

**Studies on cortisol and prolactin concentrations in umbilical cord
blood, amniotic fluid, maternal blood, and breast milk related to
perinatal factors**

Summary of the thesis

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Chapter 1: Introduction

Prolactin is known as a hormone that promotes the production of breast milk in mammals. In addition, prolactin shows a variety of physiological functions such as induction of maternal behavior, enhancement of stress tolerance, osmoregulation, and immune regulation. Cortisol is an adrenocortical hormone which regulates energy metabolism. The level of cortisol increases in response to stress. During the delivery, mother undergoes labour pain stress. The fetus is also thought to be undergoing high stress by uterine contractions during the delivery. In this study, based on the stress response of prolactin and cortisol, the correlations between the concentrations of both hormones in umbilical cord blood and amniotic fluids were studied during delivery. In lactation period, prolactin is secreted with high concentration in breast milk as well as maternal blood. It has recently been reported that breast milk prolactin taken at neonatal stage affects on the maternal behavior and stress tolerance at adult stage in rats, suggesting importance of physiological role of the breast milk. In human, however, relationship of prolactin concentrations in mothers' blood and breast milk is not known. In this study, the relationships between the prolactin concentrations in mothers' blood and breast milk were also studied.

This study was reviewed and approved by the ethics committee at Fujinomiya City General Hospital in Shizuoka prefecture. The subjects also signed a consent form after receiving an oral explanation and written documents regarding the study.

Chapter 2: Correlations of cortisol and prolactin levels in umbilical cord blood with the modes of delivery and duration of delivery

Umbilical cord blood was collected after vaginal delivery and plasma was separated by centrifugation. The concentration of cortisol was determined by Radioimmunoassay using Cortisol kit: FTA (TFB). The concentration of prolactin was measured by Electro Chemiluminescent Immunoassay using ECLusys Prolactin III kit (Roche-diagnostics). All statistical analyses were performed by Spearman's rank correlation coefficient test using SPSS v15 for Windows.

In human, delivery consists of three stages. The first stage is the period from the beginning of regular uterine contractions until full cervical dilation; the second stage is

from full cervical dilation to the birth of a neonate, and the third stage is from the birth to completion of expulsion of the umbilical cord, placenta and foetal membrane. First, relationship between delivery modes and cortisol and prolactin concentrations in the umbilical cord blood were examined. The cortisol concentration, but not prolactin concentration, of the vacuum-assisted delivery group was significantly higher than that of the spontaneous delivery group. Since vacuum-assisted delivery is employed in difficult delivery, neonate may suffer from stronger stress during the delivery. Then, the correlations between cortisol concentrations and delivery duration among the spontaneous delivery groups were analysed. Positive correlations were observed between cortisol concentration and duration of full delivery with stronger extent during the second phase of parturition. These findings suggest that fetuses suffer from the stress caused by uterine contraction during delivery. On the other hand, the prolactin concentration showed no significant correlation with the duration of delivery. Since stress response of prolactin is temporal, individual prolactin levels in cord blood may fluctuate during delivery.

Chapter 3: Correlations of cortisol and prolactin concentrations in amniotic fluid with the modes of delivery and duration of delivery

Umbilical fluid was collected at spontaneous or artificial membrane rupture during delivery. Concentrations of cortisol and prolactin were determined by the methods described in Chapter 2. Both the cortisol and prolactin concentrations in amniotic fluid of the vacuum-assisted delivery group were significantly higher than those of the spontaneous delivery group. There was a positive correlation between the cortisol concentration in amniotic fluid and the duration of delivery as observed in the umbilical cord blood, whereas, prolactin concentration showed no correlation with the duration of delivery. The origin of cortisol in the amniotic fluid is considered to be the urine of the fetuses and therefore, the increase of cortisol concentration may reflect the stress response of fetuses during delivery. Prolactin in the amniotic fluid derived from endometrial decidua where stress response of prolactin is unclear.

Chapter 4: Correlation of prolactin concentrations between maternal plasma and breast milk

Prolactin concentrations in breast milk are markedly increased during the first few

days after delivery. Although prolactin is known to be transferred from maternal blood to breast milk, the correlation between prolactin concentration in breast milk and those in maternal blood is not clear. In this study, we investigated the correlation between prolactin concentrations in maternal blood and breast milk collected 4 days after the delivery. First, prolactin concentrations in foremilk collected before breast milk feeding were compared with those in hindmilk collected after breast milk feeding. There was no significant difference in prolactin concentrations between foremilk and hindmilk. Then, prolactin concentrations in maternal plasma were compared with those in hindmilk of primiparas and multiparas. There was a positive correlation between prolactin concentrations in plasma and hindmilk, with a stronger correlation in primiparas than in multiparas. Breastfeeding is known to have many benefits such as enhancement of immune function in neonate, decrease of the risk of atopic diseases, and mortality in infants, and decrease breast and ovarian cancer risk in mother. In addition, recent reports have demonstrated the epigenetic effects of breast milk prolactin on the nutritional status and brain function of adult offspring. Therefore, it is important to clarify the maternal factors affecting on prolactin concentration in breast milk.

Chapter 5: Overall recapitulation

In this study, we first examined relationship between delivery modes and cortisol and prolactin concentrations in the umbilical cord blood. The cortisol concentration was significantly higher in the vacuum-assisted delivery than in the spontaneous delivery and positively correlated with the duration of delivery, with higher extent during the second phase of parturition. These findings suggest that fetuses as well as mothers suffer from the stress caused by uterine contraction during delivery. On the other hand, the prolactin concentration showed neither significant correlation with the duration of full delivery nor the second stage of delivery. Since the stress response of prolactin is temporal, individual prolactin concentrations in cord blood may fluctuate.

Cortisol concentration in amniotic fluid of the vacuum-assisted delivery group was significantly higher than those of the spontaneous delivery group. There was a positive correlation between the concentration of cortisol in amniotic fluid and the duration of parturition as observed in the umbilical cord blood. The origin of cortisol in the amniotic fluid is considered to be the urine of the fetus, and therefore, the increase of cortisol concentration may reflect the stress response of fetus during delivery. Prolactin

concentration in amniotic fluid of the vacuum-assisted delivery group was significantly higher than those of the spontaneous delivery group but no correlations was observed between the prolactin concentration and the duration of delivery. Prolactin in the amniotic fluid is derived from endometrial decidua where stress response of prolactin is unclear.

There was a positive correlation between prolactin concentrations in plasma and hindmilk supporting that prolactin in breast milk is mother-blood origin. Recent reports suggest epigenetic effects of breast milk prolactin on the nutritional status and brain function of adult offspring. Therefore, it is important to clarify the maternal factors affecting on prolactin concentrations in breast milk.