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RESEARCH ARTICLE

Hair Cortisol as an Indicator of Intrauterine Cortisol Exposure in Newborn Akkeçi Goat Kids: Effects of Sex, Birth Type, Parity, and Birth Weight

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Abstract

This study aimed to determine the effects of macro-environmental factors such as sex, birth type, parity, and birth weight on the hair cortisol concentrations of newborn Akkeçi goat kids. Hair samples were collected from 44 Akkeçi goat kids within the first 24 hours following birth and cortisol concentrations were quantified using an ELISA assay kit. Statistical analysis revealed that sex, birth type, parity, and birth weight had no significant effect on the hair cortisol concentrations ($p>0.05$). Although not statistically significant, male kids tended to exhibit higher cortisol levels than females; singleton kids showed higher levels than twins or triplets; and kids born to multiparous does had higher levels than those born to primiparous does. These findings suggest that hair cortisol collected at birth reflects intrauterine hormonal exposure but is not strongly influenced by macro-environmental factors. Overall, the hair matrix appears to be a useful indicator for assessing intrauterine hormonal exposure in newborn Akkeçi goat kids.

Introduction

Mammalian farm animals play a crucial role in meeting the global demand for animal protein, and throughout their productive lifespan, they undergo recurring physiological states such as estrus, pregnancy, parturition, and lactation. Among these, the pregnancy period represents a critical stage during which substantial physiological adjustments occur within the maternal system to support healthy fetal development (Davis and Sandman, 2010), and during which environmental influences experienced by the dam can exert lasting effects on the genotype and, consequently, the phenotype of the developing offspring (Rakers et al., 2020).

Maternal cortisol concentrations fluctuate throughout gestation, typically characterized by a gradual increase that peaks around parturition, followed by a sharp decline to basal levels postpartum (Thompson and Trevathan, 2008). Although excessive maternal cortisol can be harmful to the fetus, the marked rise observed during late gestation is essential for fetal maturation. Transfer of maternal cortisol to the fetus is largely prevented by the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisol into its inactive metabolite, cortisone (Brown et al., 1996; Duthie and Reynolds, 2013; Rakers et al., 2020). This mechanism serves as a partial protective barrier during critical developmental periods (Murphy and Clifton, 2003). However, when maternal cortisol secretion exceeds the buffering capacity of this placental barrier, cortisol can reach the fetus and induce short- or long-term alterations in the programming and postnatal activity of the fetal hypothalamic–pituitary–adrenal (HPA) axis (Challis et al., 2001; Rakers et al., 2020).

Studies in several species have reported that glucocorticoid concentrations in the fetal circulation increase during late gestation and form part of the biochemical cascade that initiates fetal maturation and the onset of birth (Challis et al., 2001). Indeed, during the final 2–3 weeks of gestation, elevated concentrations of adrenocorticotropic hormone and cortisol have been observed in the circulation of healthy lamb fetuses (Challis et al., 2001), and it has been reported that approximately 40–50% of fetal cortisol originates from maternal sources (Gitau et al., 1998).

The perinatal period is considered one of the most critical phases for newborn survival in mammalian farm animals and is associated with the highest rate of neonatal losses (Ayağ and Konyalı, 2019). Although numerous factors influence neonatal survival during this phase, it is well established that the intrauterine environment to which the fetus is exposed also plays a significant role (Vonnahme, 2018). Indeed, studies have reported that offspring born to dams exposed to stress during the prenatal period exhibit suppressed immune function, and that such offspring tend to have lower birth weights accompanied by impaired postnatal growth performance (Merlot et al., 2013; Otten et al., 2015).

Recent research has highlighted the potential of the hair matrix as a biomarker for assessing fetal stress during late gestation (Bacci et al., 2014; Sawyer et al., 2019; Zeinstra et al., 2023). Indeed, the hair matrix functions as a direct indicator of intrauterine hormonal exposure to the fetus (Meyer and Novak, 2021), primarily reflecting the cumulative glucocorticoid deposition that occurs during the last trimester of pregnancy of animals. Consistent with this, studies conducted in lambs (Zeinstra et al., 2023), piglets (Roelofs et al., 2019), and foals (Comin et al., 2012) have demonstrated that intrauterine stress can be quantified using cortisol accumulated in the hair. To date, however, there has been no comparable research conducted in goats. Therefore, the present study aimed to determine hair cortisol concentrations (HCC) at birth in Akkeçi goat kids and to evaluate the effects of sex, birth type, parity, and birth weight on intrauterine cortisol exposure.

