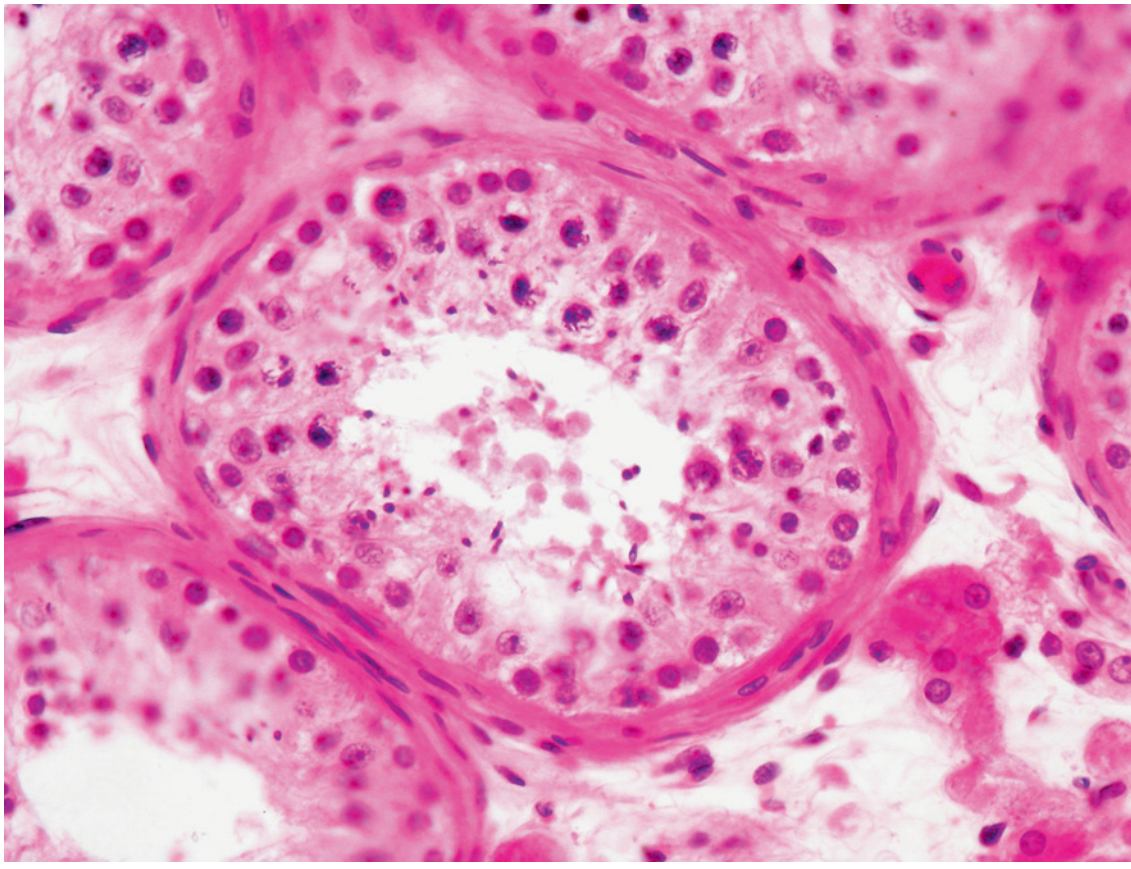
The European Academy of Andrology
University of Zagreb School of Medicine

Andrology update 2015'

mini-Symposium



Zagreb, October 16, 2015

Final program

Co-organizers and sponsors

Clinical Hospital Center "Zagreb"

Croatian Medical Association

Croatian Medical Chamber

Center of Excellence (Medical School University of Zagreb)
for Reproductive and Regenerative Medicine

Unit for Biomedical investigation of reproduction and development

Programme

EAA accreditation of the training centre in Zagreb mini-Symposium "Andrology update 2015' "

Friday, Oct. 16, 2015

9.30 – pick up at the hotel

10.00 – **School of Medicine University of Zagreb**, meeting with Vice-Dean for Int. Affairs, Prof. Dr. Davor Ježek and colleagues (new venues of co-operation with EAA; Croatian Medical Association; new edition of Atlas on the Human Testis /Springer, London/; participation in the centre for Advanced Postgraduate Studies Dubrovnik /IUC etc.);

