# Theses of doctoral (PhD) dissertation

# *Biochemical background of Feline Idiopathic Cystitis*

Dr. Patrícia Hatala Kissné

Supervisors: Dr. Zsuzsanna Neogrády, Csc
Dr. Gábor Mátis, PhD

UNIVERSITY OF VETERINARY MEDICINE
Veterinary Doctoral School

Budapest, 2024.

**Supervisor:**

.....

*Dr. Zsuzsanna Neogrády, CSc*

Department of Physiology and Biochemistry

University of Veterinary Medicine, Budapest

.....

*Dr. Gábor Mátis, PhD*

Department of Physiology and Biochemistry

University of Veterinary Medicine, Budapest

.....

Dr. Patrícia Hatala Kissné

# Introduction

Lower urinary tract disease is one of the most common disorders in domestic cats (*Felis silvestris catus*), with symptoms including variable combinations of hematuria, stranguria, dysuria, periuria, pain, and hypersensitivity during urination. The differential diagnosis of the clinical signs may include urolithiasis, urinary tract infection, neoplasia, or parasites, but there are some cases when no specific underlying cause can be diagnosed by clinical evaluation, so the disease is often referred to as feline idiopathic cystitis (FIC). The possible cause(s) and the pathogenesis of the disorder are widely studied; FIC should be considered as a complex, multifactorial disease. It is strongly suggested that stress may have an important role in the history of FIC, thus the examination of this factor is essential to gain a deeper knowledge concerning the development of the disease. Notwithstanding that the clinical sings of the disease can resolve within a few days without treatment, the reappearance of these symptoms is very common. Based on former studies up to 50% of cats with acute FIC will experience recurrence in one year, which can be related to the altered stress response of cats suffering from FIC. The disease is very similar to the human interstitial cystitis (IC) in both its symptoms and development, therefore the examination of its pathomechanism is important not only in veterinary, but in human medicine as well, and FIC could also serve as an animal model of IC.

According to previous research the activity of tyrosine hydroxylase – which is the main regulatory enzyme of catecholamine production in the central nervous system – was increased in the norepinephrinergic nucleus, *locus coeruleus* and in the paraventricular nuclei of the hypothalamus of the cats with FIC. Consequently, increased norepinephrine (NE) level could also be measured permanently in the blood. The described increased tyrosine hydroxylase activity can be observed in chronically stress-exposed, but otherwise healthy cats as well; however, the plasma NE concentration decreased over time due to the inhibitory effect of the chronic stress-related elevated cortisol production. This inhibitory effect is missing in cats with FIC, as well as they have smaller adrenal gland, thus the cortisol production is lower than in healthy animals. Therefore, examination of the direct role of NE in the pathogenesis of FIC is of major importance.

The bladders of cats with FIC undergo both functional and anatomical changes compared to healthy animals; however, in order to understand these changes, it is necessary to have an overview of the structure of the bladder. The uroepithelium consists of multiple cell layers including a basal, a one-two layers thick intermediate and a differentiated superficial cell layer. The latter consists of the so-called umbrella cells with an asymmetrical apical plasma membrane, containing uroplakins as special uroepithelial differentiation products. According to our recent knowledge, four types of uroplakins exist: Ia., Ib., II., and III., from which uroplakin III. can be detected only in urothelial cells of the urinary bladder, ureter and renal pelvis.

Therefore, uroplakin III. is suitable to characterize differentiated uroepithelial cells in cell cultures. The umbrella cells are connected by tight junctions, which provide an effective barrier against ion-, ammonia-, bacteria- and urea-flux, and make the uroepithelium suitable to form a protective barrier between the urine and blood flow. These tight junctions are consisting of cytoplasmic proteins, cytoskeletal elements and transmembrane proteins. The most characteristic proteins of tight junctions are *zona occludens*, occludin and claudins, being suitable to indicate the barrier function of the epithelium. In addition, the apical membrane of umbrella cells is covered by a layer of glycosaminoglycan (GAG), which play a role not only in improving the barrier function but can help to prevent bacterial adhesion as well, however, the uroepithelial GAG content is decreased in FIC-affected cats. In addition, electron microscopic examination of the bladder tissue revealed thinning of the urothelium and modulated expression and distribution pattern of tight junction proteins resulting in increased bladder permeability to water, urea, and various ions. Due to altered permeability of the bladder, hypertonic urine and its cytotoxic substances can easily reach the less resistant cell layers of the bladder, which are strongly innervated by the sensory nerves, hence inducing neurogenic inflammation, pain and the symptoms of FIC. Therefore, investigation of the barrier function of uroepithelial cells is essential to the more accurate understanding of the pathogenesis of FIC.