Materials and Methods

The Local Ethics Committee for Animal Experiments of Ankara University approved this study (Decision No: 2025-07-62).

Material

This study was conducted in 2025 on a total of 44 newborn Akkeçi goat kids (22 males and 22 females) born to clinically healthy does aged 2–7 (3.68±1.85) years. All does were raised under standard husbandry conditions at the Livestock Research Farm of the Department of Animal Science, Faculty of Agriculture, Ankara University. Descriptive information regarding the birth weights of the experimental goat kids is summarized in Table 1.

Table 1. Descriptive statistics of birth weights according to sex, birth type, and parity in goat kids

Factors		**n**	**ABW (kg)**	**Min. (kg)**	**Max. (kg)**
Sex	Female	22	3.68	2.00	4.30
	Male	22	4.10	3.10	5.30
Birth Type	Singleton	5	4.12	3.70	4.60
	Twin	33	3.96	2.70	5.30
	Triplet	6	3.32	2.00	4.00
Parity	Primiparous	11	3.96	2.70	4.60
	Multiparous	28	3.85	2.00	5.30

ABW: Average Birth Weight

Method

Collection Of Hair Samples from The Goat Kids and Preparation for Cortisol Analysis

Within the first 24 hours after birth, a 3 × 3 cm area on the left shoulder of each goat kid was shaved as close to the skin as possible using an electric clipper (Philips BT3206/14). The collected hair samples were wrapped in aluminium foil, placed into vacuum-sealed bags, and stored at room temperature in a manner that protected them from light and moisture until laboratory analyses were performed.

Hair sample preparation was carried out according to the method described by Ghassemi Nejad et al. (2020). Briefly, 250 mg of hair was weighed from each sample and washed three times for 3 minutes each using 5 mL of 96% isopropanol. The washed samples were then air-dried at room temperature for one week. From the dried material, 50 mg of hair was cut into pieces shorter than 1 mm using surgical scissors and transferred into Eppendorf Tubes®. For cortisol extraction, 1 mL of methanol was added to each tube, and the tubes were shaken at 30 rpm for 24 hours. Following the shaking process, the samples were centrifuged at 2,500 rpm for 30 seconds using a microcentrifuge. After centrifugation, 0.6 mL of the resulting supernatant was transferred into clean Eppendorf Tubes®, which were then placed in an incubator (Heraeus) at 38°C with their caps open until completely dried. Once fully evaporated, 0.4 mL of phosphate-buffered saline (PBS; pH 7.5) was added to each tube and the samples were vortexed thoroughly (Wiggen Hauser, VM Vortex Mixer) to ensure complete dissolution. At this stage, the samples were ready for cortisol analysis.

Cortisol Analysis

Laboratory analyses were carried out at the Reproductive Biology and Animal Physiology Laboratory of the Department of Animal Science, Faculty of Agriculture, Ankara University. Cortisol concentrations in hair samples were determined using the ELISA method (Microplate Reader, Biotek Epoch, USA; Microplate Washer, RAYTO, China) following the manufacturer's instructions with a commercial kit (Cayman Chemical, USA; Cat. No: 500360).

Statistical Analysis

For cortisol concentration analysis, the explanatory variables sex, birth type, and parity were modelled as fixed factors and birth weight as a covariate. The model was constructed as a generalised linear model using the "lme4" package in R (Bates et al., 2015). The results were compared using Tukey's post hoc test ($p < 0.05$). All statistical analyses were performed with R version 4.4.1 (R Core Team, 2021).

Results and Discussion

Least squares means for birth HCC in Akkeçi goat kids are presented in Table 2. According to the results of the analysis of variance conducted for the mean values of birth HCC, no statistically significant differences were detected among the factors of sex, birth type, parity, or birth weight ($p > 0.05$) (Table 2).

Although the differences were not statistically significant, the results of the study showed that birth HCC were higher in male kids compared with females, in singleton kids compared with twins and triplets, and in kids born to multiparous does compared with those born to primiparous does (Table 3).