13.00 – transfer to **Clinical Hospital Centre "Zagreb", Clinic for Urology**, Kišpatićeva 12, Zagreb

13.30 – refreshment, light lunch, preparing for lectures

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14.00 **mini-Symposium "Andrology update 2015'"** (registration free)

14.00 – 14.15 **Welcome** (Dean of School of Medicine, University of Zagreb, Prof. Dr. Marijan Klarica; Director of the Clinical Hospital Centre "Zagreb", Prof. Dr. Hrvoje Vrčić; President of Croatian Medical Association, Prof. Dr. Željko Krznarić; President of Croatian Medical Chamber, Dr. Trpimir Goluža)

14.15-14.30 Csilla Krausz: **"EAA-mission, present activities and future perspectives"**

14.30-14.50 Hans-Christian Schuppe: **"The impact of urogenital infections and inflammation on male infertility"**

14.50-15.00 *Discussion*

15.00-15.20 Davor Ježek **"Macrophages and Leydig cells in testicular biopsies of infertile men with azoospermia: friends or enemies?"**

15.20-15.30 *Discussion*

15.30-15.40 Coffee break

15.40-16.00 Dinko Hauptman: **"Conventional TESE - Croatian experience"**

16.00-16.10 *Discussion*

16.10-16.30 Branko Zorn: **"mTESE- Slovenian experience"**

16.30-16.40 *Discussion*

16.40-17.00 Ewa Rajpert-De Meyts: **"Germ cells turning malignant: bad genes or bad environment?"**

17.00-17.10 *Discussion*

17.10-17.30 Csilla Krausz: **"Fertility and cytostatic therapies: when is the right timing for natural conception?"**

17.30-17.40 *Discussion*

17.40-17.50 Ewa Rajpert-De Meyts: **"Andrology – the first European & American journal in the area of andrology: scope & journal profile"**

17.50-18.00 Davor Ježek: **"Foundation of Croatian Society for Andrology"**

18.00 Concluding remarks

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19.00 – dinner with hosts (Ježek, Kaštelan) (or departure, upon individual plans, transfer to the airport)

Saturday, Oct. 17, 2015

09.00 – coffee in the lobby of the hotel; launching the new andrology project with University of Padua (project partners only)
10.00 – meeting in the lobby of the hotel "Panorama" (all participants)
10.15 – walk through the city centre, sightseeing;
12.00 – refreshment in the upper town

Free time and departure

Andrology update 2015'

EAA – mission, present activities and future perspectives

The European Academy of Andrology (EAA) was founded in 1992 with the purpose to raise the scientific and clinical standards of andrology in Europe by encouraging basic/translational research in all fields of andrology and focusing this work on areas of clinical importance.

At the global European level, the EAA is the main organization dealing with the promotion of education in andrology. The EAA aims to provide education in the prevention, diagnosis, treatment (medical and surgical) and rehabilitation of ALL andrology-related diseases or pathological conditions, including male infertility, contraception, sexual dysfunction, endocrine and metabolic diseases, genital tract infections/ inflammations, as well as testis and prostate cancers. To accomplish this objective, EAA directs 25 Training Centers in Europe, one in Egypt and one in the USA (Los Angeles) The education is based on 18 months of clinical training in a certified EAA training center, followed by an exit exam which qualifies the fellow as "EAA Clinical Andrologist".

The Academy also offers post-graduate courses and workshops on a regular basis and these educational activities are highly attended by young clinicians and research fellows. The EAA educational courses focus on one or more of the following macro areas: fertility and infertility, genetics/epigenetics and embryology in andrology, hypogonadism and other endocrine and metabolic diseases of andrological interest, andrological cancers, surgical andrology, sexually transmitted diseases, and sexual medicine.

The EAA is not only an organization devoted to education, it is also a platform for multicentre studies among them the highly successful European Male Aging Study (EMAS). Currently, a multicentre study focusing on "Standardization of the male genital tract colour-Doppler ultrasound parameters in healthy, fertile men" is ongoing and will allow to define normal and pathological ultrasound parameters.

