Hormone levels in breast milk

Doctoral (Ph.D.) thesis

Réka Anna Vass M.D.

University of Pécs

Medical School

Clinical Medical Sciences Doctoral School

Mentor: Prof. Tibor Ertl M.D., Ph.D., D.Sc.

Program leader: Prof. Péter Gőcze M.D., Ph.D., D.Sc.

Head of Doctoral School: Prof. Lajos Bogár M.D., Ph.D., D.Sc.

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1. Introduction

Breastfeeding provides irreplaceable nutritional and nonnutritional factors that influence the postnatal adaptation and development of the infant. In neonatal intensive care practice, three options are available for enteral nutrition: the infant's own mother's milk, banked donor milk, and commercial infant formula. Breastfeeding has numerous protective effects compared to formula.

The fetal and neonatal periods are of the utmost importance in human development because they encompass critical periods for normal development. Early nutrition influences growth, functional development of organs, and impacts health status in later life (Buckleyet et al, 2005). Breastfeeding protects against necrotizing enterocolitis, obesity, acute otitis media, atopic dermatitis, type I and II diabetes (Ip et al, 2007; Gage et al, 2013; Binns et al, 2016; Victora et al, 2016) and protects later obesity (Silfverdal et al, 1999; Nobili et al, 2009; Ortega-García et al, 2018).

Studies were focusing on the growth factor, cytokine, fatty acid, and hormones content of breast milk produced for term and preterm infants. Results show that breast milk of mother's gave birth to preterm infants, has richer in oligosaccharides, peptides, and fat (ESPGHAN, 2013; Gabrielli et al, 2011; Bauer et al, 2011; Nommsen et al, 1991).

The hormones we measured are leptin, insulin, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), cortisol, progesterone, and testosterone. Each of these hormones plays a role in maternal and fetal metabolism and they are linked with adult metabolic syndrome. They have overlapping roles in developmental biology, and it is critical to measure them simultaneously to develop an endocrine profile, and subsequently link that signature to maternal health and nutrition.

We investigated pleiotropic hormones, like the peptide hormone leptin playing various role in development (Erkonen et al, 2011; Steinbrekera & Roghair, 2016). Plasma leptin level was increased in obes mothers (prepregnancy BMI > 30 kg/m2). Obese mothers had two-fold higher breast milk insulin and leptin concentration compared to non-obese volunteers (Lemas et al, 2016). Leptin promotes cognitive development (Meyer et al, 2014), and has proved protective effect on neurodevelopment (Erkonen et al, 2011). Preterm and term male infants have higher plasma leptin concentration compared to female newborns (Ertl et al, 1991). Previous study revealed that breastfed infants have elevated plasma leptin level compared to formula fed newborns (Savino et al, 2016). Preterm delivery leads to the infant's early separation from mother, and maternal circulation was a source of leptin, and preterm birth predisposes postnatal leptin deficiency (Steinbrekera et al, 2019).

Insulin is an anabolic hormone, which promotes carbohydrate absorption and increases leptin absorption in the brain (Kastin and Akerstrom, 2001). Insulin plays a role in dendritic arborization, neuronal survival, and also in short- and long-term memory consolidation in the hippocampus (Werner and LeRoith, 2014). Endogen insulin and leptin impacts blood glucose level, in this way ensure protection against hypo- and hyperglycemia (Kastin and Akerstrom, 2001).

Hormones produced by the pituitary gland are essential coordinators of development, from cell growth and differentiation to tissue metabolism. Hormones of thyroid gland is controlled by TSH, influencing metabolism and playing essential role in physiological neurodevelopment (Lignell et al, 2016).

Gonadotrophins, like folliculus stimulating hormone (FSH) and luteinizing hormone (LH) controlling reproductive functions.

Steroid hormones have diverse physiological effects, their role in early development is unrevealed. Cortisol controls gluconeogenesis, acts as a diuretic (McKay and Cidlowski, 2003). Glucocorticoids play a role in cell growth and differentiation (Hinde et al, 2015). Progesterone considered a neurosteroid, impacts remyelination, neuro- and behavior development and suppresses maternal immune responses to fetal antigens and potentiates T-cell differentiation (Schumacher et al, 2004; Arck et al, 2007). Testosterone is a key hormone of the male reproductive system and influences neurodevelopment (Bramen et al, 2012). Early postnatal androgen elevation, or minipuberty, contributes to human neurobehavioral sexual differentiation (Pasterski et al, 2015).

Feeding preterm infants with human donor milk is the choice when own mother's milk is not available (AAP, 2012; AAP, 2017). Pasteurization is necessary to assure the safety of donor milk, although it may affect milk quality by reduction of bioactive components (Wesolowska et al, 2018; Bertino et al, 2018; Escuder-Vieco et al, 2018; Donalisio et al, 2018). Holder pasteurization, the most frequently used technique, reduces the levels of cortisol and cortisone in breast milk (van der Voom et al, 2017). In the past few years numerous research groups investigated the concentration of several factors such as erythropoietin and IL-10 in fresh milk samples before and after Holder pasteurization (Untalan et al, 2009). Insulin, adiponectin, and erythropoietin concentrations were all reported to be decreased significantly after Holder pasteurization, although the paucity of the reports does not allow any conclusion on this issue to be generalized (Peila et al, 2016).

In everyday practice, breast milk often stored in refrigerator in home and neonatal intensive care practice as well. However, our knowledge is limited about the impact of refrigeration in milk hormone levels (Hanna et al, 2004; Bertino et al, 2013). Hormones in breast milk previously were examined with the semiquantitative radioimmunoassay. The applied biological assays are the preferred methods to measure the biologically active form of hormones.