In addition to the barrier integrity of uroepithelial cells, it is also important to study the molecular mechanisms of the inflammatory response in FIC. Based on earlier studies, the levels of the pro-inflammatory cytokine interleukin- (IL-6) and the pro-inflammatory chemokine stromal-cell derived factor-1 (SDF-1) were increased in the urine and uroepithelial cells in case of experimentally induced cystitis in rats. Further, concentrations of IL-12, IL-18 and SDF-1 in blood plasma were elevated in cats with FIC. Besides the connection between inflammatory processes and the release of stress hormones, the redox state of the uroepithelial cells is also important to investigate. Inflammatory stimuli are able to produce reactive oxygen species, and the excessive production of the pro-inflammatory cytokines and chemokines may cause oxidative stress, and thereby damage of cellular DNA, membrane lipids and proteins. The oxidative stress can further increase the amount of inflammatory mediators, and also the permeability of the cells due to membrane damage.

# Aims of the study

Briefly summarized, the main aims of this PhD study were:

**Ad 1,** to develop and characterize a novel primary uroepithelial cell culture from feline origin, which contains differentiated uroepithelial cells, as FIC is a complex multifactorial disease, a primary uroepithelial cell culture from feline origin would be a very useful tool to study the effect of different factors separately or in targeted combination.

**Ad 2,** to examine the possible effects of acute sympathetic stress (1h NE treatment) on metabolic status, oxidative stress, pro-inflammatory processes and barrier integrity of the uroepithelial cells *in vitro*, applying the established cell culture model.

**Ad 3,** to examine the pathological role of chronic intermittent stress mimicked by 3x1 h NE treatment in the pathogenesis of FIC, and the comparison of the effects of acute and chronic stress in case of oxidative stress, pro-inflammatory processes and barrier integrity of the uroepithelial cells.

In order to reach these goals, three studies were designed as follows:

| Study No. | Applied cell culture and treatment procedure | Aim of the study | Laboratory analyses (measured parameters) |
|---|---|---|---|
| **Study I.** | Feline primary uroepithelial cell culture | Development and characterization of novel feline primary uroepithelial cell culture model | Characterization with Giemsa staining and immunocytochemistry (pan-cytokeratin and uroplakin III) |
| **Study II.** | Feline primary uroepithelial cell culture, short term (1h) NE exposure | Investigation on the molecular effects of acute stress on uroepithelial cells | Metabolic activity (CCK-8), extracellular LDH release, redox parameters (MDA, PC, 8-OH-dG, GRP78), pro-inflammatory cytokine concentration (IL-6, SDF-1), barrier function parameters (GAG, claudin-4, TER) |
| **Study III.** | Feline primary uroepithelial cell culture, intermittent, pulsative (3x1 h) NE exposure | Investigation on the molecular effects of chronic, intermittent stress on uroepithelial cells, and comparing the effects of acute and chronic stress in the pathogenesis of FIC | Metabolic activity (CCK-8), extracellular LDH release, redox parameters (MDA, $H_2O_2$), pro-inflammatory cytokine concentration (IL-6, SDF-1), barrier function parameters (GAG, claudin-4, TER) |

LDH=lactate dehydrogenase, MDA=malondialdehyde, PC=protein carbonyls, 8-OH-dG=8-hydroxy-2-deoxy guanosine, GRP78= glucose-regulated protein 78, TER=transepithelial electrical resistance

