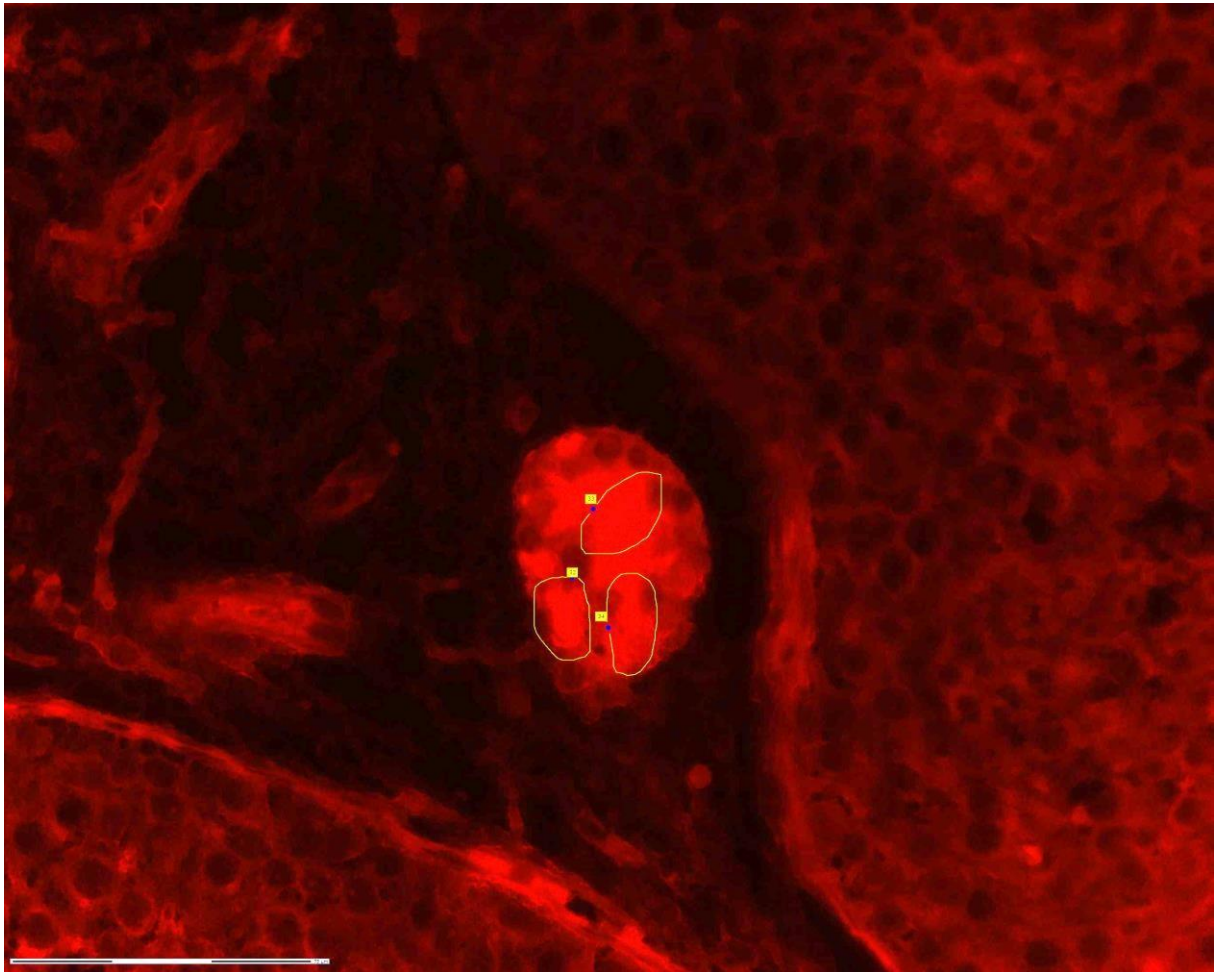




Rigshospitalet



*EAA Centre Zagreb and EAA Centre Copenhagen
under the auspices of the European Academy of Andrology (EAA)*