Besides education, another important mission of the EAA is related to the "diffusion of knowledge in andrology". This is accomplished through: i) publishing a journal ("International Journal of Andrology" followed by "Andrology" in 2013); ii) is the organization of the EAA/EMQN External Quality control program on Y chromosome microdeletion screening; iii) organizing the European Congress of Andrology every second year.

Since 2015 among the EAA priorities are: i) the enlargement of the society mainly by increasing the number of affiliated members, especially young andrologists; ii) establishment of sponsored travel grants and EAA scholarships; iii) publication of EAA Guidelines on male infertility and sexual dysfunction-related topics; iv) accreditation of novel EAA Centers with special focus on Eastern countries and those countries which do not have yet such centers; v) promote and finance novel multi center studies and submit projects in the frame of Horyzon 2020. In November 2015 will start the EAA Ultrasound school which offers an intensive 4 weeks hand-on training to andrologists, interested in this diagnostic test.

Finally, the EAA and ESAU have a strong interest in the recognition of andrology as an independent discipline at the European level. The process for the recognition as a "Multidisciplinary Joint Committee" of the UEMS has been undertaken by the EAA President and hopefully will be achieved in the near future. This recognition is of the outmost importance in order to increase the number of fully trained andrologists e.g. specialists with global andrology knowledge.

Csilla Krausz,
President of the European Academy of Andrology

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Csilla Krausz obtained her MD in 1990 and her specialization in Endocrinology and Metabolic Diseases in 1995 at the University of Florence, Italy; and her PhD in Human Genetics in 2001 at the Institute Pasteur/ University Paris 7, France. Currently she is appointed as Professor in Endocrinology and research group leader at the University of Florence, Italy. Her first research field was spermatology with special interest in oxidative stress and sperm function tests, whereas from 1995 she is focusing on genetics and epigenetics of male infertility, cryptorchidism, testis tumor and genomic instability of the male gamete in relationship with recurrent abortion and cytotoxic therapies. She is author of more than 120 scientific publications in international peer-reviewed journals and her H citation index is 43. She has been chairperson of the Scientific Committee of the European Congress of Andrology (ECA2014) and the chairperson of three editions of Florence-Utah International Symposium of Genetics of Male infertility. She is officer of the International Society of Andrology (ICA) since 2009 and has been elected as the President of the European Academy of Andrology (EAA) in October 2014.

Fertility and cytostatic therapies: when is the right timing for natural conception?

Csilla Krausz MD, PhD,

Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy; e-mail: csilla.krausz@unifi.it

The most frequent malignancies among young men in reproductive age are testis cancer, Hodgkin's and non-Hodgkin's lymphoma and leukemia. Advances in treatment for these diseases lead to the same curative result with much lesser toxicity with respect to the initial drugs regimens. The relatively low gonadotoxicity allows to reverse temporary azoospermia few months after the therapy and in about 50% of patients sperm count may reach to several millions spermatozoa after the first year. The future welfare of the offspring conceived by a father treated with cytotoxic therapy remains a major concern for patients undergoing cytotoxic therapy and the right timing for natural conception is still uncertain and based only on few

studies. The largest epidemiological study to date, reports a significantly increased frequency of malformations in children of cancer survivals (conceived after 2 years from the cytotoxic therapy). Genetic studies on spermatozoa reported an increased frequency of chromosomal abnormalities even 24 months after the cytotoxic treatments. In addition, radio- and chemotherapy also alter sperm chromatin quality, resulting in a higher DNA damage in the male gamete that may persist for several years after the treatment; therefore, a theoretical risk of inducing pregnancies with genetically compromised sperm does exist.

The major limitation of human data on chromosomal anomalies is that there is still a limited number of studies (on relatively small study populations) with a follow up beyond 2 years. A longer follow up period would be auspicial since a persistency of high aneuploidy rate after 24 months for some chromosomes has been reported in the literature. In addition, an increased DNA fragmentation in spermatozoa after cytotoxic treatments have been also observed, persisting after 2 years in some patients.