# Materials and methods

## Establishment and characterization of novel primary uroepithelial cell culture from feline origin (Study I.)

In **Study I.**, a urinary bladder was obtained from a euthanized European short hair cat. The cat did not suffer from any urinary tract disease, and the cadaver was offered by the owner for scientific purposes. The uroepithelial cells were gained from the bladder, being excised and washed followed by overnight incubation at 4°C in dispase containing medium. The epithelial cells were separated from the underlying connective tissue, resuspended and digested in trypsin-EDTA solution, and incubated at 37°C for 30 min. Thereafter, following multistep centrifugation, the cells were seeded on collagen-coated culture dishes and cultured for 6 days. The cell cultures were characterized by Giemsa and immunofluorescence staining. For proving the epithelial origin of the cultured cells eFluor conjugated pan-cytokeratin specific antibody binding was used, and to determine the rate of differentiation the uroepithelial cells were labelled with specific fluorescein isothiocyanate (FITC) conjugated uroepithel-binding anti-uroplakin antibodies, further, the cell nuclei (DNA) were stained with DAPI.

## Investigation of the molecular effects of acute stress mimicked by short term (1h) NE treatment on the newly established primary feline uroepithelial cell culture model (Study II.)

The aim of **Study II.** was to examine the molecular effects of acute NE-triggered stress on the established cell culture which served as an *in vitro* model for studying the neurohumoral and biochemical background of FIC. Cell isolation process and culturing conditions have been carried out in accordance with the developed methodology explained in **Study I.** For monitoring pro-inflammatory mediators (IL-6 and SDF-1), oxidative stress parameters (MDA (malondialdehyde), 8-OHdG (8-hydorxy-2-deoxy-guanosine), PC (protein carbonyl), GRP78 (glucose-regulated protein 78), cell injury (LDH [lactate dehydrogenase]) and GAG concentration, cells were plated on 24-well plates. Further, 96-well plates were applied for assessing the cellular metabolic activity (by the CCK-8 assay) and barrier function by claudin-4 abundance. For measuring the TER, cells were seeded on 24-well, high-density polyester membrane inserts. In **Study II.** after 6 days of culturing, the cells were exposed to NE dissolved in defined keratinocyte medium at concentrations of 10, 100, and 1000 µM at 37 °C for 1 h (n=3/group on 24-well plates and n=6/group on 96-well plates), followed by a 24 h regeneration time (culturing without NE supplementation). For measuring the above-mentioned factors and parameters ELISA tests (IL-6, SDF-1, PC, GRP78, 8-OHdG, claudin-4), colorimetric assays (CCK-8, LDH, MDA GAG) and EVOM2 epithelial Volt/Ohm meter (TER) were used.

## Investigation of the molecular effects of chronic, intermittent stress mimicked by pulsative (3x1h) NE exposure on primary feline uroepithelial cell culture model (Study III.)

In **Study III.** similarly, as in **Study II.**, the cell isolation process and culturing conditions have been carried out in accordance with the developed methodology explained in **Study I.** The cell cultures were challenged to NE dissolved in defined keratinocyte medium at the same concentrations (10, 100, and 1000 µM) as in **Study II**. at 37 °C, but instead of 1 h treatment the NE solutions were applied for 3x1 h to mimic *in vitro* chronic recurrent stress (n=3/group on 24-well plates and n=6/group on 96-well plates). Between the pulsative NE treatments simple defined keratinocyte medium without NE was added to the cultures for 1 h at 37°C. The last dose of NE treatment was followed by a 24 h regeneration time (culturing without NE supplementation). The molecular effects of NE were examined by assessing the cellular metabolic activity (CCK-8) and measuring the concentrations of the inflammatory mediators IL-6 and SDF-1, the oxidative stress marker MDA and $H_2O_2$ in cell culture media and cell lysates. To investigate the effect of stress on barrier function, the abundance of the tight junction protein claudin-4, GAG concentration of the cell culture media and the TER of cultured cells were measured after NE treatment. For measuring the above-mentioned factors and parameters ELISA tests (IL-6, SDF-1, claudin-4), colorimetric- (CCK-8, LDH, MDA, GAG) and fluorometric ($H_2O_2$) assays were used, further, the TER values were measured with EVOM2 epithelial Volt/Ohm meter.