2. Aims

After birth in neonatal intensive care and home practice, newborns have two options to get breast milk:

1. Preterm newborn's own mother is breastfeeding,
2. Donor milk, which is pasteurized.

This thesis summarizes the result of two investigations:

- Distribution of insulin, cortisol, leptin, progesterone, and testosterone in breast of milk of mothers gave birth to preterm infants and in donor milk, before and after Holder pasteurization.

- Investigation of FSH, LH, and TSH in breast milk produced for preterm infants, and after 24h long refrigeration. We also measured these pituitary hormones in milk of MMBI donor mothers before and after Holder pasteurization. Modelling everyday intensive care practice, pasteurized donor milk was refrigerated for 24h.

We studied:

- the presence of insulin, leptin, cortisol, progesterone, testosterone, FSH, LH, and TSH in breast milk
- the effect of Holder pasteurization on hormone content of breast milk
- the impact of 24h refrigeration on hormone levels in human milk
- the combined effect of Holder pasteurization and 24h refrigeration on pituitary hormones
- whether hormone content is influenced by maternal BMI
- the differences between raw preterm milk and pasteurized donor milk

3. Methods

The donors from the milk bank were selected from among those who had met the criteria to donate and were screened and tested based on the protocol of the Human Milk Banking Association of North America. The study was approved by the institutional review board of the University of Iowa and the Mother's Milk Bank of Iowa (IRB ID: 201805935). The preterm mothers and milk donors consented to the use of their milk samples for this study. To avoid sampling colostrum or transitional milk, we collected samples from mothers who were able to continuously breastfeed and whose infants were 3−5 weeks old.

The milk samples were sonicated to disrupt milk fat globules and allow proteins to enter the aqueous phase. One aliquot was pasteurized in a preheated water bath at 62.5 °C for 30 min. Then, the pasteurized and nonpasteurized aliquots were centrifuged at 15,000 × g at 4 °C. Consistent with recommendations from prior investigations, the fat layer was discarded, and the skim milk was only been described for mothers that delivered at term. The effects of preterm delivery and specific breast milk processing practices, such as HoP, on the non-nutritive components of breast milk have not been defined.

The tests were performed in duplicate. The amounts of leptin and insulin were determined by customized magnetic bead assay "Human Metabolic Hormone Magnetic Bead Panel" (HMHEMAG-34K) (END Millipore Corporation, Billerica, MA, USA). The levels of cortisol, progesterone and testosterone were measured with a separate customized array "Multi-Species Hormone Magnetic Bead Panel" (MSHMAG-21K) (END Millipore Corporation, Billerica, MA, USA). To measure the FSH, LH, and TSH concentrations, we used "Human Pituitary Magnetic Bead Panel 1" (HPTP1MAG-66K) (END Millipore Corporation, Billerica, MA, USA) kits. Kits were performed based on the company's instructions.

Statistical analysis

For statistical analyses, we utilized GraphPad (GraphPad Software, La Jolla, CA, USA), IBM SPSS Statistics v20.0 (IBM's Corporate, New York, USA), and R-Program (R-project, http://www.r-project.org) with normal distributions confirmed by Shapiro −Wilks tests. Data were analyzed by ANOVA or paired Student's t test, as appropriate, and the sample size was chosen to provide >80% power to detect moderate effect sizes (Cohen's d = 0.6) with significance set at p <

0.05. Data are shown as mean ± SEM or mean ± SD or median values with interquartile ranges. The intraassay and interassay coefficients of variation were less than 10%.

4. Results

Hormone levels in preterm and term human milk before and after Holder pasteurization

Maternal data

We recruited 26 mothers who gave birth to preterm infants who were hospitalized in the neonatal intensive care unit of the University of Iowa Stead Family Children's Hospital. The donor samples ($n=31$) were collected from mothers who delivered at term. The donor mothers expressed their milk manually or by pump at their homes and later donated the samples to the Mother's Milk Bank of Iowa (Iowa City, IA, USA). The donors from the milk bank were selected from among those who had met the criteria to donate and were screened and tested based on the protocol of the Human Milk Banking Association of North America. The study was approved by the institutional review board of the University of Iowa and the Mother's Milk Bank of Iowa. The preterm mothers and milk donors consented to the use of their milk samples for this study.

Maternal age and infant sex did not significantly differ between the two cohorts. As expected, the milk bank donors delivered at later gestational ages and they were further removed from the time of delivery when the samples were collected (Table 1.).

Table 1. Maternal and infant characteristics

	Donor mother	**Preterm mother**	**p-value**
Participants	n=31	n=26	
Age (years)	30.8 ± 0.9	30.3 ± 1.1	0.70
interval	23 - 41	19 - 41	
Gestational age (weeks)	39.5 ± 0.2	30.1 ± 0.8	<0.0001
interval	38 - 41	23 - 35	
Postpartum age at collection (days)	164 ± 13	28 ± 4	<0.0001

Continuous variables, expressed as mean ± SEM, were analyzed by ANOVA while infant gender was analyzed by Fisher's Exact Test.

Hormone levels in breast milk

Nonpasteurized preterm milk contained threefold more leptin than nonpasteurized term donor milk. We found no significant differences in insulin, cortisol, progesterone, or testosterone concentrations between nonpasteurized term donor and preterm milk samples (Table 2.).

Table 2. The concentration of hormones in non-pasteurized term donor milk and preterm infant's own mother's milk

Hormone	**Term donor milk**	**Preterm milk**	**p-value**	**Changes**
Insulin (pg/ml)	1269 ± 208	1396 ± 302	0.72	Term Donor ≈ Preterm
Leptin (pg/ml)	198 ± 28	586 ± 121	0.002	Term Donor < Preterm
Cortisol (pg/ml)	6866 ± 2613	1471 ± 433	0.06	Term Donor „>” Preterm
Progesterone (pg/ml)	145 ± 49	361 ± 190	0.25	Term Donor ≈ Preterm
Testosterone (pg/ml)	108 ± 39	106 ± 63	0.97	Term Donor ≈ Preterm

Values are expressed as mean ± SEM.