EAA WINTER SCHOOL

**Testis Histology and Pathology
for Clinical Andrologists and Embryologists**

Zagreb, 3-6 December 2019

**WELCOME TO THE EAA WINTER SCHOOL:
Testis Histology and Pathology
for Clinical Andrologists and Embryologists**

Dear colleagues,

It is our pleasure to welcome you to the *Testis Histology and Pathology for Clinical Andrologists and Embryologists* winter school to be held from 3 to 6 December 2019 at the University of Zagreb School of Medicine. The winter school is jointly organised by the European Academy of Andrology (EAA) training centres Copenhagen and Zagreb.

The idea of the winter school is to bring together experts from various fields of the booming science of andrology and unite them in the various theoretical and practical aspects of their profession.

The objectives of this winter school will address the following:

- Refreshing the knowledge of clinical andrologists and clinical embryologists/biologists on normal testis morphology, in particular spermatogenesis;
- Enhancing the knowledge on various degrees of damage of spermatogenesis; make them familiar with the current classifications of spermatogenesis damage;
- Demonstrating open biopsy of the testis – TESE and mTESE, spermatozoa harvesting from testicular biopsy;
- Genetic and epigenetic factors influencing male fertility/infertility (essentials);
- Delivering basic knowledge on testicular dysgenesis syndrome and the early detection of germ cell neoplasia in situ (GCNIS) testis;
- Presenting novel tumour markers and modern classification of testicular tumours;
- Presenting the current EU regulations regarding sperm and testicular tissue bio-banking.

On behalf of the EAA Centre Zagreb and our colleagues from the EAA Centre Copenhagen, we wish you a successful winter school and enjoyable stay in Zagreb, the oldest centre of academic and research excellence in this part of Europe, hoping you will appreciate its multicultural atmosphere, vibrant life and picturesque scenery. We also hope you will enjoy the atmosphere of the famous Zagreb Christmas market that has already won the title of the best Christmas market in Europe three times.



Davor Ježek, MD, PhD
Director of EAA Centre Zagreb



Niels Jørgensen, MD, PhD
Director of EAA Centre Copenhagen

COURSE PROGRAMME

Monday, 2 December 2019

Location: University of Zagreb School of Medicine, Šalata 3

18.00 **Welcome Reception**
Venue: Biology hall

Tuesday, 3 December 2019

Location: Department of Histology and Embryology, University of Zagreb School of Medicine, Šalata 3

08.30 – 08.40 **Introduction: Programme Overview**
Davor Ježek

08.45 – 09.30 **Initial test**

09.30 – 10.15 Lecture 1: **Essentials of testis histological techniques**

Tissue fixation; dehydration, embedding; tissue sectioning, standard staining hemalaun eosin; basic principles of immunohistochemistry techniques (IHC); markers of various testis cell types

Davor Ježek

10.15 – 10.30 **Coffee break**

10.30 – 13.00 **45-min practical rotations**

Practicals 1: **Back to the lab - Department of Histology and Embryology**

Step-by-step demonstration of testis tissue fixation, dehydration, embedding, sectioning, staining etc. (participants will be able to section their own sections and stain them; "see my sections")

Davor Ježek, Ana Planinić and laboratory technicians

Practicals 2: **Back to the lab - Department of Medical Biology**

Immunohistochemistry (IHC) reactions; section pre-conditioning; section incubation; buffers; application of primary antibody; use of secondary antibody and augmentation complex; application of the chromogen, visualization of the IHC reactions; "see my IHC slide"; epigenetic analysis (pyrosequencing) - essentials

Ana Katušić Bojanac, Marta Himmelreich, Tihana Marić and laboratory technicians

Practicals 3: **Back to the lab - Department of Chemistry and Biochemistry**

Y chromosome microdeletions; clinical chemistry and molecular diagnostics; introduction to principles of molecular laboratory analyses; multiplex PCR/electrophoresis - AZF basic and extension markers; EAA/EMQN - European molecular genetics quality network; CFTR mutations - methods, result and interpretation.

