

UMBILICAL CORD CORTISOL LEVELS IN NEWBORNS DEPENDING ON GENDER AND MODE OF DELIVERY

Iskra Martinovska^{1,2}, Nevenka Laban Gucheva², Silvana Naunova Timovska^{3,4},
Pranvera Izairi^{1,5}, Ana Kocevski^{1,4}

¹Specialized Hospital for Gynecology and Obstetrics "Mother Teresa" Skopje,
North Macedonia;

²Faculty of Medical Sciences, Goce Delchev University, Shtip, North Macedonia.

³University Clinic for Pediatric Diseases, Neonatal Intensive Care Unit, Skopje,
North Macedonia

⁴Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia

⁵Faculty of Medical Sciences, State University of Tetovo, North Macedonia

Abstract

One of the most stressful experiences for a newborn is the act of childbirth, and multiple studies have confirmed increased levels of cortisol and catecholamines in the blood of newborns immediately after spontaneous vaginal delivery (SVD). In contrast, completing the birth by elective cesarean section (SC) should be far less stressful for the newborn, presenting with lower cortisol levels.

A prospective clinical study measuring serum cortisol levels in umbilical cord blood samples was conducted at the Mother Teresa Hospital in Skopje, and 88 samples were obtained for analysis.

The aim was to compare cortisol levels in healthy and appropriate for gestational age (AGA) newborns, depending on gender and mode of delivery.

The results showed a mean cortisol level of 93.41 [95%CI 82.72 – 104.10]. In infants born via SVD, the mean cortisol level was significantly higher, with a mean value of 118.13 [95%CI 102.77 – 133.50], than in those born via SC in which the mean cortisol level was 70.83 [95%CI 59.19 – 82.47], with a statistically significant difference ($p < 0.0001$). There was no significant difference between cortisol levels when grouping was done by gender ($p = 0.424$).

According to the results and comparative literature, we can say that the level of cortisol in the umbilical cord, as well as its dependence on the method of delivery, can be a good early indicator of the stress experienced during pregnancy and birth process.

Keywords: cortisol, newborn, umbilical cord, delivery, gender

Introduction

Cortisol is a steroid hormone in the class of glucocorticoids. It is produced mainly in the zona fasciculata of the adrenal cortex. Cortisol synthesis begins with the conversion of cholesterol through multiple metabolic pathways involving a multitude of enzymes.

In response to stress signals or circadian rhythm, the hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH). ACTH, through upregulation of LDL receptors and increased activity of cholesterol desmolase, which converts cholesterol to pregnenolone, is the major rate-limiting step in cortisol synthesis. The majority of glucocorticoids circulate in an inactive form, bound either to corticosteroid-binding globulin (CBG) or to albumin.

Thus, cortisol circulates in its inactive form, cortisone, and is converted to its active form by 11-beta-hydroxysteroid dehydrogenase 1 (11 β -HSD1) in most tissues, while 11 β -HSD2 inactivates cortisol back to cortisone in the kidneys and pancreas. Cortisol levels in the body are regulated by the hypothalamic-pituitary-adrenal (HPA) axis.

During pregnancy, the HPA axis shows increased activity, accompanied by the appearance of hypercortisolemia. As pregnancy progresses, a gradual increase in serum cortisol levels is observed in pregnant women, reaching 3-fold higher values in the third trimester compared to non-pregnant women [1].

In the fetus, cortisol concentrations are lower in the first weeks of pregnancy, and in the period around 10 weeks of gestation, an increase in this hormone is observed as a result of the activity of the

newly formed fetal adrenal tissue. In the perinatal period, cortisol concentrations reach a maximum level due to increased synthesis by the fetal adrenal glands [2].

Increased cortisol levels during pregnancy have anti-inflammatory and catabolic effects in both the pregnant woman and fetus. Cortisol helps in the metabolism of fats, proteins and carbohydrates, participates in the regulation of blood glucose levels and blood pressure. It plays a role in the normal development of the fetus and stimulates the production of fetal pulmonary surfactant [3].