## Statistics

For statistical analysis, R v. 4.2.2 (R Core Team, 2022) software was used. Differences between means were determined by one-way ANOVA, and Dunnett's post-hoc tests were used for pairwise comparisons. Data were checked for normal distribution by Shapiro-Wilk test before the statistical analysis was performed. Differences were considered to be significant at $p<0.05$.

# Results and discussion

## Establishment and characterization of novel primary uroepithelial cell culture from feline origin (Study I.)

In **Study I.** of the recent thesis, a novel primary uroepithelial cell culture of feline origin was successfully established as an *in vitro* model suitable for investigating the pathomechanism of FIC. To the best of our knowledge, this model is the first cell culture from a cat which has been proven to contain differentiated uroepithelial cells, verified by the immunofluorescent detection of uroplakin III. As uroplakins, such as uroplakin III are specific urothelial differentiation products, uroplakin III is suitable to characterize differentiated uroepithelial cells in cultures. Cytokeratins are characteristic of epithelial cells, therefore, the positivity confirms the epithelial origin of the cultured cells. The cytokeratin positivity of the cells could be detected from the first day after plating, but the uroplakin III positivity could be observed only on the sixth day after seeding, which may suggest that several days are required to obtain differentiated uroepithelial cells in cell cultures.

## Investigation of the molecular effects of acute stress mimicked by short term (1h) NE treatment on the newly established primary feline uroepithelial cell culture model (Study II.)

In **Study II.**, it was investigated if there is a direct molecular link between the presence of NE and the appearance of the lesions in the bladder of cats with FIC. Hence, the molecular effects of an acute NE treatment on cultured feline uroepithelial cells were examined, whereby the applied treatment induced a pro-inflammatory response, oxidative stress and barrier function impairment in cell cultures, confirming a direct role of NE in the pathogenesis of the disease. After the acute NE exposure, increased metabolic activity of the cells, further, elevated extracellular concentration of the pro-inflammatory cytokine IL-6 and pro-inflammatory chemokine SDF-1 were measured, suggesting the direct pro-inflammatory role of NE in the stress response of the uroepithelial cells. In addition, the modulatory action of the NE in the oxidative status of the cultured cells was also examined, and elevated PC level was measured; however, the concentrations of MDA, GRP78 and 8-OHdG were not increased. These results suggest that the acute NE exposure can induce oxidative stress by the oxidative damage of proteins without triggering lipid peroxidation, endoplasmic reticulum stress or DNA damage. Regarding the barrier function of the uroepithelial cells after the short term NE treatment, it was found that NE was able to decrease the abundance of the tight junction protein claudin-4 and also the GAG concentration (in case of 100 µM NE treatment) and the TER values of the cultured cells (in case of 100 and 1000 µM NE treatment), suggesting that an acute NE challenge might diminish the barrier integrity of uroepithelial cells.

## Investigation of the molecular effects of chronic, intermittent stress mimicked by pulsative (3x1h) NE exposure on primary feline uroepithelial cell culture (Study III.)

Since the recurrence of symptoms in response to repeated stress is very common in FIC-affected cats we investigated whether the onset of symptoms might be related to the presence of repeatedly elevated NE, or whether uroepithelial cells might be able to adapt to chronic stress. There are numerous studies in the literature describing the ability of certain cell types to adapt to the persistent presence of different stressors, for example hepatocytes can successfully adapt to heat stress, or endothelial cells to mechanical stress. In other studies, it was described that acute and also chronic NE exposure could induce oxidative stress and elevated pro-inflammatory cytokine expression in rat myocardial cells, however, until now no information could be found regarding the effect of chronic presence of NE on uroepithelial cell adaptation. Hence, in **Study III**. the effects of intermittent stress were mimicked by 3x1 h NE exposure of the cultured uroepithelial cells. Regarding our results, the response of the repeatedly stressed cells was similar to the consequences of acute exposure: 3x1 h NE exposure resulted in elevated extracellular SDF-1 production, however in contrast to the acute treatment, IL-6 concentrations remained unchanged suggesting that SDF-1 might be more involved in disease progression than IL-6 in case of chronic FIC cases. Further, the pulsative NE treatment induced oxidative stress by increasing $H_2O_2$ production and it was also able to disturb the barrier integrity of the uroepithelial monolayer by decreasing the GAG concentration, the claudin-4 content and the TER values of the cell cultures. Moreover, compared to acute treatment, lower NE concentrations were already capable to reduce GAG levels and TER values suggesting that the cultured uroepithelial cells are more sensitive to repeated exposure to NE than to a single acute treatment.