When the effect of sex on birth weight and HCC at birth in goat kids was evaluated, it was observed that mean birth HCC increased as mean birth weight increased. Indeed, compared with female kids, male kids exhibited both higher mean birth weights and

Table 2. Least-squares means (LSM) and standard errors (SE) for hair cortisol concentrations (HCC), covariates, and regression coefficients in goat kids

Factors		**n**	**LSM ± SE**
Sex	Female	22	852.73 ± 155.71
	Male	22	1021.79 ± 198.72
Birth Type	Singleton	5	764.35 ± 382.57
	Twin	33	1145.45 ± 125.25
	Triplet	6	901.98 ± 302.17
Parity	Primiparous	11	861.53 ± 215.33
	Multiparous	28	1012.96 ± 136.35
Covariate		**n**	**Regression Coeff. ± SE**
Birth Weight		39	121.39 ± 166.67

Table 3. Hair cortisol concentrations (HCC) at birth in goat kids

Factors		n	$\bar{x} \pm S\bar{x}$ (pg/ml)	Min. (pg/ml)	Max. (pg/ml)
Sex	Female	22	1022.66 ± 428.81	363.3	1746.5
	Male	22	1072.06 ± 440.04	583.7	2068.6
Birth Type	Singleton	5	1207.42 ± 340.49	831.0	1659.2
	Twin	33	1037.72 ± 463.69	363.3	2068.6
	Triplet	6	966.93 ± 296.86	690.8	1408.4
Parity	Primiparous	11	976.64 ± 305.06	591.0	1579.6
	Multiparous	28	1100.15 ± 472.56	363.3	2068.6

higher mean birth HCC (Table 1, Table 3). Reports on the effect of sex on HCC in mammals are inconsistent, and it has been suggested that the direction and magnitude of this effect may vary depending on species, breed, age, and various environmental factors. In agreement with the findings of the present study, higher HCC in males compared with females have been reported in horses (Medill et al., 2015), black bears (Lafferty et al., 2015), lambs (Zeinstra et al., 2023), humans (Staufenbiel et al., 2015), and Egyptian mongoose (Azevedo et al., 2019). Conversely, studies in cattle (Heimbürge et al., 2020), goats (Dulude-de Broin et al., 2019), pigs (Roelofs et al., 2019; Heimbürge et al., 2020), and brown bears (Cattet et al., 2014) have reported the opposite pattern, with females exhibiting higher HCC than males. The observation that male goat kids had higher HCC and higher birth weights at birth has been attributed to factors such as greater intrauterine space and nutrient competition, as well as differences in gonadal steroid hormone metabolism during fetal development (Ghassemi Nejad et al., 2022). Moreover, it has been reported that as fetal birth weight increases, the developing fetus requires greater intrauterine space and nutrient availability (Casellas and Caja, 2014).

When the effect of birth type on HCC at birth in Akkeçi kids was examined, the highest mean concentrations were observed in singleton kids, whereas the lowest mean concentrations were found in triplet-born kids (Table 3). The mean birth weights of the experimental kids were measured as 4.12 kg for singletons-born, 3.96 kg for twins-born, and 3.32 kg for triplets-born (Table 1). As the number of offspring born per litter increased, a decrease was observed both in mean birth HCC (Figure 1) and in mean birth weight (Table 1). In a study conducted in cattle, Sano et al. (2023) reported that HCC at calving were significantly higher in cows delivering oversized calves or twins compared with cows delivering normal-sized singleton calves, and Zeinstra et al. (2023) similarly observed that ewes giving birth to larger litters exhibited higher wool cortisol concentrations than those giving birth to smaller litters and that neither litter size nor ewe parity affected wool cortisol levels in the lambs. Likewise, Alon et al. (2021) reported that litter size did not have a statistically significant effect on wool cortisol concentrations in sheep, and Roelofs et al. (2019) reported in pigs that although litter size did not directly affect HCC, increases in litter size were associated with reduced birth weight and elevated HCC. It has been suggested that as litter size increases, the dam may become unable to supply adequate nutrient resources to all fetuses, leading to intrauterine competition for nutrients and space (Casellas and Caja, 2014), which in turn may result in reduced birth weights and elevated birth HCC in the offspring. Furthermore, increasing litter size has been reported to accelerate maturation of the fetal HPA axis and lead to higher fetal cortisol concentrations (Rutherford et al., 2013), as well as to elevate maternal cortisol levels as the number of fetuses increases (Alon et al., 2021).