Cortisol is also known as the "stress hormone" and its levels in the blood increase in response to that stress. Certainly, one of the most stressful experiences is the act of childbirth, or more precisely spontaneous vaginal delivery (SVD) and the passage of the newborn through the birth canal where uterine contractions exert enormous pressure on the newborn's body, which pressure is interpreted by its nervous system as stress. This is also confirmed by the increased concentrations of cortisol and catecholamines in the newborn's blood immediately after birth. In contrast, these levels, especially when the birth is completed by elective cesarean section, are much lower, which also means that the newborn child has experienced much less stress.

Aim

To compare cortisol concentrations in healthy and appropriate for gestational age (AGA) newborns, depending on gender and mode of delivery.

Materials and Methods

A prospective clinical study was conducted measuring serum cortisol levels in umbilical cord samples.

Group

The study was conducted at the Special Hospital for Gynecology and Obstetrics "Mother Teresa" - Skopje, a level 2 maternity hospital. The study contains data from 88 newborns who met the inclusion criteria.

Healthy, AGA newborns from mothers with regularly monitored singleton pregnancies and with APGAR ≥ 7 were included.

Exclusion criteria were designed to avoid all possible conditions that could alter and disrupt the normal physiological cortisol levels in both the mother and the newborn. Exclusion criteria involved: mothers with high-risk pregnancies, those receiving corticosteroids, genitourinary infections in the last trimester, and diagnosed psychological conditions.

All necessary data on the course of pregnancy were provided from the mothers' obstetric histories and a standardized questionnaire. Informed consent was obtained for the inclusion of newborns in the study, after every mother was informed of the purpose of the research and notified that the sampling protocol would not cause additional stress or adverse effects to the newborn.

Material collection protocol

After delivery, either by spontaneous birth or cesarean section (SC), the umbilical cord was clamped, and before the delivery or extraction of the placenta, 2 ml of arterial blood was extracted from the umbilical cord using a sterile syringe and a 21G (0.8 mm) needle.

The aspirated blood was immediately transferred to a prepared serum tube with a red cap, which contained a gel separator and a coagulant.

The sample was left at room temperature to coagulate for at least 30 minutes and then centrifuged at 3000-3500 rpm for 10-15 minutes.

The separated serum remained above the gel, ready for analysis. Until 2 pm, the cortisol analysis was performed, and after 2 pm, the serum was separated and stored in a refrigerator at a temperature of 2-8 °C until next morning for analysis.

The cortisol level measurement itself was performed on an Access 2 Immunoassay System [4], operating by CLIA [5] method, with paramagnetic particles. All measured values were in $\mu\text{g/L}$.

Statistical analysis

Data processing was performed using the statistical software programs Microsoft Excel, MedCalc 23.0, and JASP.

Data are presented with their mean, standard deviation (SD), standard error (SE) and 95% CI, and for the descriptive parameters of the populations of interest with absolute numbers and percentages.

Mann-Whitney U-test and ANOVA tests were used when comparing and testing hypotheses.

The statistical significance level was set at $p < 0.05$.

Results

The characteristics of the study group, including maternal age, parity, mode of conception, cigarette, alcohol and drug consumption are presented in Table 1. Some of the more important medical data for the mothers such as hospitalizations during pregnancy, as well as some data from the birth process are presented in Table 2.