Effect of Holder pasteurization

HoP significantly decreased leptin levels in both donor and maternal breast milk. HoP significantly decreased insulin concentration in breast milk produced for preterm infants. Cortisol, progesterone, and testosterone levels were not significantly influenced by HoP (Table 3.).

Table 3. Impact of Holder pasteurization on breast milk hormone levels

Hormone	**Source**	**Raw milk**	**Pasteurized milk**	**p-value**
Insulin (pg/ml)	donor	1269 ± 208	1203 ± 205	0.105
	mother	1396 ± 302	1093 ± 222	0.003
Leptin (pg/ml)	donor	198 ± 28	34 ± 9	<0.0001
	mother	586 ± 121	52 ± 15	<0.0001
Cortisol (pg/ml)	donor	6866 ± 2613	4063 ± 707	0.815
	mother	1471 ± 433	1418 ± 533	0.301
Progesterone (pg/ml)	donor	145 ± 49	230 ± 68	0.103
	mother	361 ± 190	102 ± 50	0.199
Testosterone (pg/ml)	donor	108 ± 39	109 ± 14	0.978
	mother	106 ± 63	113 ± 39	0.811

Results are expressed as mean ± SEM.

Holder pasteurization significantly decreased insulin and leptin levels, by 13 and 81% respectively. Cortisol, progesterone, and testosterone levels were not significantly influenced by HoP (Table 4.).

Table 4. Hormones concentrations in breast milk samples obtained from both term donors and preterm mothers were determined before and after pasteurization

Hormone	Holder pasteurization	Concentration (pg/ml)	p-value
Insulin	before	1328 ± 178	<0.001
	after	1152 ± 149	
Leptin	before	382 ± 64	<0.0001
	after	46 ± 8	
Cortisol	before	4376 ± 1459	0.29
	after	2843 ± 485	
Progesterone	before	245 ± 92	0.44
	after	174 ± 45	
Testosterone	before	107 ± 35	0.85
	after	111 ± 19	

Results expressed as mean ± SEM.

Body mass index

We examined how maternal BMI is associated with the hormonal content of breast milk of mothers gave birth to preterm infants. Cortisol, progesterone, and testosterone were not influenced by maternal BMI. However, the milk samples of obese mothers (BMI 30 or above) had significantly increased insulin and leptin levels compared to samples from mothers without obesity, and those associations persisted after HoP (Figure 1).

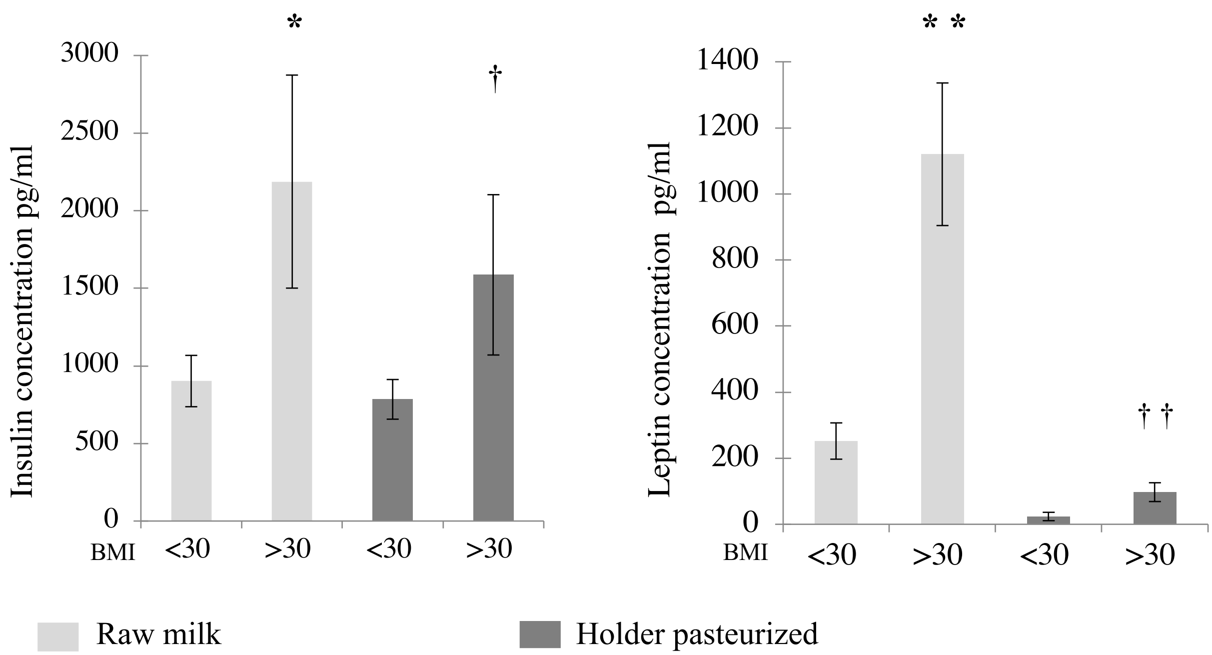

Figure 1. Women providing breast milk for the own premature infant were classified as obese if their body mass index (BMI) was 30 or higher. Using two-way ANOVA, breast milk insulin levels and leptin levels, determined before (light gray bars) and after (dark grey bars) Holder pasteurization, were compared for mothers with or without obesity. *$p < 0.05$ or **$p < 0.001$ for raw milk from mothers with BMI < 30 versus BMI 30 or higher; †$p < 0.05$ or ††$p < 0.001$ for pasteurized milk from mothers with BMI < 30 versus BMI 30 or higher.

Neonatal intensive care practice

During hospitalization preterm infants receiving human milk are fed their own mother's milk or pasteurized donor milk. While the insulin, progesterone, and testosterone concentrations do not statistically differ between those two options, the preterm infant's own mother's milk contains 17 times as much leptin as donor milk and about one-third as much cortisol as donor milk (Table 5.).