## Conclusions

Based on the results of the present PhD thesis it can be concluded that short term NE exposure mimicking acute stress can provoke an inflammatory response and decrease the barrier integrity of cultured feline uroepithelial cells. Hence, it is highly expected that stress-associated NE release may play an important mediatory role in the pathogenesis of FIC. Furthermore, the chronic recurrent NE exposure also resulted in inflammatory response; however, a different cytokine profile was detected compared to acute stress exposure. The oxidative status and barrier function of the cultured cells were also diminished by intermittent NE treatment. According to these results it can be concluded that NE may play a key role in the pathogenesis of the disease in both acute and chronic recurrent cases, and that the presence of the elevated NE concentrations in the body of the FIC affected cats may have a direct impact on the changes in the bladder that are involved in the development of disease symptoms.

Information provided by the present study contribute to the better understanding of the pathogenesis and biochemical background of FIC; further, the newly established primary uroepithelial cell culture from feline origin can serve as a useful tool for investigating not only the pathomechanism of FIC, but also for examining other urinary tract disorders in cats.

# New scientific results

**Ad 1,**

A novel primary uroepithelial cell culture, which contains differentiated uroepithelial cells from feline origin has been successfully established and characterized by Giemsa staining and immunocytochemistry. This newly established cell culture can serve as proper tool for studying the biochemical background of FIC and other urinary tract diseases.

**Ad 2,**

Acute stress mimicked by short term (1h) NE treatment was able to increase the metabolic activity of the cultured uroepithelial cells and elevated the extracellular concentrations of the pro-inflammatory mediators IL-6 and SDF-1, confirming that NE can trigger an inflammatory response in the uroepithelium. Oxidative stress markers such as cellular PC levels were elevated by NE exposure, while the concentration of MDA, 8-OHdG and GRP78 were not increased, indicating that NE may provoke the oxidative damage of proteins without inducing lipid peroxidation, DNA damage or endoplasmic reticulum stress. Further, the acute NE challenge might diminish the barrier function of uroepithelial cells, as reflected by the decreased GAG concentration, claudin-4 protein expression, and reduced TER values of the NE-treated cell cultures. Hence, it is highly expected that stress-associated NE release may play an important mediatory role in the pathogenesis of FIC.

**Ad 3,**

Chronic intermittent stress mimicked by pulsative (3x1 h) NE treatment was also able to trigger pro-inflammatory response and oxidative-stress in the uroepithelial cells by increasing the level of SDF-1 and $H_2O_2$ in cell culture media. In addition, similarly to the acute stress exposure, NE increased the permeability of the uroepithelium, since decreased GAG concentration, claudin-4 abundance, and TER values were measured after the NE treatments. The obtained results suggest that bladder epithelial cells are unable to adapt to recurrently elevated NE levels, further supporting the important role of NE in the pathogenesis of FIC.

# Own scientific publications

## Publications related to the topic of the present dissertation

### Full text papers in peer-reviewed journals

Hatala, P., Lajos, A.; Orbán, K., Kulcsár, A.; Mátis, G.; Neogrády, Zs.: **Neurohumoral and biochemical background of feline idiopathic cystitis.** *Magyar Állatorvosok Lapja* 142:367–376, 2020. (Impact factor: 0.143)

Hatala, P.; Lajos, A.; Mackei, M.; Sebők, C.; Tráj, P.; Vörösházi, J.; Neogrády, Z.; Mátis, G.: **Feline Uroepithelial Cell Culture as a Novel Model of Idiopathic Cystitis: Investigations on the Effects of Norepinephrine on Inflammatory Response, Oxidative Stress, and Barrier Function.** *Veterinary Sciences*, 10, 132. 2023. (Impact factor: 2.4)

Hatala P, Sebők C, Mackei M, Kárpáti K, Gálfi P, Neogrády Z, Mátis G. **Molecular effects of intermittent stress on primary feline uroepithelial cell culture as an *in vitro* model of feline idiopathic cystitis.** *Frontiers in Veterinary Science*, 6;10:1258375, 2023. (Impact factor: 3.471)

### Oral and poster presentations on international conferences

Hatala, P.; Lajos, A.; Orbán, K.; Mátis, G. and Neogrády, Zs. **Investigating the inflammatory effect of noradrenaline in a feline urinary bladder cell culture.** Clinical/Research Abstracts Poster Presentation at ISFM European Feline Congress, Cavtat, Croatia 2019.