Table 1. Statistical demographic data of mothers

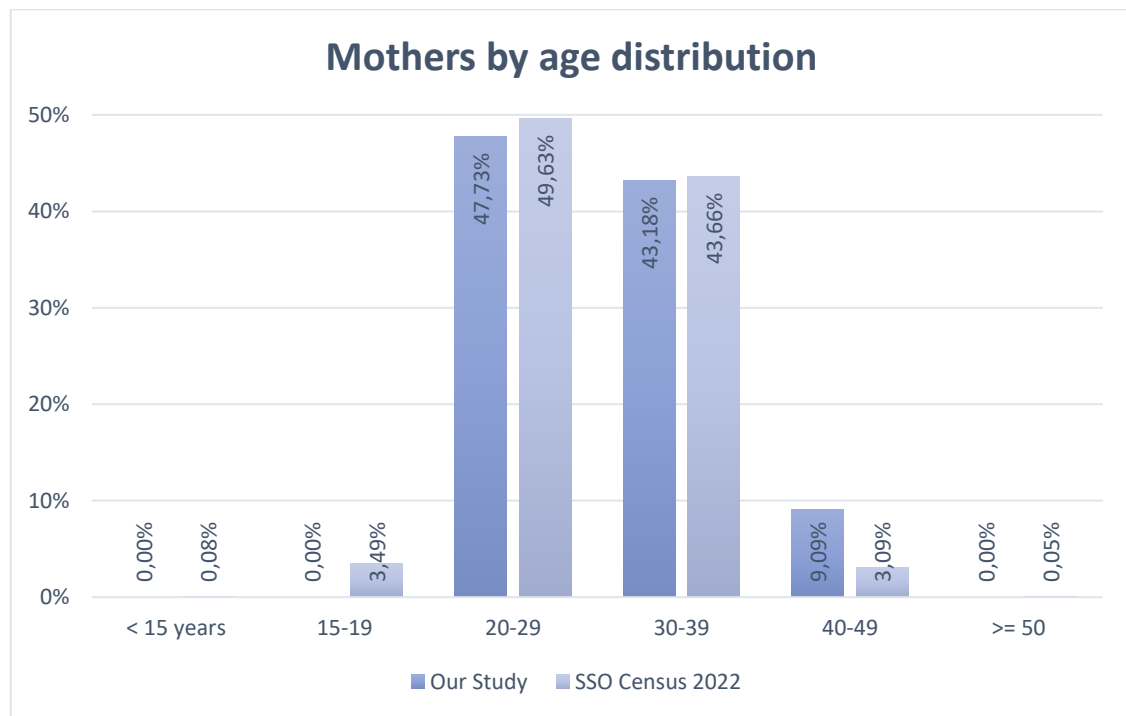
Mothers	n	Mean (SD) or %
Age	88	31.69 (6.56)
Ethnicity	88	
Albanian	40	45.46%
Bosnian	2	2.27%
Macedonian	38	43.18%
Roma	5	5.68%
Turkish	3	3.41%
Pregnancy in row	88	2.56 (1.91)
Way of getting pregnant	88	
In vitro	2	2.27%
Spontaneous	86	97.73%
Smoking	88	
Yes	14	15.91%
No	74	84.09%
Alcohol	0	0%
Drugs	0	0%

Table 2. Medical and obstetric data of mothers

Mothers	n	Mean (SD) or %
Previous hospitalizations	88	
Yes	11	12.50%
No	77	87.50%
Rupture of membranes (hours)	88	3.18 (6.47)
Duration of birth (hours)	88	4.15 (4.01)
Method of delivery	88	
SVD	42	47.73%
SC	46	52.27%
Presentation of fetus	88	
Head-first	78	88.64%
Breech	7	7.96%
Feet	3	3.41%

The maternal age ranged from 21 to 48 years, or an average of 31.69 (6.56). As shown in Figure 1, the age distribution, according to the methodology of the State Statistical Office used in the Census from 2021, is consistent with national data from that census. This suggests that our sample quite realistically reflects the maternal age distribution in the general population.

Figure 1. Distribution of mothers by age compared to national data from the State Statistical Office



We would like to emphasize that, although labor duration for vaginal delivery lasted from 3 to 11 hours (6.64 hours on average), regional analgesia was not used, and cortisol levels were not affected.

Basic characteristics of newborns, such as birth weight and length, gestational age, and APGAR scores at 1 and 5 minutes, are shown in Table 3.