Table 5. Hormone concentrations are contrasted for the two main types of preterm infant enteral nutrition, pasteurized term donor milk and raw preterm mother's own milk.

Hormone	**Pasteurized donor milk**	**Preterm milk**	**p-value**	**Differences**
Insulin (pg/ml)	1203 ± 205	1396 ± 302	0.59	↔
Leptin (pg/ml)	34 ± 9	586 ± 121	<0.0001	**17 X**
Cortisol (pg/ml)	4063 ± 707	1471 ± 433	<0.01	**0.36 X**
Progesterone (pg/ml)	230 ± 68	361 ± 190	0.50	↔
Testosterone (pg/ml)	109 ± 14	106 ± 63	0.96	↔

The results are expressed as mean ± SEM.

Hypophysis hormones in breast milk

Measurement protocol

The donor milk samples were analyzed in 3 different forms: the raw, freshly thawed samples, which are presumed to represent the native hormone levels; the pasteurized samples, which showed the effect of pasteurization; and samples that were pasteurized, frozen at −20 ◦C, then thawed and stored at 4 ◦C, which is the usual clinical practice. This last group represents samples typically fed to infants in the neonatal intensive care unit (NICU). The preterm milk samples were divided into 2 different groups (raw samples and refrigerated samples) to represent the general practice applied at the hospital and home. The day before the measurements, we thawed one aliquot of the donor milk samples to room temperature, pasteurized an aliquot, and then placed an aliquot in a refrigerator. For the preterm milk samples, one aliquot was first allowed to reach room temperature. We then placed an aliquot in polypropylene tubes in the refrigerator (Figure 2.).

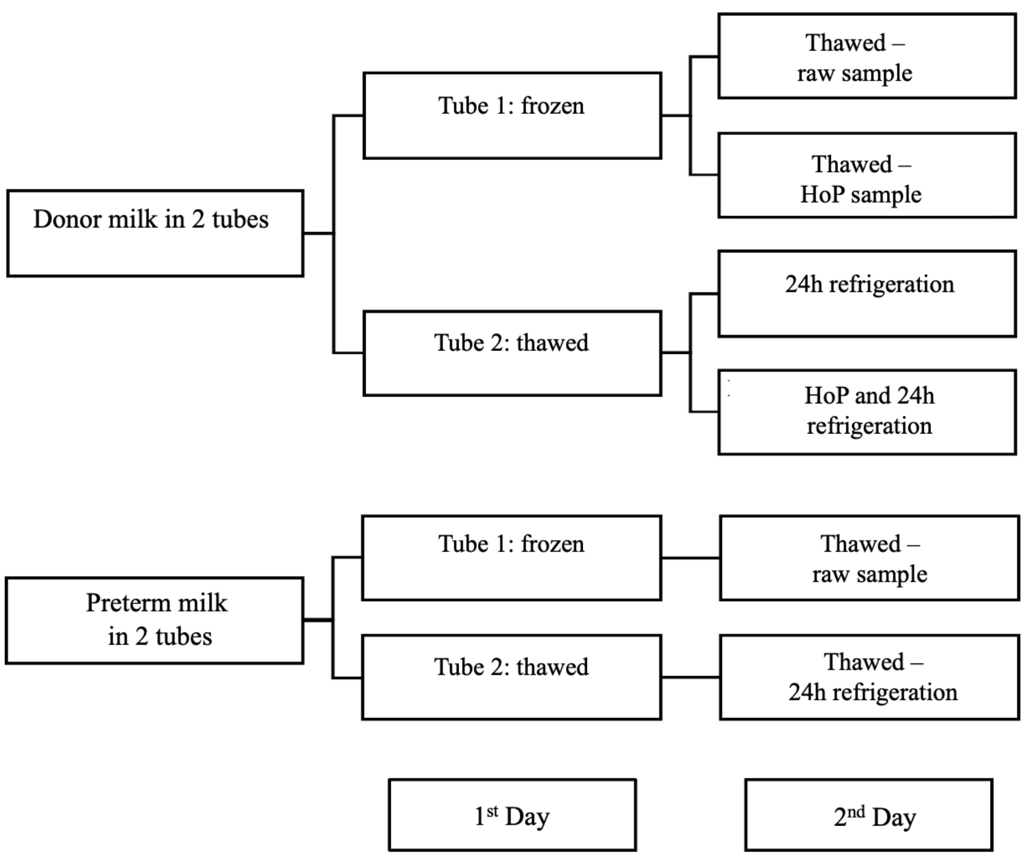

Figure 2. Measurement protocol

Maternal and infant characteristics

The donors of Mother's Milk Bank of Iowa and the mothers of preterm infants were approximately at the same age. At the time of birth, gestational age of the preterm infants was significantly lower compared to the donor's gestational age at the time of delivery. Of the NICU mothers, 14 gave birth to male and 13 to female infants. Among donor mothers 15 had a male child and 15 had a female infant. On average, collected donor milk samples represent the fifth month of lactation, while breast milk produced for preterm infants was from the first month (Table 6.).

Table 6. Maternal and infant characteristics

	Donors	**Preterm mothers**	**p-value**
Recruited mothers	30	27	
Age (years)	30.8 ± 0.8	30.3 ± 1.1	0.70
Gestational age at delivery (weeks)	39.4 ± 0.2	30.1 ± 0.8	<0.0001
Gender of infant			
female	15	13	
male	15	14	
Postpartum at milk collection (days)	159.3 ± 13	28 ± 4	<0.0001

Values are expressed as mean ± SD.

Pituitary hormones in breast milk

Prior to HoP or refrigeration, hormone levels did not differ in the milk provided by the mothers of preterm or term infants. LH was decreased by 24% after HoP, 29% after refrigeration, and 41% after combined HoP and refrigeration (all $p < 0.05$). FSH was unaltered by HoP, but significantly increased by refrigeration. Finally, TSH levels significantly increased after HoP, but this was matched by a significant decrease after refrigeration such that levels were unchanged after combined processing and storage (Table 7.).

