

**Objective:** Craniofacial development is a complex process which requires an expression of various genes and factors for proper facial embryogenesis. One such group of genes essential for craniofacial development is the Homeobox genes, and we have focused on the detection of DLX4, HOXB3, and MSX2 along with analyzing their role in promoting local-site inflammation using NF- $\kappa$ B and tissue remodeling of PTX3 in cleft lip and palate (CLP) affected tissue.

**Methods:** Material was obtained from 15 children (aged 3–8 months) during the lip plastic. Seven controls were obtained from the lip during upper labial frenectomy. Informed consent was obtained from parents, Research Ethics Committee approval no. 5/26.08.2018. DLX4, HOXB3, MSX2 and PTX3 were detected by in situ hybridization simultaneously with the immunohistochemistry for the genes and NF- $\kappa$ B.

**Results:** We found a residual gene and protein expression of DLX4 in cleft mucosa, whilst no differences in gene expression levels of HOXB3 and MSX2 were noted. A significant increase in protein expression for these genes was noted in cleft mucosa, coupled with a significant increase in NF- $\kappa$ B expression. No differences in PTX3 expression were noted.

**Conclusion:** Residual expression of DLX4 via upregulation of NF- $\kappa$ B pathway in CLP mucosa participates in the increased cellular proliferation (stimulated also by the high HOXB3 expression) and promotion of pro-inflammatory environment. Persisting appearance of MSX2 and NF- $\kappa$ B in the cleft-affected lip seems to dysregulate the hard tissue formation in CLP. PTX3 gene plays a crucial role in regulation and fine-tuning the persisting inflammation in postnatal cleft-affected lip tissue.

**Keywords:** cleft, genes, inflammation, immunohistochemistry, in-situ hybridization

#### **P-04**

##### **Pro-, anti- and inflammatory cytokines and antimicrobial peptides in the milk of healthy and mastitis-affected cows**

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**Objective:** The bovine mastitis is extremely important due to its economic and environmental impact. There are contrary data on mastitis fast indicators like pro-inflammatory, regulatory and anti-inflammatory cytokines and antimicrobial peptides. The aim of the work was the diagnostic marker evaluation

from the different milk cytokines, and defenses in healthy and cows with mastitis.

**Methods:** Milk was taken from 15 healthy, and Holstein Friesian cows with subclinical and clinical mastitis, kept in a typical agricultural setting in Northern Poland. Milk smears were prepared and stained with IL-10, IL-17A, TGF $\beta$ 1, IL-8, IL-12, IL-4, NF $\kappa$  $\beta$ , IFN $\gamma$  and  $\beta$  defensin 3 by immunohistochemistry during the week. Results were evaluated semi-quantitatively.

**Results:** IL-10 showed 90%, but  $\beta$  defensin 3–55% positive cells in the healthy cows with the following decrease during the acute inflammation. IFN $\gamma$  and IL-8 demonstrated individual variations. IL-12 showed 50% of cells in the healthy group, while indices overlapped for clinical and subclinical cows. The most stable factors with the highest number of positive cells in all the groups were IL-2, IL-4, IL17A, TGF $\beta$ 1. NF $\kappa$  $\beta$  demonstrated a small number of positive cells in milk smears.

**Conclusion:** The IL-10 and  $\beta$  defensin 3 positive milk cells are the fastest most sensitive markers for the mastitis-affected cows. IFN $\gamma$ , IL-12 and IL-8 should be removed from the diagnostic prognostic markers due to their individual variations. The stable high number of IL-2, IL-4, IL-17A and TGF $\beta$ 1 reveals the phenomenon to be researched in future studies. NF $\kappa$  $\beta$  is not valid for diagnostic purposes due its small number of positive cells.

**Keywords:** cytokines, defensins, mastitis, milk, cows

#### **P-05**

##### **The modulatory role of curcumin and quercetin on Drosophila GSK-3: a potential therapeutic intervention in Parkinson's disease**

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**Objective:** We explored the mechanistic interactions and potential therapeutic benefits of curcumin and quercetin co-administration with a specific focus on Glycogen synthase kinase 3 GSK-3 activity.

**Methods:** We hypothesize that excess GSK-3 accumulation in the substantia nigra is driven by oxidative stress and aim to test the effects of these compounds on the localisation and activity of GSK-3 in the well-established model organism *Drosophila melanogaster*. We probed the dopaminergic neurons characterisation via Tyrosine Hydroxylase Confocal microscopy, Locomotion and lifespan test were also assayed for

**Results:** The antioxidant properties of curcumin with quercetin mediated an anti-inflammatory response, ameliorat-