

HRVATSKO MIKROSKOPIJSKO DRUŠTVO

POZIV NA 237. SASTANAK

Hrvatskog mikroskopijskog društva, koji će se održati u prostorijama
Biološkog odsjeka Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu,
Rooseveltov trg 6, dvorana Vijećnica (predvorje desno), u

utorak, 13. ožujka 2018. u 16:00 sati

u organizaciji Gorana Kovačevića, dopredsjednika HMD-a

uz sljedeći

Dnevni red:

1. Crtica iz povijesti:

Ognjen Milat: Logotip HMD-a; što, tko, kada, gdje, kako, i zašto?

2. Izlaganja stipendista za MCM2017:

Viviana Kozina: Mast cells of the human foetal testis

Nikola Bijelić, MeF Osijek: A method for measuring area and surface-related parameters on microphotographs by using free and open-source image processing software

Ivana Lovrić, MeF Osijek: Growth plate and trabecular bone histomorphometry in wild-type and TFF3 knock-out mice (izlaganje će održati Nikola Bijelić)

Edi Rođak, MeF Osijek: A histological analysis of glycogen content in hepatocytes of trefoil factor family 2 and trefoil factor family 3 knock-out mice

3. Sponzorsko izlaganje:

Igor Pongrac, Merck d.o.o.

4. Plan aktivnosti popularizacije mikroskopije u 2018.

5. Razno

Tajnica:
Jelena Macan

Predsjednica:
Andreja Gajović

Ognjen Milat:

Crtice iz povijesti HMDa - Logotip; što, tko, kada, gdje, kako, i zašto?

Sažetak:

Logotip našega društva kreiran je pred 28 godina. Usprkos međuvremene promjene i samog naziva društva, ostao je isti. Ukratko ću izložiti prigodna događanja i okolnosti iz toga vremena; mlađima na znanje a starijima za prisjećanje. O nastanku *loga* postoji pisani spomen na stranicama 86-7 monografije *Elektronska mikroskopija u Hrvatskoj* D.Baumana i S.Gajovića.



Mast cells of the human foetal testis

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Keywords: human foetal testis, mast cells, sex cords

Data about the mast cells of human foetal testis are lacking in the literature. The aim of this study is to gain knowledge about the presence of mast cells within the testis during foetal period and how their number is changing with the gestational age. Our hypothesis was that the number of this cells in foetal testis is increasing with the volume of testis ie size of the sex cords and intersititial volume. Specific aims were to visualize the presence of mast cells using histological and immunohistological methods and quantify their number during the intrauterine development of the whole testis. The material was obtained during the routine paedopathological autopsy of 39 spontaneously aborted/stillborn fetuses between 15 and 36 gestational weeks. Qualitative and quantitative analysis was performed. During gestational weeks 15-29, mast cells were exclusively located within the tunica albuginea. After 30th gestational week, besides the tunica albuginea, mast cells populated the loose connective tissue of the tunica vasculosa and the connective tissue of septa/interstitium within the parenchyma. The total number of mast cells increased over the investigated period. Quantitative stereological analysis showed that the development of mast cells coincided with the increase of the volumes of the testis. In contrast to increase of the volume and number of mast cells, diameter of the sex cords remains similar. The development of mast cells in the foetal testis is probably regulated by paracrine factors secreted by cells of the interstitium and sex cords.

**References:**

1. Sharpe RM., Clin Endocrinol Med (1986) 185-207.
2. Young JC et al., Semin Cell Dev Biol.(2015) 94-1030.
3. Raz E., Curr Opin Cell Biol (2004) 169-73.