Table 7. Effect of Holder pasteurization and/or refrigeration on breast milk levels of pituitary hormones

Hormones	Source	Raw	Pasteurized (% Raw milk)	Refrigerated	Pasteurized and Refrigerated (%Raw milk)
LH	Donor	0.05 (0.03-0.06)	76 * (60-100)	259 ** (169-392)	59 ** (39-74)
(mIU/ml)	Preterm mother	0.04 (0.02-0.10)		71 * (37-86)	
FSH	Donor	0.18 (0.14-0.24)	100 (89-125)	189 ** (139-298)	121 ** (108-134)
(mIU/ml)	Preterm mother	0.18 (0.08-0.32)		127 (100-147)	
TSH	Donor	0.05 (0.04-0.06)	117 * (88-158)	168 * (102-313)	100 (65-136)
(µIU/ml)	Preterm mother	0.06 (0.04-0.09)		79 * (47-100)	

Values are expressed as median (interquartile range) with *$p < 0.05$ ** $p < 0.001$ versus Raw milk.

Impact of Holder Pasteurization

In milk banks, HoP is the usual method applied to ensure microbiological safety. In our study, by paired analysis, HoP reduced LH levels by 24% (IQR 0–40%) and increased TSH levels by 17% (IQR −12–58%), both $p < 0.05$, without significantly influencing the concentration of FSH (Figure 3.).

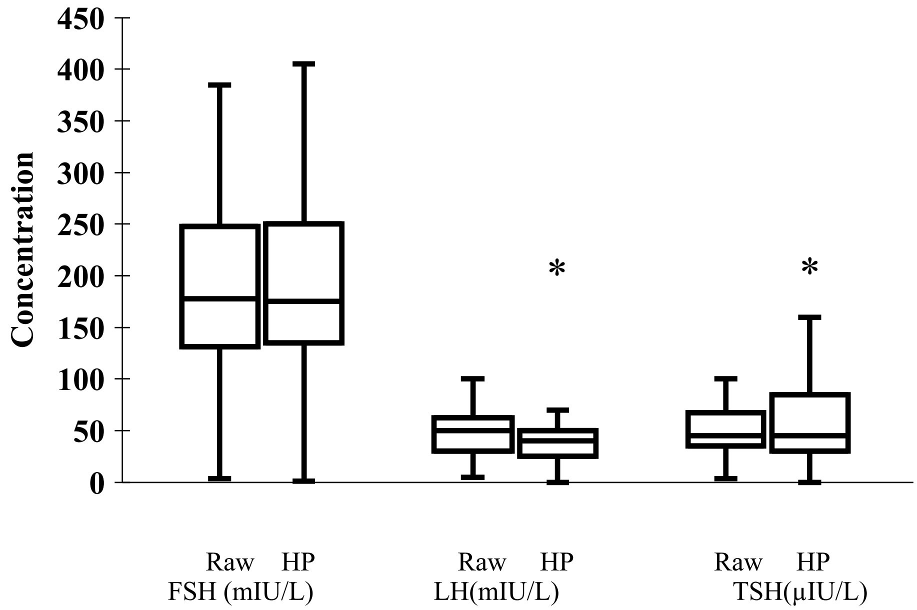

Figure 3. Effect of Holder pasteurization (HP) on the concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) on donor milk * $p < 0.05$ versus raw milk

Impact of refrigeration

We next investigated whether refrigeration, an everyday home and hospital practice for milk storage, influenced breast milk hormonal composition. In a combined cohort of donor and maternal samples, 24 h refrigeration increased the level of FSH by 21% (IQR 0–35%) and reduced the level of LH by 39% (IQR 17–62%), both $p < 0.05$, without significantly altering TSH content (Figure 4.).