Table 3. Statistical data and results for newborns

		$\bar{x}$	SD	95% CI
Gestational age (weeks)	Preterm n=13)	35.92	0.28	35.92±0.15 [35.77- 36.07]
	Term (n=75)	39.43	0.87	39.43±0.06 [39.37- 39.49]
Birth weight (gr.)	Preterm (n=13)	2796.15	409.01	2796.15±222.34 [2573.81 – 3018.49]
	Term (n=75)	3353.60	453.57	3353.60±102.651 [3250.95 – 3456.25]
Birth length (cm)	Preterm (n=13)	47.77	2.09	47.77±1.14 [46.634 – 48.906]
	Term (n=75)	49.89	2.12	49.89±0.480 [49.410 – 50.370]
APGAR Score 1 min	Preterm (n=13)	8.69	0.63	8.69±0.342 [8.348 – 9.032]
	Term (n=75)	8.47	1.00	8.47±0.226 [8.244 – 8.696]
APGAR Score 5 min	Preterm (n=13)	9.15	1.07	9.15±0.582 [8.568 – 9.732]
	Term (n=75)	9.17	0.67	9.17±0.152 [9.018 – 9.322]

It is worth noting that the relatively high gestational age among preterm newborns (35.92 [95%CI 35.77- 36.07] gestational weeks) is largely due to the fact that SHGO Mother Teresa is a level maternity hospital as defined in the Master Plan for Regionalization of Perinatal Care adopted by the Government of the Republic of North Macedonia in 2024, and accordingly accepts mothers who have full 34 weeks of pregnancy for childbirth.

When analyzing cortisol levels by gender of the newborns (Table 4), no statistically significant differences were observed ($p = 0.424$), indicating that cortisol secretion in the immediate perinatal period was not significantly associated with baby's gender.

Table 4. Cortisol level by gender of the newborns ($\mu\text{g/L}$)

	n	$\bar{x}$	SD	95% CI
Average of whole group	88	93.41	51.185	93.41 ±10.694 [82.716 – 104.104]
Male	49	89.49	52.161	89.49 ±14.605 [74.886 – 104.096]
Female	39	98.33	50.167	98.3277 ±15.745 [82.583 – 114.073]
p(M/F) = 0.424				

On the other hand, the mode of delivery appeared to be a critical factor (Table 5). Infants born by spontaneous vaginal delivery (SVD) had significantly higher cortisol levels compared to those born by cesarean section (CS), with a highly significant p value (< 0.0001).

Table 5. Cortisol level by mode of delivery ($\mu\text{g/L}$)

	n	$\bar{x}$	SD	95% CI
Average of whole group	88	93.41	51.185	93.41 ± 10.694 [82.716 – 104.104]
P.S.	42	118.13	50.801	118.13 ± 15.364 [102.768 – 133.496]
SC	46	70.83	40,278	70.83 ± 11.639 [59.193 – 82.472]
p(SVD/SC) < 0.0001				

This finding is consistent with the fact that childbirth is a stressful process, activating the hypothalamic-pituitary-adrenal (HPA) axis in the fetus. Thus, an adaptive increase in cortisol may be beneficial in preparing the newborn for life outside the womb. This aligns with results obtained in similar studies [6,7,8].

Discussion

Given that the act of childbirth is a painful experience for the mother, and the growing evidence suggests that the fetus is capable of feeling pain before birth, it is logical that the newborn is also subjected to stress during this process. There is also growing evidence that increased levels of stress have far-reaching consequences for the subsequent development of the newborn many years after birth [9].

It has already been established that spontaneous vaginal delivery (SVD) is associated with an increase in the stress hormone cortisol in the fetus [10,11,12].

On the other hand, studies have demonstrated that cesarean section (SC) is less stressful for the fetus than spontaneous vaginal delivery, because cortisol levels in cord blood are much lower [13,14].