Microsatellite loci show a relatively high spontaneous mutation rate of germline mutations and have been reported to be sensitive targets for detecting germline mutations induced by radiation and chemotherapy. Microsatellite instability (MSI) is a phenomenon characterized by small deletions or expansions within short tandem repeats at a particular microsatellite locus, relative to the "control" tissue/condition. There are a number of evidences that irradiation can lead to mini/microsatellite instability, but these data are mainly based on studies dealing with populations exposed to radioactive fallout (the Chernobyl accident) or are on murine models. Only few studies have used mini- and microsatellite to evaluate the genomic instability induced by radio/chemotherapy treatments and only one of them have measured MSI in the sperm DNA.

An overview of the literature and the presentation of unpublished data from a longitudinal and a cross-sectional study focusing on sperm DNA fragmentation (Tunel and Comet assays) and on sperm microsatellite instability will be the topic of this talk.

According to current data, the timing for natural conception should be postponed from 12 months to at least 24 months. Given that some patients show persisting genetic anomalies in their gamete even after two years, sperm DNA testing should be proposed to couples prior conception.

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Hans-Christian Schuppe earned his medical degree from Albert-Ludwigs-University in 1985. After training in immunology and internal medicine, he completed his clinical residencies in dermatology and andrology. Since 2000, he is senior physician and head of the Clinical Andrology Unit & Laboratory at the University Hospital Giessen and Marburg (Giessen), which is now part of the Dept. of Urology, Pediatric Urology and Andrology. He is associate professor of dermatology and andrology of the Justus Liebig University and member of the Hessian centre of reproductive medicine. With his special research interest in sperm morphology and function, testicular histology and immunopathology, and male genital tract infection and inflammation, he is project partner in the local research network and the international research training group Giessen-Melbourne. H.-Chr. Schuppe is a former secretary of the German Society of Andrology and president-elect of the German Society of Reproductive Medicine, moreover he is academician of the European Academy of Andrology (EAA).

Affiliation: Andrology Unit, Department of Urology, Pediatric Urology & Andrology,
Justus-Liebig-University of Giessen, Gaffkystr. 14, 35385 Giessen, Germany,
Phone: +49-641-985-43251/-43288, Fax: +49-641-985-43259
Email: hans-christian.schuppe@derma.med.uni-giessen.de

The Impact of Urogenital Infections and Inflammation on Male Fertility

Hans-Christian Schuppe[1], Adrian Pilatz[1], Hamid Hossain[2], Thorsten Diemer[1], Florian Wagenlehner[1], Martin Bergmann[3], Andreas Meinhardt[4], Wolfgang Weidner[1]

[1]Dept. of Urology, Pediatric Urology and Andrology, [2]Institute for Medical Microbiology, [3]Dept. of Veterinary Anatomy, Histology and Embryology, and [4]Dept. of Anatomy and Cell Biology, Justus Liebig University Giessen, Germany

Urogenital infections and inflammatory disorders are considered as prevalent causes of male infertility. The majority of affected patients are asymptomatic. Diagnosis is therefore based on the identification of microbial pathogens, leukocytes, and in-

flammatory mediators in semen, prostate secretions, and urine. According to these criteria, however, organ-specific localization of infection/inflammation in the male urogenital tract remains difficult. Chronic post-infectious and non-infectious low-grade inflammation in the testis and/or epididymis are most likely to be neglected. Ascending, canalicular bacterial infections with sexually transmitted bacteria or common uro-pathogens represent the most frequent etiology of inflammatory conditions within the male genital tract. Whereas the impact of chronic prostatitis on semen parameters seems to be limited, acute epididymitis results in persistent sub- or infertility in up to one third of affected patients. Apart from obstruction of the excurrent ductal system, spermatogenic failure due to testicular inflammation has to be taken into account. For the latter condition, infectious orchitis associated with systemic infections represents a hallmark. In contrast, the etiology of focal inflammatory lesions dominated by T lymphocytes and frequently encountered in testicular biopsies of infertile men remains largely unclear.

With regard to diagnostic work-up, a systematic search for microbial pathogens by means of conventional and molecular methods as well as functional characterization of leukocyte populations in urogenital secretion appears mandatory. Notably, disturbances of sperm integrity and –function may even persist after adequate antimicrobial therapy. Concerning origin and pattern of testicular inflammation associated with male infertility, assessment of cytokine gene expression profiles provides a new perspective on putative functions of immune cells and other somatic cells involved.