### Oral presentations on Hungarian national conferences

Hatala Patrícia, Lajos Andrea, Mackei Máté, Orbán Kata, Kulcsár Anna, Neogrády Zsuzsanna, Mátis Gábor: ***Macskák ismeretlen oktanú cisztitiszének biokémiai háttere*****,** MTA Akadémiai beszámolók, 2019. január, Budapest

Hatala Patrícia, Kárpáti Karina, Sebők Csilla, Mackei Máté, Mátis Gábor és Neogrády Zsuzsanna: ***Az intermittáló stressz molekuláris hatásainak vizsgálata macska eredetű primer húgyhólyaghámsejt-tenyészeten*****,** MTA Akadémiai beszámolók, 2024. január, Budapest

## Publications not related to the topic of the present dissertation

### Full text papers in peer-reviewed journals

Mátis Gábor, Hatala Patrícia, Kulcsár Anna, Kulcsárné Petrilla Janka, Neogrády Zsuzsanna **A Kupffer-sejtek szerepe a máj gyulladásos és metabolikus folyamatainak szabályozásában: Irodalmi áttekintés: Role of Kupffer-cells in the regulation of hepatic inflammatory and metabolic processes** *Magyar Állatorvosok Lapja* 137:(9) pp. 569-575, 2015.

### Oral and poster presentations on international conferences

Anna Kulcsár, Dénes Dudás, Gábor Mátis, Patrícia Hatala, Hedvig Fébel, Zsuzsanna Neogrády **The effect of age and diet type on the hepatic and intestinal CYP activity in broiler chicken** XVth European Poultry Conference, Dubrovnik, Horvátország, 2018.

Gábor Mátis, Anna Kulcsár, Patrícia Hatala, Máté Mackei, Zsuzsanna Neogrády **Investigations on the effects of heat stress on hepatic cell culture models of chicken origin** XVth European Poultry Conference, Dubrovnik, Horvátország, 2018.

### Oral presentations on Hungarian national conferences

Kulcsár Anna, Sebők Csilla, Mátis Gábor, Talapka Petra, Hatala Patrícia, Petrilla Janka, Fébel Hedvig, Neogrády Zsuzsanna **Az inzulin és a glukagon jelpálya különböző takarmányozási faktorok segítségével történő szabályozása brojlercsirkében** MTA Akadémiai Beszámolók, Budapest, Hungary, 2018.

Mátis Gábor, Kulcsár Anna, Hatala Patrícia, Tóth Adrienn, Mackei Máté, Neogrády Zsuzsanna **A T-2 toxin sejtkárosító hatásainak összehasonlító vizsgálata csirke primer bélhámsejt- és májsejttenyészeten** MTA Akadémiai Beszámolók, Budapest, Hungary, 2018.

Mátis Gábor, Kulcsár Anna, Kulcsárné Petrilla Janka, Hatala Patrícia, Kővágó Csaba, Neogrády Zsuzsanna **Bakteriális lipopoliszacharidok által kiváltott gyulladás vizsgálata sertés hepatocyta – Kupffer-sejt ko-kultúra modellen** MTA Akadémiai Beszámolók, Budapest, Hungary, 2015.

Mátis Gábor, Kulcsár Anna, Kulcsárné Petrilla Janka, Hatala Patrícia, K_vágó Csaba, Csikó György, Neogrády Zsuzsanna **A Kupffer-sejtek arányának meghatározása sertés primer májsejttenyészeten** MTA Akadémiai Beszámolók, Budapest, Hungary, 2014.

