forkhead box-O transcription factors (FoxO), and increases the expression of antioxidant enzymes (especially MnSOD). It also strengthens antioxidant defenses by stimulating the Nrf2 pathway. These mechanisms facilitate the scavenging of reactive oxygen species and reduce oxidative DNA damage (Yamamoto et al 2005). α-Klotho reduces the inflammatory response by suppressing NF-κB and the NLRP3 inflammasomes in both membrane-bound and soluble forms. It also limits fibrotic processes by blocking the TGF-β receptor and Wnt ligands. In experimental models, Klotho treatment has been reported to reduce inflammation and oxidative stress, increase Nrf2 activity, and suppress NF-κB activity (Ma et al 2020, Prud'homme and Wang 2024). Klotho regulates metabolic balance by inhibiting insulin/IGF-1 signaling; it supports β-cell health, increases lipid oxidation in the liver and adipose tissue, and suppresses gluconeogenesis. However, human studies have reported that high α-Klotho levels may be positively associated with insulin resistance (Landry et al 2021, Liu and Chen 2023). Because toxemia of pregnancy is a metabolic disorder characterized by oxidative stress, inflammation, and insulin resistance, it is thought that α-Klotho may have a regulatory or reflective role in these processes. However, more comprehensive mechanistic studies are needed to confirm this hypothesis.

α-Klotho protects mitochondrial DNA integrity and biogenesis in muscle stem cells; its deficiency is associated with mitochondrial damage, disrupted cristae, and decreased energy production. It also enhances mitochondrial biogenesis and energy metabolism by activating the AMPK/PGC-1α pathway. Klotho supplementation has been reported to restore mitochondrial function to youthful levels in aged muscles. Furthermore, it has been shown to support cellular viability and mitochondrial function by increasing PGC-1α transcription under oxidative stress conditions (Sahu et al 2018, Zhou et al 2023). α-Klotho modulates the activity of POMC and NPY/AgRP neurons by activating the FGFR-PI3K signaling pathway via the arcuate nucleus (ARC) in the hypothalamus. Through these mechanisms, it regulates appetite, energy expenditure, and glucose homeostasis. In experimental studies, central α-Klotho administration has been shown to reduce appetite, increase energy expenditure, and improve glucose regulation (Landry et al 2020, Landry et al 2021).

Research by Sigurdsson (1991), Brozos et al (2011), and Hefnawy et al (2011) have shown that sheep and goats with pregnant toxemia had lower levels of calcium in their blood. Schlumbohm et al (1997) assert that hypocalcemia, along with diminished insulin sensitivity in target tissues during late pregnancy, exacerbates the disease by impeding hepatic gluconeogenesis and elevating insulin resistance in target tissues. Schlumbohm and Harmeyer (2003) also asserted that the coexistence of hypocalcemia and hyperketonemia imposes an extra inhibitory influence on the homeostatic glucose system in pregnant sheep. Klotho is responsible for placental calcium and phosphorus transport (Mangos et al 2012). In sheep, inhibition of chorionic somatomammotropin hormone results in lower calcium intake and decreased expression of α-Klotho in the fetal component of the placentome (Stenhouse et al 2022). In the present study, it is suggested that the low Klotho levels may be caused by hypocalcemia associated with pregnancy toxemia. α-Klotho plays various roles in the regulation of calcium levels, including the regulation of parathyroid hormone secretion and the signaling of fibroblast growth factor 23 (FGF23), which adjusts calcium concentrations by affecting vitamin D metabolism (Imura et al 2007, Nabeshima and Imura 2008).

In the present study, a negative correlation was identified between α-Klotho levels and both BHBA and NEFA concentrations. This finding suggests that while increased concentrations of BHBA and NEFA are associated with liver damage, decreased α-Klotho concentrations may also serve as an indicator of hepatic injury. Elevated BHBA and NEFA levels have been found to be associated with severe liver damage in sheep and goats with pregnancy toxemia (Cal et al 2009, Hassan et al 2024). Furthermore, lower hepatic Klotho expression and reduced FGF19/FGF21 levels have been linked to more severe liver damage in pediatric non-alcoholic fatty liver disease (NAFLD) (Alisi et al 2013, Liu and Chen 2023).

There are some limitations in our study. As a clinical study, the α-Klotho level was evaluated with only a single measurement and a limited number of animals. Therefore, a specific α-Klotho value range for pregnancy toxemia could not be determined. Additionally, since this is the first study in this area, the limited literature available prevented a comprehensive discussion of the topic from all aspects.

Conclusion

In conclusion, our findings suggest that α-Klotho concentrations may serve as a promising biomarker for the diagnosis of both clinical and subclinical forms of pregnancy toxemia in goats. Future studies should investigate α-Klotho concentrations throughout the entire course of the disease using larger cohorts and experimentally induced pregnancy toxemia models, which will provide a clearer understanding of its dynamic role. Moreover, evaluating the influence of other late-pregnancy complications on α-Klotho levels could help determine its specificity and sensitivity for pregnancy toxemia.

Declarations

Competing Interests

The authors have no conflict of interest to declare.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

This study was conducted in accordance with the approval of the Harran University Local Ethics Committee for Animal Experiments (HRÜ-HADYEK) under the permit number 2025/001/07.

Author Contributions

Motivation/Concept: OY/TA; Design: OY/TA; Control/Supervision: OY/TA; Data Collection and Processing: OY/TA; Analysis and Interpretation: OY/TA; Literature Review: OY/TA; Writing the Article: OY/TA; Critical Review: OY/TA

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