Unravelling the complex mechanisms underlying the pathogenesis of infection and inflammation in the male genital tract as well as dissecting their impact on fertility-related parameters is a pre-requisite for the development of innovative diagnostic tools as well as evidence-based therapeutic strategies.

**Supported by LOEWE (excellence initiative of the state government of Hessen, Germany) focus group MIBIE (Male Infertility during Infection and Inflammation; 2011-2014).*

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Davor Ježek has a double appointment: he is a full professor at School of Medicine University of Zagreb; he is also acting as a Head of Testicular Biobank at Dept. of Transfusion Medicine and Transplantation Biology, Clinical Centre "Zagreb". His main areas of research/interest are: male infertility, andrology, testicular sperm extraction (TESE), biobanking of testicular biopsies as well as genital ridge development. Currently, Dr. Ježek is a co-chair of Scientific Centre of Excellence for Reproductive and Regenerative Medicine at School of Medicine University of Zagreb. He is a project partner in the FP7 BIOCOMET project and was a principal investigator of many international and national projects. Dr. Ježek is also a project partner in newly approved BIOCHIP Horizon 2020 project. He published more than 60 publications in internationally renowned journals. Dr. Ježek is a member of many international societies in the area of reproductive medicine, including European Academy of Andrology.

Macrophages and Leydig cells in testicular biopsies of infertile men with azoospermia: friends or enemies?

Davor Ježek[1,2]

[1]School of Medicine, University of Zagreb, Department of Histology and Embryology, Zagreb, Croatia [2]Clinical Hospital Centre "Zagreb", Dept. of Transfusion Medicine and Transplantation Biology, Zagreb, Croatia

A number of studies have indicated that testicular macrophages play an important role in regulating steroidogenesis of Leydig cells and maintain homeostasis within the testis. Since non-obstructive azoospermia (NOA) is rather difficult to treat, the current paper deals with macrophages and Leydig cells in patients with that particular disorder. Methods employed included histological analysis on semi and ultra-thin sections, immunohistochemistry, morphometry and hormone analysis in the blood serum. Histological analysis pointed out certain structural changes of macrophages and Leydig cells in NOA group of patients when compared to controls. An increased presence of macrophages in the testis interstitium and, based on their ultrastruc-

tural characteristics, activation of these cells have been noted. Leydig cells in NOA patients displayed a kind of a mosaic picture: both normal and damaged Leydig cells with pronounced vacuolisation and various intensity of expression of testosterone have been observed. Stereological analysis indicated a significant increase in volume density of both macrophages and vacuolated Leydig cells, and a positive correlation between the volume densities of these cell types. The continuous gonadotropin overstimulation of Leydig cells, together with a negative paracrine action of macrophages, could result in the damage of steroidogenesis and deficit of testosterone *in situ*.

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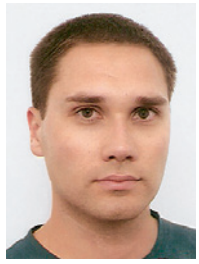

Dinko Hauptman obtained his MD in 2004 at the School of medicine, University of Zagreb, Croatia.
In 2011 he became urologist at Department of urology, University hospital center Zagreb, Croatia where he still works.
His fileds of interest are diagnosis and treatment of male infertility, reconstructive uretral surgery and kidney transplantation.

Conventional TESE – Croatian experience

Dinko Hauptman
Department of Urology, University Hospital Center Zagreb, Croatia

Testicular sperm extraction (TESE) is used in diagnosis and treatment of infertile man. Almost 15% of couples are infertile and half of them refers to man. Unfortunatelly, after thorough diagnostic in up to 30% of man we cannot find reason for infertility. After complete evaluation of infertile man which includes medical history, physical status, hormonal status, genetics, urin and sperm microbiacterial findings, we decide to perform TESE with cryopreservation.
In last two and half years we perfomred 90 TESE with cryopreservation at University Hosiptal Center Zagreb, Department of Urology in colaboration with School of Medicine. Almost all patients were operated under spinal anesthesia and only one complication was noticed as scrotal haemathoma with fever. Complete patohistological examination with immunohistochemistry is performed. Immediately after testicular tissue extraction cryopreservation is performed in controlled conditions.
In 52% cases sperms were found. Successful fertilisation after ICSI procedure was obtained in 26% and in 18% healthy born babies were achieved.
TESE in man with azoospermia represents the most complex treatment of infertile couple. It is mandatory to involve all necessary specialities, technical and legal support for sucessful infertility program. We confirm with our results that it is possible even in the most complex infertility cases to isolate good sperms for sucessful IVF/ICSI procedure.