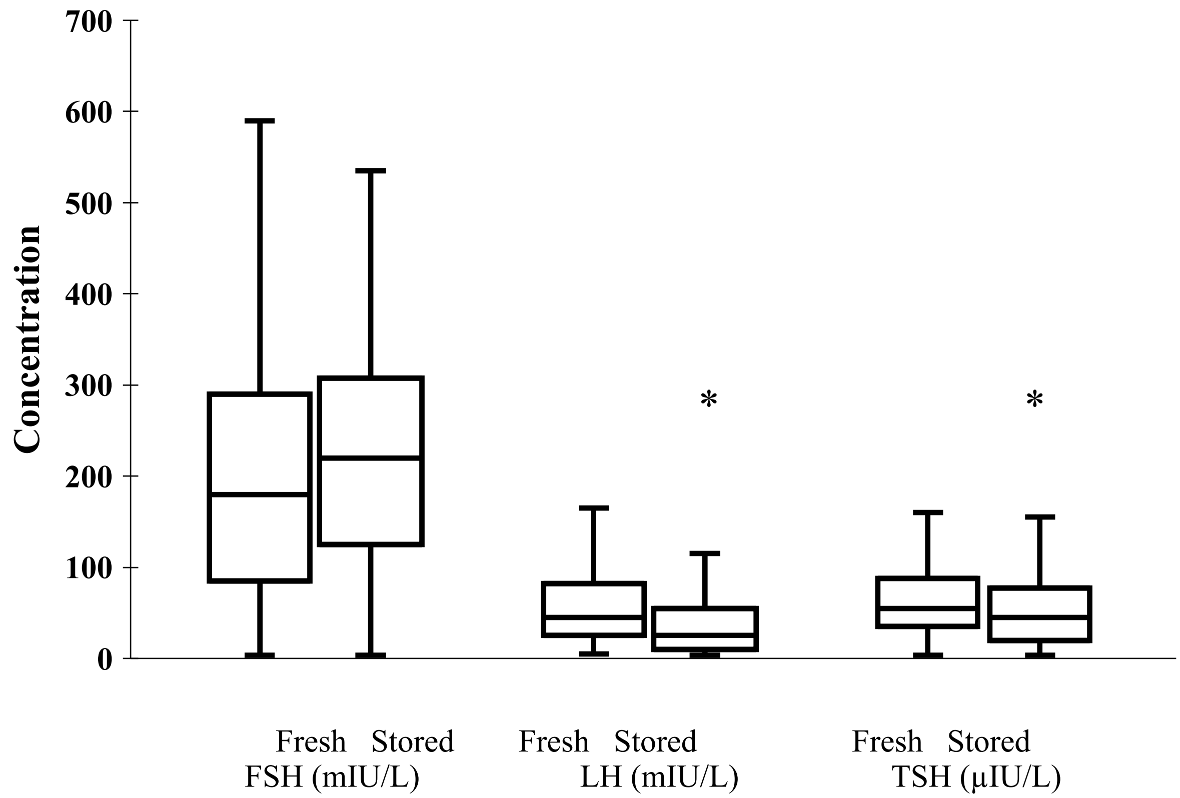

Figure 4. Impact of 24 h refrigerated storage on the concentrations of FSH, LH, and TSH in a combined cohort of freshly pasteurized donor and freshly pumped maternal milk

* $p < 0.05$ versus fresh milk

Clinical and everyday practice in milk handling

When donor milk was subjected to combined HoP and refrigerated storage, the level of FSH increased by 21% (IQR 8-34%), reflecting the HoP-independent consequence of prolonged storage. In contrast, the content of LH decreased by 41% (IQR 26-61%) within those same samples, reflecting the combined effects of HoP and storage. Finally, related to the counterbalancing effects of HoP and storage on TSH level, TSH content in donor milk was unaffected after combined HoP and storage (Figure 5).

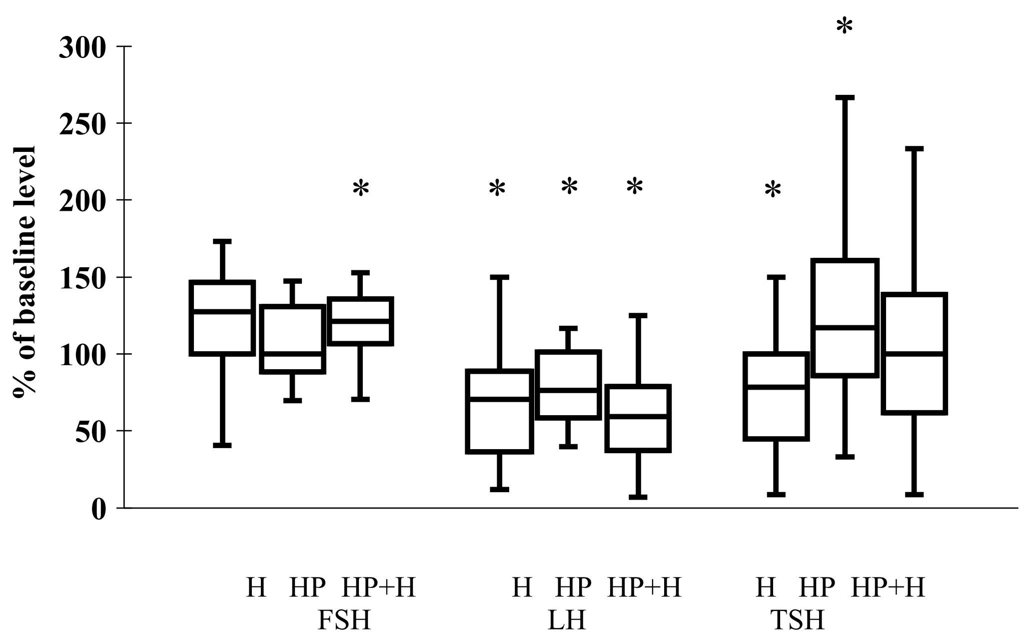

Figure 5. Summary of the changes that occur in the concentrations of pituitary glycoproteins after refrigerated storage, holder pasteurization, or both h storage and holder pasteurization (N = 30, all donor) * p < 0.05 versus fresh raw milk.

FSH:LH ratio in breast milk

The contrasting effects of HoP and refrigeration on the two gonadotropins after combined HoP and storage (Figure 6.).

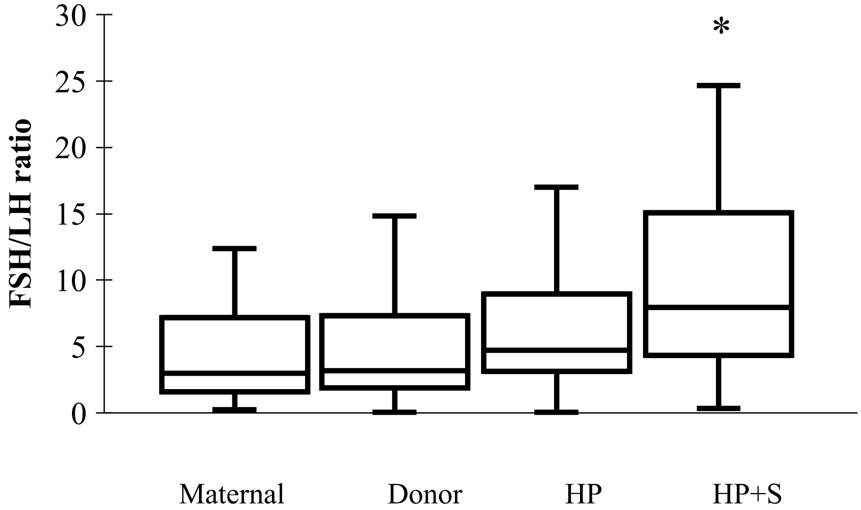

Figure 6. FSH:LH ratio in fresh maternal milk (Maternal), fresh donor milk (Donor), pasteurized donor milk (HP), and pasteurized donor milk after refrigerated storage (HP+S).
* $p < 0.05$ versus fresh donor milk or HoP donor milk

5. Discussion

Breast milk provides not only the nutrients needed by the newborn, but including a myriad of bioactive compounds, such as cytokines, immunoglobulins, growth factors, and hormones (Ballard and Morrow, 2013; Vass et al, 2019).