## Supervision of DVM theses

Sebők Csilla: **Egyes takarmányozási faktorok inzulin jelpályára gyakorolt hatásának vizsgálata brojlercsirkében** TDK thesis, supervisors: Kulcsár Anna, Hatala Patrícia, Budapest, 2017.

Lajos Andrea: **A noradrenalin gyulladáskeltő hatásának vizsgálata macska eredetű primer húgyhólyaghámsejt-tenyészeten** TDK thesis, supervisors: Hatala Patrícia, Mátis Gábor, Budapest, 2018

Kárpáti Karina: **A krónikus stressz molekuláris hatásainak vizsgálata macska eredetű primer húgyhólyaghámsejt-tenyészeten** TDK thesis, supervisors: Hatala Patrícia, Mátis Gábor, Budapest, 2023

# Acknowledgement

First of all, I would like to express my greatest gratitude and sincere appreciation to my supervisors, **Dr. Zsuzsanna Neogrády**, for all her help, for her motivation and for all her incredible and persistent faith in me during our work together, and **Dr. Gábor Mátis**, who is one of the smartest and kindest people I know, who introduced me to the beauty of research work already during my university years as my TDK supervisor, for all the help, corrections and realistic vision during our work together. I am particularly grateful for their absolute patience and positive energy through these years.

I would like to give special thanks to my colleagues and to all my former fellow workers at the Division of Biochemistry who assisted me perfectly during the years: **Dr. Anna Kulcsár,** who helped me a lot and taught me a lot about statistics and showed me many useful practices in manual lab work; **Dr. Máte Mackei**, who was always a pleasure to work with both in the lab and at the PhD school, who always had time for brainstorming with me; **Dr. Csilla Papp-Sebők**, for being such a wonderful workmate not only as a colleague, but as a TDK student during her university years and for her help in statistical analysis; for **Dr. Kata Orbán, Júlia Vörösházi, Dr. Patrik Tráj, Dr. Rege Márton** for their kind help in the laboratory measurements.

I am very thankful to our assistants **Márta Tolnainé Hinka**, and **Gabriella Petrovics**, for their essential help regarding the plenty of devices and background work in the lab, and to our secretary, **Júlia Seprődi,** for the administrative work, and for always having a kind, encouraging word for me.

I would like to thank **Professor Dr. Tibor Bartha** for the possibility to do my research in Department of Physiology and Biochemistry. Special thanks have to be given to **Zsuzsanna Szikora,** who always knows where something is or how something is working, for her always quick and professional help.

For the fluorimetric measurements of extracellular hydrogen peroxide concentrations, Victor X2 reader, and for the measurements of transepithelial electrical resistance, EVOM2 epithelial Volt/Ohm meter were kindly provided by the Department of Pharmacology and Toxicology. I highly appreciate the kind help of the department's colleagues in any phase of our research project. By name: **Professor Dr. Péter Gálfi**, **Dr. Orsolya Farkas** and **Dr. Csaba Kővágó**.

I would like to express my gratitude to the **Palavet Veterinary Clinic**, namely **Dr. Attila Pallagi** and **Dr. Zsófia Kántás** for their cooperation, flexibility, and professional assistance.

I want to express special thanks to my graduate students: **Dr. Andrea Lajos,** who became not only my student but also my friend during our work together, and **Karina Kárpáti** for her hard work and enthusiasm.

I can be also very grateful to my high school teachers, my chemistry teacher, **Dr. Endre Borissza**, who made me love biochemistry and my form-teacher, and biology teacher, **Katalin Czédulás** who gave me critical, realistic, independent thinking.

Finally, I am infinitely grateful to my **family** and **friends**, especially my husband, **Dr. Bence Kiss,** who has stood by me in the most difficult times, encouraging, supporting and looking after our beautiful children, giving me peace of mind for the long hours of writing this thesis, and to my children, who always cheer me up and sleep soundly in the afternoon, helping me in my work.

This study was founded, by the **NKFIH grant No. 124586** of the Hungarian National Research, Development and Innovation Office; further the work was also supported by the OTKA**-FK grant No 134940**. The project was partly funded by the University of Veterinary Medicine Budapest, **IK-UK-2017 grant No. 49P11AIO3**.