Jadranka Sertić, Hana Ljubić, Ana Merkler and laboratory technicians

13.00 – 14.00 **Lunch**

14.00 – 14.45 Lecture 2: **Normal histology of the testis**

Emphasis on normal spermatogenesis; regular structure of seminiferous tubules; seminiferous epithelium; Sertoli cells and spermatogenic cells; spermiogenesis; round late spermatids and spermatozoa; testicular interstitial tissue; "excretory" part of the testis: straight tubules, rete and mediastinum testis, efferent ducts, epididymis

Ana Katušić Bojanac

14.45 – 15.00 **Coffee break**

15.00 – 17.00 Practical 4: **Normal histology of the testis**

Real-time microscopy, "see my slide"; parallel projection of slides and discussion

Davor Ježek, Ana Katušić Bojanac, Ana Planinić

17.00 – 17.45 Lecture 3: **In vitro spermatogenesis: current state and future developments**
Stefan Schlatt

- 17.45 - 17.55 Discussion
17.55 - 18.00 **Wrap-up of Day 1; Take-home messages; Programme overview of Day 2**
Davor Ježek

Wednesday, 4 December 2019

Location: University Hospital Zagreb Department of Urology, Kišpatićeva 12

- 08.30 – 09.15 **Lecture 4: From Testicular Biopsy to Human Embryo**
Željko Kaštelan, Dinko Hauptman, Davor Ježek
Location: University Hospital Zagreb Department of Urology, Lecture Hall
- 09.30 – 11.00 **Practicals 5: “Open biopsy” of the testis**
Patient pre-treatment; local anaesthesia; biopsy procedure; tissue transfer into the tissue transfer medium; tissue processing: immersion into the cryomedium; tissue fixation for histology
Dinko Hauptman, Zoran Zimak, Davor Ježek and other medical staff
Location: Operation Tower, University Hospital Zagreb Department of Urology, Operational Theatre No.2
- 11.15 – 13.00 **Practicals 6: Programmed freezing of testicular tissue**
Step-wise freezing of testicular biopsy; manipulation of cryotubes and liquid nitrogen; tissue storage into cryocontainers; testicular tissue bank
Davor Ježek and co-workers
Location: Andrology Unit - Green Building 1st floor, University Hospital Zagreb Department of Urology
- 13.00 – 14.00 **Lunch**
- 14.00 – 14.45 **Lecture 5: Current EU regulation on tissue banking**
Emphasis on semen and testicular tissue banks; EU directives, tissue encoding, tissue transfer within EU
Branka Golubić Čepulić and co-workers
Location: University Hospital Zagreb Department of Urology, Lecture Hall
- 14.45 – 15.00 **Coffee break**
- 15.00 – 17.50 **Practicals 7.1: Testicular sperm extraction (TESE)**
Thawing of the frozen tissue; tissue manipulation and microdissection, spermatozoa visualization, isolation and extraction (for ethical reasons, pre-frozen rat tissue will be used)
Patrik Stanić, Davor Ježek
Location: Andrology Unit - Green Building 1st floor, University Hospital Zagreb Department of Urology
- Practicals 7.2: ICSI Station**
Intracytoplasmic sperm injection after spermatozoa isolation/extraction
Nina Gelo and co-workers
Location: Andrology Unit - Green Building 1st floor, University Hospital Zagreb Department of Urology
- 17.50 – 18.00 **Wrap-up of Day 2; Take-home messages; Programme overview of Day 3**
Davor Ježek

Thursday, 5 December 2019

Location: Department of Histology and Embryology, University of Zagreb School of Medicine, Šalata 3