We studied the levels of insulin, leptin, cortisol, progesterone, and testosterone in preterm and term donor breast milk and also assessed the effect of Holder pasteurization. We were able to detect - utilizing the latest technology - the biologically active forms of the investigated hormones - insulin, leptin, cortisol, progesterone, and testosterone - and demonstrate the capacity to quantify essential hormones with emerging roles in metabolic adaptation and neurological development. These hormones may act directly in the gastrointestinal tract or through the microbiome - gut - brain axis throughout influencing the composition of microbiome, which affects development. Presumably, the protective factors of breast milk both influence the composition of gut microbiota and contribute to maturation of gut-associated lymphatic tissue (Lemas et al, 2016; Pärnänen et al, 2018). Furthermore, these hormones can act like growth factors, influencing the differentiation of gut epithelia (Georgiev et al, 2003).

Premature birth disrupts pregnancy, for preterm infants - after birth - breast milk is the exclusive source of maternal hormones, and they are the main recipients - during their neonatal intensive care - of pasteurized donor human milk. Remarkable differences were detected in the concentration of leptin and cortisol between pasteurized term donor milk and breast milk produced by the mothers of preterm infants. It is not established that preterm milk hormone levels are ideal or that low levels of cortisol and relatively high levels of leptin are preferred. Previous study has demonstrated profound leptin deficiency in premature infants, with plasma leptin levels below the lowest limits of detection (41 pg/ml) at a time when this peptide hormone is believed to have an important role in neuromaturation (Steinbrekera et al, 2019). Serum cortisol levels of preterm infants are variable, but generally was detected above 10,000 pg/ml (al Saedi et al, 1995). The measured plasma ratios are approximated by the concentration values of donor but not preterm milk.

Monkeys and human studies have correlated lower milk cortisol levels with social withdrawal and increased impulsiveness (Dettmer et al, 2018), an outcome reminiscent of the behavioral phenotype described for preterm infants (Johnson and Marlow, 2011). Our results highlight extensive differences in the delivery of leptin and cortisol to preterm infants based on the source of human milk with potential implications for breast milk processing techniques and feeding guidelines.

The applied bioassays in these studies are the preferred methods of analyzing the biologically active forms of hormones in human milk, providing precise data about the effects of everyday milk handling practices, Holder pasteurization and refrigeration on hormone levels.

Our findings demonstrate, for the first time, that FSH, LH, and TSH, three pituitary glycoprotein hormones, are present in the same amounts in term and preterm breast milk. Moreover, that everyday milk handling practices influence the milk concentration of FSH, LH, and TSH in different, hormone-specific ways. Further investigations should reveal the implications of an imbalance in the provision of these hormones, with special attention now drawn to the detected relatively low intake of LH that follows the Holder pasteurization and 24h refrigeration of donated human milk.

6. Conclusion

- We detected all hormones of interests - FSH, LH, TSH, insulin, leptin, cortisol, progesterone, testosterone - in term donor and preterm milk.
- Breast milk of mothers have birth to preterm infants have higher leptin and lower cortisol level than the donor milk.
- Levels of insulin, progesterone, testosterone, FSH, LH, and TSH do not differ in preterm and donor milk.
- Holder pasteurization extremely decreases leptin in preterm and donor milk.
- Obese mothers gave to preterm infants have higher insulin and leptin concentration in their breast milk.
- Holder pasteurization does not affect cortisol, progesterone, and testosterone levels in human milk.
- Storage in refrigerator for 24h increases the TSH, LH, and FSH levels in donor milk.
- Holder pasteurization changes the FSH, LH, and TSH concentrations in donor milk.
- Refrigeration followed by Holder pasteurization the FSH, LH, and TSH concentrations in breast milk.

Summary of new results:

1. FSH, LH, and testosterone present in breast milk.

2. Preterm infant own mother's milk has significantly higher leptin and lower cortisol level compared to term donor milk.

3. Preterm infant own mother's milk has similar insulin, progesterone, testosterone, FSH, LH, and TSH content to donor milk.

4. Holder pasteurization dramatically decreases leptin concentration in milk of mothers gave birth to preterm infants.

5. Obese mothers of preterm infants have elevated insulin and leptin breast milk levels.

6. Holder pasteurization has no influence on cortisol, progesterone, and testosterone concentration in breast milk.

7. Refrigeration increases FSH, LH, and TSH in donor milk.

8. Holder pasteurization impacts FSH, TSH, and LH concentration in donor milk.

9. Refrigeration followed by Holder pasteurization affects FSH, TSH, and LH in human milk.

Impact on everyday practice

After birth, breastfeeding is the exclusive biological connection between mother and infant. During intrauterin development maternal hormones constantly influences infant development, and preterm birth disrupts this connection. Although in breast milk these hormones were found in low concentrations, they ensure constant supply for the infant, and it is also known that leptin does not present in breast milk.

Hormones have innumerous physiological impact on development, their supplementation is an option in infant feeding and nutrition. Ng and coworkers recently published a study investigating the effect of early thyroxine supplementation revealing that it improves long-term neurodevelopment in infants born below 28 weeks' gestation at the age of 3 or 4 (Ng et al, 2020). This observation highlights the importance of studies focusing on early supplementation, especially in donor milk breast fed preterm infants.