Branko Zorn is an assistant Professor of Obstetrics and Gynaecology. He graduated from the School of Medicine in Paris in 1979.
He is a gynaecologist, specializing in andrology and assisted reproductive technology, head of the Andrology Unit, Division of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Slovenia.
His fields of interest are diagnosis and treatment of male infertility.
Dr Zorn is a member of the Slovenian Society for Reproductive Medicine, the Société d'Andrologie de Langue Française and the European Academy of Andrology.

Affiliation: Division of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Šlajmerjeva 3, 1000 Ljubljana. branko.zorn@kclj.si, 00 386 1 522 60 72.

Micro-TESE-Slovenian experience

Branko Zorn

Andrology Unit, Division of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Slovenia

Microdissection testicular sperm extraction (micro-TESE) has been proposed in non-obstructive azoospermia (NOA), where conventional TESE has been unsuccessful.
Micro-TESE technique has been introduced by Schlegel in 1999: a microscope (15-20x power) identifies the dilated tubules with foci of intact spermatogenesis.
FSH value and the surgical procedure (micro-TESE vs. conventional TESE) are the two variables significantly predicting a positive surgical sperm retrieval (SSR) rate.
Moreover, the longer the duration of micro-TESE, the higher the chances of sperm retrieval.
In maturation arrest lesions (MA), late MA and higher FSH levels were positively associated with a successful SSR.
Prospective data have shown more favourable SSR in NOA for micro-TESE, especially in histological patterns of patchy spermatogenesis such as Sertoli-cell only syndrome. However, in patients with uniform histological patterns, such as MA, outcome of micro-TESE seems less favourable.

In nonmosaic Klinefelter syndrome, SSR might be higher when using hormonal stimulation by aromatase inhibitors followed by micro-TESE.
Micro-TESE performed on the day of or the day before oocyte collection permits successful ICSI outcome. In azoospermics after chemotherapy, treatment by gonadotropins associated to micro-TESE has enabled SSR.
In infertile men undergoing orchiectomy for testicular cancer micro-TESE is superior to TESE.
Hormonal follow-up after micro-TESE SSR was recommended, particularly in patients with Klinefelter's syndrome to prevent hypogonadism.
For diagnostic purpose we performed 15 micro-TESE in 15 NOA. Four patients were affected by Klinefelter's syndrome and two have previously been biopsied by TESE without retrieved sperm. Sperm and late spermatids were retrieved in the 4 patients (28%). Histology was Sertoli-cell only syndrome and late MA.
Provided that the operating room is equipped with a microscope, micro-TESE should be considered the gold standard for SSR in NOA, especially in cases with previously unsuccessful attempts. However, classical TESE must be attempted beforehand, rendering micro-TESE unnecessary in more than 50% of men.

Ewa Rajpert-De Meyts, MD, PhD, DMSc is a research group leader in the Department of Growth & Reproduction, Copenhagen University Hospital (Rigshospitalet), Denmark. She directs a molecular laboratory performing analyses relevant for andrology and paediatric endocrinology, and a histology laboratory where she evaluates testicular biopsies. Her main research interests are: pathogenesis of germ cell tumours with focus on carcinoma in situ testis, developmental aspects of human reproduction, disorders of sex development and genetics of male infertility. She contributed to more than 200 scientific publications, most of them in international peer-reviewed journals. Her publications have been cited >8000 and her H citation index is 51.

She is a member of several societies in the field of endocrinology, biology of reproduction and andrology, and serves on editorial boards of several scientific journals. She is currently co-Chief-Editor of *Andrology*, an official journal of European Academy of Andrology (EAA) and since 2009 has been a member of the Executive Council of EAA.