In this study, we compared cord cortisol levels between two groups of newborns, those delivered via spontaneous vaginal birth and those delivered via cesarean section. Spontaneous vaginal births were performed without the use of regional analgesia (epidural) and without the use of devices and methods for assisted spontaneous labor. Cesarean section (SC) deliveries included in this study were elective so as not to affect cortisol levels, because those that are urgent almost always affect cortisol levels due to fetal distress.

The results showed a statistically significant difference between newborns born by spontaneous vaginal delivery and those by cesarean section, if we take as a baseline the mean cortisol level (93.41 ± 51.18) of the entire group of newborns. In those born by SVD, the mean cortisol level of 118.13 ± 50.80 was higher than in those born by SC, in which the mean cortisol level was 70.83 ± 40.28 . The difference between the two groups was statistically significant ($p < 0.0001$).

This is consistent with results from similar studies [15-17], and with the growing evidence that infants born via different types of delivery have different responses to stress and pain both during the act of birth and in the early postnatal period. Those delivered via elective cesarean section show the least measurable response to stress and stressful situations, while those born via spontaneous delivery have a much stronger stress response [15], as reflected in our results.

We also wanted to find out whether the gender of the newborn plays a role in the cortisol levels and in the stress response at birth.

The results of previous studies showed that it did not play a statistically significant role ($p=0.424$), i.e. the intervals of cortisol levels in male and female newborns were statistically very close. In male newborns, the level with a 95% confidence interval was within 89.49 ± 14.605 [74.886 – 104.096], and in female 98.3277 ± 15.745 [82.583 – 114.073], which shows that the intervals largely overlap.

The lack of difference between male and female newborns has also been shown by other studies [16,17].

We would like to emphasize that this investigation is only part of a larger study involving a larger number of newborns than the 88 studied here, and additional parameters that could play a role in increasing fetal stress and, consequently an increase in cortisol levels in an umbilical cord sample.

Further research, both in our country and in the region and beyond, is needed in order to determine a reference level of cortisol in newborns, depending on various parameters.

Thus, healthcare professionals, particularly neonatologists in the early postnatal period and later pediatricians in the subsequent period of growth and development of children, could have a relevant indicator of the stress experienced before and during birth. Growing evidence for the cortisol role in various ways and to varying degrees may play a significant role in a child's development and in the emergence of various health conditions in later life.

Conclusion

According to the results obtained, as well as experiences in the world literature, we can conclude that the cortisol level in the umbilical cord, as well as levels obtained according to the mode of delivery can serve as a good early indicator of the stress experienced during pregnancy and childbirth.

Particular attention should be paid to increased and especially high cortisol concentrations, which can have a strong negative effect on children's growth as well as the possible occurrence of HTA in early childhood, i.e. it is associated with the so-called hypertensive crisis phenotype [9].

Hence, measures to avoid high concentrations of cortisol in the umbilical cord, such as regularly controlled pregnancy, prevention of prematurity, timely indication of the method of termination of birth, etc. would lead to a reduced incidence of the hypertensive and stress phenotype.