Remarkable differences detected in leptin, insulin, and cortizol between preterm infant own mother's milk and donor milk, proposes improvement of new nutritional guidelines in clinical practice.

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8. Publications

- **Publications related to thesis**

Vass RA, Bell EF, Colaizy TT, Schmelzel ML, Johnson KJ, Walker JR, Ertl T, Roghair RD. Hormone levels in preterm and donor human milk before and after Holder pasteurization. Pediatr Res 2020 Jan 30 [Online ahead of print] doi: 10.1038/s41390-020-0789-6. (Q1; IF: 2,747)

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Impact Factor: 7.293

- **Publications not related to thesis**

Turai R, Schandl MF, Dergez T, Vass RA, Kvárik T, Horányi E, Balika D, Gyarmati J, Fónai F, Vida G, Funke S, Reglődi D, Ertl T. [Early and late complications of hyperglycemic extremely low birth-weight infants.] Az extrém alacsony születési súlyú koraszülöttek hyperglycaemiájának korai és késői szövődményei. Orv Hetil. 2019;160(32):1270-1278. (IF: 0,536)

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Cumulative Impact Factor: 12.345

- **Conference presentations related to thesis**

Intézményi ÚNKP Konferencia Pécs, 2020. május 19-20.
Hormonok vizsgálata az anyatejben
Vass Réka Anna

60th Midwest Society for Pediatric Research Meeting 2019 Chicago, IL, USA, 2019. október 24-25.
Effects of Holder pasteurization on levels of metabolic hormones in human milk
Réka A. Vass; Edward F. Bell; Tarah T. Colaizy; Karen J. Johnson; Mendi L. Schmelzel; Jacky R. Walker; Tibor Ertl; Robert D. Roghair

RECOOP 10th Annual Project Review Meeting 2019 Wroclaw 2019. október 10-13.
Understanding the risks of obesity during pregnancy — plus steps to promote a healthy pregnancy
Réka A. Vass, Sandor G. Vari; Timea Takacs; Anna A. Kiss; Flora Dombai; Tibor Ertl

RECOOP 10th Annual Project Review Meeting 2019 Wroclaw 2019. október 10-13.
Associations of pre-pregnancy body mass index and gestational weight gain with the outcome of pregnancy
Tibor Ertl; **Réka A. Vass**, Sandor G. Vari

Magyar Perinatológus Társaság XXVII. Kongresszusa Siófok 2019. szeptember 26-28.
A mindennapi anyatejtárolási és kezelési technikák hatása az anyatej hormontartalmára
Vass Réka Anna; Edward F. Bell; Robert D. Roghair; Tibor Ertl

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Effects of holder pasteurization on levels of metabolic hormones in human milk
Réka A. Vass; Edward F. Bell; Tarah T. Colaizy; Karen J. Johnson; Mendi L. Schmelzel; Jacky R. Walker; Tibor Ertl;Robert D. Roghair

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- **Conference presentations not related to thesis**

RECOOP 10th Annual Project Review Meeting 2019 Wroclaw 2019. október 10-13.
Metabolic hormones in breast milk
Flóra Dombai; **Réka A. Vass**; Gariella Kiss; Attila Miseta; Anna A. Kiss; Timea Takács; Tibor Ertl

RECOOP 10th Annual Project Review Meeting 2019 Wroclaw 2019. október 10-13.
Pituitary hormones in breast milk
Timea Takács; **Réka A. Vass**; Gariella Kiss; Attila Miseta; Anna A. Kiss; Flóra Dombai; Tibor Ertl

HMAA Summer Conference in Balatonfüred, Balatonfüred 2019. augusztus 30-31.
Metabolikus hormonok az anyatejben
Anna A. Kiss; Timea Takács; **Réka A. Vass**; Gabriella Kiss; Attila Miseta; Tibor Ertl

14th Bridges Annual Scientific and 2nd RECOOP-KFSD Student Conferences, 2019. április 10-14. Pozsony
Renal functional effects of early transient postnatal hyperglycaemia and oxidative stress in rats – a preliminary study
Balika D, Horányi E, Kvarik T, **Vass R**, Kiss G, Miseta A, Szabó E, Fonai F, Gyarmati J, Ertl T

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Reka Anna Vass, Tibor Ertl, Sándor G. Vári

14th Bridges Annual Scientific and 2nd RECOOP-KFSD Student Conferences, 2019. április 10-14. Pozsony
IgG N-glycosylation in human milk and serum of obese women with milk cow allergy and in serum of their formula-fed infants

Anna Farkas, Apolka Domokos, Oksana Matsura, Zita Gyurkovics, **Réka Vass**, András Guttman, Sándor G. Vári

14th Bridges Annual Scientific and 2nd RECOOP-KFSD Student Conferences, 2019. április 10-14. Pozsony
Cardiovascular effects of neonatal hyperglycemia – an experimental study
Márton F. Schandl, **Réka A. Vass**, András Czigler, Tímea Kvárik, Dorottya Balika, Eszter Horányi, Dóra Reglődi, Tibor Ertl

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Proapoptotic effects of a PARP inhibitor on cervical carcinoma cells
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Olaparib treatment of HeLa cells- molecular biological changes in the background
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Secretory IgG and IgA N-glycans alteration in human milk of obese pregnant women with gestational diabetes mellitus
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XXII. Nemzetközi és XIX. Országos Interdiszciplináris Grastyán Konferencia Pécs 2019. április 4-5. – Legjobb előadás díj
A korai hiperglikémia kardiovaszkuláris hatásai újszülött patkányban
Vass Réka A., Schandl Márton F., Czigler András, Balika Dorottya, Horányi Eszter, Kvárik Tímea, Reglődi Dóra, Ertl Tibor

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Balatonfüred Hatásos-e a PARP gátló Olaparib méhnyakrák sejteken?
Vass Réka, Boronkai Árpád, Kovács Krisztina

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