Testicular Germ Cells Turning Malignant: Bad Genes or Bad Environment?

Ewa Rajpert-De Meyts

Department of Growth & Reproduction, Copenhagen University Hospital (Rigshospitalet), Denmark, e-mail: erm@rh.dk

Testicular cancer is predominantly derived from germ cells. Testicular germ cell tumours (TGCT) are rare in childhood and in older men (spermatocytic tumour), when the aetiology is genetic. However TGCT is the most common malignancy among young men (age 15-45), with the incidence dramatically rising in many countries. These tumours are derived from a precursor lesion, known as carcinoma in situ (CIS) or germ cell neoplasia in situ (GCNIS). The current model for the pathogenesis of TGCT postulates a three-hit scenario. The key first hit is the arrest of germ cell maturation at the fetal gonocyte stage, most likely due to disruption of testis development. The arrested gonocytes which retain a high expression of pluripotency

genes, subsequently acquire genomic changes and transform into malignant CIS/GCNIS cells (the second hit). The final third hit is the progression into an invasive tumour, either seminoma or nonseminoma.

Testicular cancer has a strong hereditary component, with an increased risk among the brothers and sons of cases. Targeted genetic studies and genome-wide association studies (GWAS) identified a group of genes strongly associated with a risk of TGCT. The susceptibility genes nearly exclusively operate within pathways regulating sex differentiation and germ cell development/survival (*DMRT1, KITLG, BAK1, SPRY4*), sex steroid hormone action (*ESR2, CYP19A1, CYP3A4*) as well as regulation of telomeres and some epigenetic modifications. However, the genetic factors explain only 15-20% of TGCT cases. The changing trends in incidence of TGCT and other disorders related to testicular dysgenesis syndrome (TDS), such as cryptorchidism and infertility, are consistent with the predominant influence of environmental/lifestyle factors that target gonadal development. Few exogenous factors so far have been associated with an increased risk of testicular cancer, e.g. maternal problems with pregnancy, exposure to some endocrine disrupters (organochlorine pesticides and polychlorinated biphenyls), cannabis smoking, heat and fire fighting. Taken together, the existing evidence suggests that testicular cancer is a complex polygenic and multifactorial disease. Disruption of multiple pathways have been associated with TGCT, including the androgen receptor signalling, SRY-directed cascade, the TGF-beta pathway (incl. Cripto/Nodal signalling), and the sex-dimorphic mitosis-meiosis switch (incl. DMRT1). Predisposing genomic variation and epigenetic modulation combined with different levels of exposures to environmental factors may explain the individual- and population-level differences in the prevalence of TGCT.

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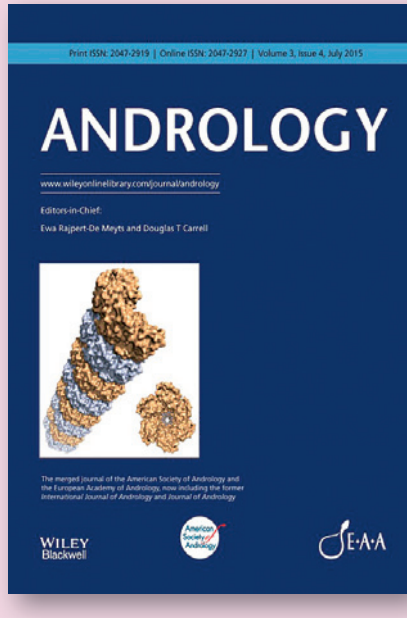

Andrology is a joint official publication of the European Academy of Andrology (EAA) and the American Society of Andrology (ASA) and replaces two previous society journals, *International Journal of Andrology* and *Journal of Andrology*, which merged together in 2012. The journal is edited jointly by Ewa Rajpert-De Meyts (EAA) and Douglas T. Carrell (ASA) and is published in collaboration with Wiley Blackwell.

The journal publishes scientific articles within the broad field of andrology and male reproductive biology, including animal models. High quality original studies, review or opinion articles and comments relevant for andrologists are welcome.

The first Impact Factor (2014) covering only one year of publications and citations is 2.298 and is expected to rise next year.

More information can be found on the *Andrology* website:
http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)2047-2927