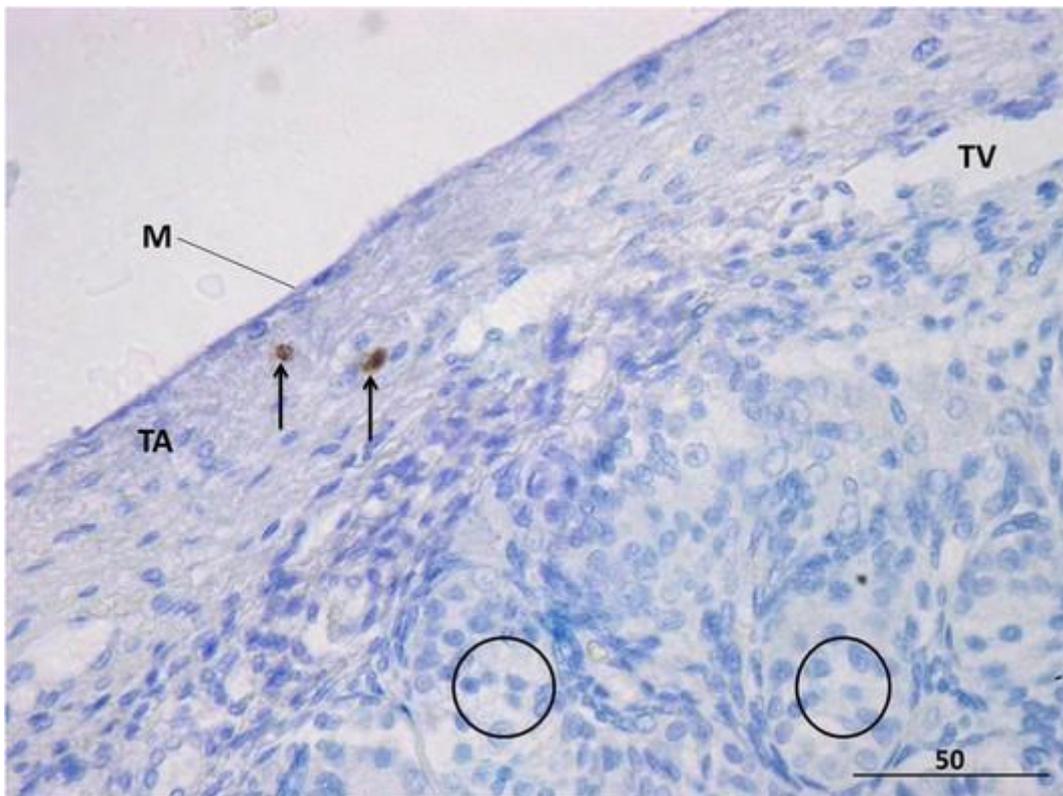


Figure 1. Human foetal testis in the 30th week of gestation. Two positive mast cells (□) are visible within the connective tissue of tunica albuginea (M – mesothelium; TV – tunica vasculosa; □ - sex cords). IHC (mast cell tryptase Ab+DAB) counterstained with hemalaun, x 400, scale bar = 50 μ m.



POSTER PRESENTATIONS L7:

A method for measuring area and surface-related parameters on microphotographs by using free and open-source image processing software

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Keywords: histomorphometry, microscopy, software, methods

There are different ways of obtaining area and surface-related parameters for describing the micromorphology of different tissues, including cancellous bone. Histomorphometry and micro computed tomography can be too costly for small laboratories and institutions, especially when proprietary software or expensive equipment is needed. Free and open-source software alternatives are available today. Some of these can be used for histomorphometry. Here we demonstrate how all image processing and morphometry can be done on freely available software. This method was inspired by the paper by Egan et al. (1), however, we further replaced all of the proprietary and expensive software by using free and open-source alternatives. For measurements of the cancellous bone, images were first processed using open-source and free imaging software GIMP (GNU Image Manipulation Program) and measurements were performed by using FIJI software (FIJI is Just ImageJ), a distribution of ImageJ open-source image processing software (2, 3). The measured parameters were: bone area (B.Ar), trabecular tissue total area (T.Ar) and trabecular bone perimeter (B.Pm). Using several mathematical formulas, we calculated the following parameters describing cancellous bone histomorphometry: trabecular bone volume (BV/TV, in %), trabecular bone surface (BS/TV, in /mm), trabecular thickness (Tb.Th, in mm), trabecular number (Tb.N, in/mm) and trabecular separation (Tb.Sp, in mm). These parameters are important in studies of bone development and quality. There are many benefits of using freely available and open-source



software for scientific research, especially the low cost, worldwide availability and transparency, and this work supports that notion.