References

1. Jung C., Ho JT, Torpy DJ, Rogers A., Doogue M., Lewis J., Czajko RJ, Inder W. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. *J. Clin . Endocrinol . Metab .* 2011 ;96:1533 –1540. doi : 10.1210/jc.2010-2395.
2. Busada JT, Cidlowski JA Mechanisms of glucocorticoid action during development. *Curr . Top. Dev. Biol.* 2017 ;125:147 –170. doi : 10.1016/bs.ctdb.2016.12.004.
3. Thau L, Gandhi J, Sharma S. Physiology, Cortisol. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538239/>
4. Beckman Counlter ACCESS <https://www.beckmancoulter.com/products/immunoassay/access-2>
5. WANG, Chen & WU, Jie & ZONG, Chen & XU, Jie & JU, Huang-Xian. (2012). CLIA - Chemiluminescent Immunoassay and Its Applications. *Chinese Journal of Analytical Chemistry*. 40. 3–10. 10.1016/S1872-2040 (11)60518-5.
6. Gitau R, Menson E, Pickles V, Fisk NM, Glover V, MacLachlan N. Umbilical cortisol levels as an indicator of the fetal stress response to assisted vaginal delivery. *Eur J Obstet Gynecology Reprod Biol.* 2001 Sep ;98 (1):14-7.
7. Mears K, McAuliffe F, Grimes H, Morrison JJ. Fetal cortisol in relation to labor , intrapartum events and mode of delivery. *J Obstet Gynecol .* 2004 Feb;24(2):129-32
8. Sameer P. Darawade , Sandhya V. Haribhakta , Dhananjay D. Vaze , Seema G., Shilpa A. Pratinidhi (2016); To study the effect of mode of delivery on umbilical cord cortisol level. *Int. J. of Adv. Res.* 4 (Jan). 1471-1477] (ISSN 2320-5407)
9. Huh SY, Andrew R, Rich-Edwards JW, Kleinman KP, Seckl JR, Gillman MW. Association between umbilical cord glucocorticoids and blood pressure at age 3 years. *BMC Med.* 2008 Aug 28 ; 6:25 . doi : 10.1186/1741-7015-6-25. PMID: 18755017; PMCID: PMC2533350.
10. Padbury JF, Roberman B, Oddie TH, Hobel CJ, Fisher DA. Fetal catecholamine release in response to labor and delivery. *Obstet Gynaecol* 1982 ;60:607 -11.
11. Economides DL, Nicolaides KH, Linton EH. Plasma cortisol and adrenocorticotropin in appropriate and small for gestational age fetuses. *Fetal Therapy* 1988 ;3:158 -64.

12. Gulmezoglu AM, Mahomed K, Hofmeyr GJ, Nikodem VC, Kramer T. Fetal and maternal catecholamine levels at delivery. *J Perinatal Med* 1996 ;24:687 -91.
 13. Goldkrand JW, Schulte RL, Messer RH. Maternal and fetal plasma cortisol levels at parturition. *Obstet Gynecol* 1976 ;47:41 -5.
 14. Bird JA, Spencer JA, Mold T, Symonds ME. Endocrine and metabolic adaptation following caesarean section or vaginal delivery. *Arch Dis Child Fetal Neonatal Ed* 1996;74:F132-4
 15. Gitau R, Menon E, Pickles V, Fisk NM, Glover V, MacLachlan N. Umbilical cortisol levels as an indicator of the fetal stress response to assisted vaginal delivery. *Eur J Obstet Gynecology Reprod Biol.* 2001 Sep ;98 (1):14-7.
 16. Mears K, McAuliffe F, Grimes H, Morrison JJ. Fetal cortisol in relation to labor , intrapartum events and mode of delivery. *J Obstet Gynecol .* 2004 Feb;24(2):129-32
 17. Sameer P. Darawade , Sandhya V. Haribhakta , Dhananjay D. Vaze , Seema G., Shilpa A. Pratinidhi (2016); To study the effect of mode of delivery on umbilical cord cortisol level. *Int. J. of Adv. Res.* 4 (Jan). 1471-1477] (ISSN 2320-5407)
 18. Taylor A, Fisk NM, Glover V. Mode of delivery and subsequent stress response. *Lancet* 2000 ;355:120 .
 19. Słabuszevska-Jóźwiak , A. et al . Does the Caesarean Section Impact on 11 β HSD2 and Fetal Cortisol? *Int. J. Environ. Res. Public Health* 2020, 17, 5566.
 20. Koskivuori J, et al. Comparative steroid profiling of newborn hair and umbilical cord serum highlights the role of fetal adrenals, placenta, and pregnancy outcomes in fetal steroid metabolism. *J Steroid Biochem Mol Biol.* 2023 Sep ;232:106357.doi: 10.1016/j.jsbmb.2023.106357. Epub 2023 Jun 28. PMID: 37390977.
-