- 08.30 – 09.15 Lecture 6: **Obstructive (OA) and non-obstructive azoospermia (NOA): damage of spermatogenesis**
Description of OA and NOA; histology of OA and NOA; current classification(s) of the spermatogenesis damage; hypospermatogenesis, maturation arrest, Sertoli cell-only syndrome, tubular sclerosis, “mixed atrophy”, interstitial fibrosis, histology of Klinefelter syndrome and AZFc microdeletion cases, etc.
Davor Ježek
- 09.30 – 11.00 Practicals 8: **OA cases**
Real-time microscopy, projection and discussion of slides; “see my slide”
Davor Ježek, Ana Katušić Bojanac, Ana Planinić
- 11.00 – 11.15 **Coffee break**
- 11.15 – 13.00 Practicals 9: **NOA cases**
Real-time microscopy, projection and discussion of slides; “see my slide”
Davor Ježek, Ana Katušić Bojanac, Ana Planinić
- 13.00 – 14.00 **Lunch**
- 14.00 – 15.30 Practicals 10: **NOA cases, continued**
“Special cases”, Klinefelter syndrome, Y chromosome AZFc, partial AZFb microdeletion; real-time microscopy, projection and discussion of slides
Davor Ježek, Ana Katušić Bojanac, Ana Planinić
- 15.30 – 15.45 **Coffee break**
- 15.45 – 16.30 Lecture 7: **Testicular dysgenesis syndrome (TDS) and germ cell neoplasia in situ (GCNIS)**
Concept of TDS; origin of GCNIS; modern IHC markers of GCNIS
Niels Jørgensen, Ewa Rajpert-De Meyts
- 16.35 – 17.45 Practicals 11: **Spermatogenesis and GCNIS**
Real-time microscopy; projection and discussion of slides; demonstration of IHC slides and markers
Ewa Rajpert-De Meyts, Niels Jørgensen
- 17.50 – 18.00 **Wrap-up of Day 3; Take-home messages; Programme overview of Day 4**
Ewa Rajpert-De Meyts, Niels Jørgensen, Davor Ježek

Friday, 6 December 2019

Location: Department of Histology and Embryology, University of Zagreb School of Medicine, Šalata 3

- 08.30 – 09.15 Lecture 8: **Testicular neoplasms/tumours, essentials**
Current classification of testicular tumours; essential morphological properties
Niels Jørgensen, Ewa Rajpert-De Meyts
- 09.15 – 09.30 **Coffee break**
- 09.30 – 10.45 Practicals 12: **Testicular neoplasms/tumours**
Main characteristics of seminoma; non-seminoma; spermatocytic seminoma; real-time microscopy; projection and discussion of slides; demonstration of IHC slides and markers
Niels Jørgensen, Ewa Rajpert-De Meyts
- 11.00 – 11.45 **Test (multiple choice)**
Davor Ježek, Ana Planinić
- 11.45 – 12.00 **Evaluation of the course (questionnaire)**
- 12.00 – 13.00 **Lunch**
- 13.00 – 13:20 **Test results, certificates, sum-up discussion**
- 13:20 **Farewell**

Literature:

Ježek D. (ed.): Atlas on the human testis: Normal morphology and pathology. Springer Verlag London, 285 pgs., London, 2013 (ISBN 987-1-4471-2763-5; DOI 10.1007/978-1-4471-2763-5)
(each participant will get a free e-version of the book)

Handouts of presentations.

PDFs of papers of interest on Google Drive.

Faculty:

University of Copenhagen, Royal University Hospital

Prof. Dr. Niels Jørgensen

Prof. Dr. Ewa Rajpert-De Meyts

University of Münster Centre of Reproductive Medicine and Andrology

Prof. Dr. Stefan Schlatt

University of Zagreb School of Medicine

Prof. Dr. Davor Ježek

Prof. Dr. Željko Kaštelan

Prof. Jadranka Sertić

As. Prof. Dr. Branka Golubić Čepulić

As. Prof. Ana Katušić Bojanac

Dr. Dinko Hauptman

Dr. Zoran Zimak

Dr. Ana Planinić

Hana Ljubić, PhD

Ana Merkler, PhD

Dr. Marta Himmelreich

Tihana Marić, mag. biol. mol.

Patrik Stanić, PhD

Nina Gelo, PhD