Animals that have previously experienced stressors may exhibit a more pronounced activation of the stress response. During gestation, elevated maternal cortisol concentrations can cross the placenta and diffuse into the uterus; therefore, the high cortisol concentrations observed in the fetus may originate from the dam, the fetus itself, or a combination of both sources (Hantzopoulou et al., 2022). In the present study, higher birth HCC were detected in kids born to multiparous does compared with those born to primiparous does (Figure 1). This finding may be attributed to the fact that multiparous does have previously experienced the stress associated with pregnancy and parturition. Reports on the effect of parity on HCC in mammalian farm

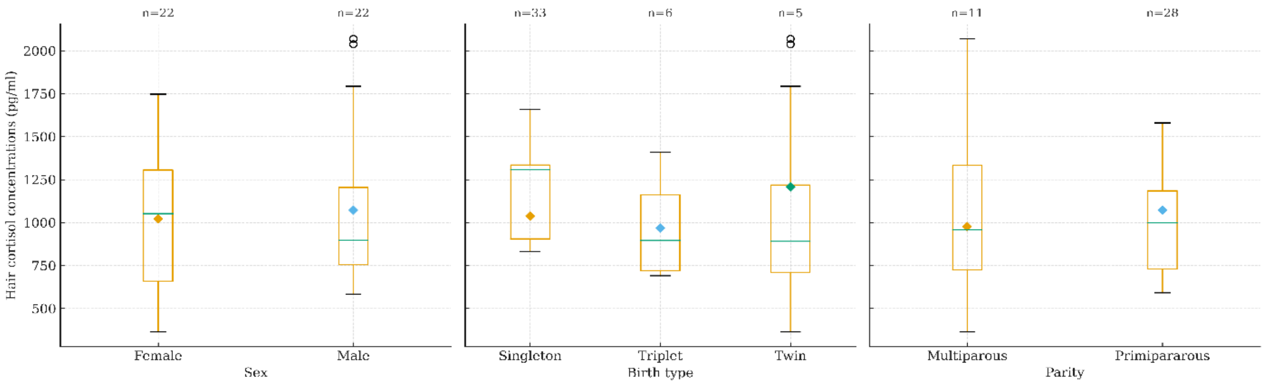

Figure 1. Distribution of hair cortisol concentrations (HCC) in goat kids. Diamonds represent group means; lines indicate medians and interquartile ranges.

animals vary considerably. Comin et al. (2012) reported that HCC in foals were not influenced by dam age; Burnett et al. (2014, 2015) observed higher HCC in multiparous cows than in primiparous cows; Roelofs et al. (2019) reported no direct effect of parity on HCC in pigs, although primiparous animals exhibited higher levels than multiparous ones; Alon et al. (2021) found no statistically significant effect of parity on wool cortisol concentrations in sheep; and Sano et al. (2023) noted that parity did not influence HCC in cows or calves, although higher concentrations were detected in multiparous cows. In addition, Aköz (2025) reported no statistically significant differences in mohair cortisol concentrations across age groups in Angora goats, whereas Güven (2024) noted higher HCC in primiparous Akkeçi goats compared with multiparous individuals. Furthermore, decreasing HCC with increasing age have been reported in cattle (del Rosario Gonzalez-de-la-Vara et al., 2011; Heimbürge et al., 2020), foals (Comin et al., 2012), goats (Dulude-de Broin et al., 2019), and pigs (Heimbürge et al., 2020).

A point that should not be overlooked is that, when evaluating the effect of parity on HCC, birth type may also contribute to this effect especially in species such as sheep and goats that commonly can produce multiple offspring per birth. This is because the likelihood of twinning increases with advancing maternal age in these species. Therefore, the effects of both parity and birth type may overlap on birth HCC.

Conclusion

In this study, the findings support the potential use of hair collected at birth as a non-invasive indicator of intrauterine hormonal exposure in newborn Akkeçi goat kids. However, future studies with larger sample sizes are needed to further validate these findings.

Author Contributions

All authors contributed to manuscript drafting, statistical analyses and critical review.

Conflict of Interest

There are no conflicts of interest for this study.

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