References:

1. K. P. Egan et al. *Histopathology* 61 (2012) 1168–1173.
2. J. Schindelin et al. *Nat Methods* 9 (2012) 676–682.
3. J. Schindelin et al. *Mol Reprod Dev* 82 (2015) 518–529.



Growth plate and trabecular bone histomorphometry in wild-type and TFF3 knock-out mice

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Keywords: TFF3, trabecular bone, primary ossification center, growth plate, histomorphometry

TFF3 peptide is present during intrauterine endochondral ossification in mice. Lack of TFF3 peptide in TFF3 knock-out mice affects histomorphometric parameters describing cancellous bone quality in secondary ossification centers of mouse tibiae, impairing bone formation. The aim of this study was to quantitatively analyze several parameters describing the growth plate and primary ossification centers in tibiae of one month old wild type and TFF3 knock-out mice. For the analysis of primary ossification centers, tibiae of one month old 5 wild type mice and 5 TFF3 knock-out mice were used. Three representative slides were used from each bone, hemalaun-eosine stained and analyzed. Digital photographs of bones were processed by open source computer programs Gimp and FIJI. Histomorphometric parameters describing growth plate were analyzed after staining with Masson's trichrome stain. Growth plate photographs were analysed by QuickPHOTO Pro software. Statistical software Statistica was used to perform Mann-Whitney U test. Morphological analysis of the TFF3 knock-out mice bone showed significantly smaller trabecular number (Tb.N.) and significantly larger trabecular separation (Tb.Sp.), compared to the wild type mice. Trabecular bone volume (BV/TV), trabecular bone surface (BS/TV) and trabecular thickness showed no significant difference between wild type and knock out mice. No significant histomorphological differences between wild-type mice and TFF3 knockout mice were found in epiphyseal plate thickness, in the



thickness of different zones of endochondral ossification, and in the chondrocyte density. There are several histomorphological differences in bone structure between the wild type and the TFF3 knock-out mice. TFF3 probably has an effect on the formation and quality of the cancellous bone in the primary ossification centers. Further research might explain the extent of TFF3 influence on bone development and the function of epiphyseal plate.



POSTER PRESENTATIONS L6:

A histological analysis of glycogen content in hepatocytes of trefoil factor family 2 and trefoil factor family 3 knock-out mice

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Keywords: Tff, liver, histology, glycogen

Trefoil factor family (Tff) peptide 2 and Tff peptide 3 are small peptides mostly present in the gastrointestinal mucosa and related to mucosal protection and restitution. Tff3 is included in hepatic glucose metabolism, and both Tff2 and Tff3 peptide stimulate beta-cell proliferation in the pancreatic islets. Tff2 and Tff3 deficient mice including appropriate wild type mice of mixed background (Sv129/C57Bl6) (N=6 per genotype) were kept on standard diet until 6 month old. Glycogen distribution was monitored in PAS stained formalin fixed and paraffin embedded tissue sections (6 μ m). Areas of strongest glycogen staining were chosen for analysis and glycogen-positive cells were counted within regions of 100 cells. Using an arbitrary semi-quantitative scale, the signal was classified as weak (0-35 positive cells), medium (36-70 positive cells) and strong (71 or more positive cells). Tff3 deficient mice had the strongest accumulation of glycogen that was statistically increased compared to wild-type mice ($p=0.005$, Mann-Whitney U test). Liver glycogen distribution in Tff2 deficient mice was heterogeneous and overall signal did not differ statistically from that of wild-type mice ($p=0.5$). Our results support the notion that Tff3 peptide is included in the hepatic glucose and glycogen metabolism. Further, metabolism-oriented studies are needed to elucidate the exact metabolic role of Tff3 peptide.

References:

1. Y. Xue et al., Plos One 8 (2013) e75240.